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Flexible Estrogen Receptor Modulators

by

Helena M. Smith

A thesis presented to the University of Dublin for the degree of Doctor of Philosophy in Pharmaceutical Chemistry.

Based on research carried out under the supervision of
Mary J. Meegan BSc., PhD. (N.U.I.), M.R.S.C., C. Chem.

at

The School of Pharmacy,
Trinity College,
Dublin.

December 2004

DECLARATION

This thesis has not been submitted as an exercise for a degree at any other University. The work described is entirely my own work except where duly acknowledged.

Helena M. Smith

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Abstract

Tamoxifen is a selective estrogen receptor modulator (SERM) with a triarylethylene structure, which has estrogenic activity in bone and endometrial tissue, yet antagonises the actions of estrogen in other tissues including breast tissue. It was proposed in the present work to develop novel diaryl and triaryl analogues with a preferable, more selective activity profile than that of tamoxifen.

This thesis is presented in three different sections. The first section covers the synthesis of a number of novel, flexible and non-flexible analogues closely related to the anti-estrogen tamoxifen. The second section includes the synthesis of dihydrochalcones and subsequently, from these, the preparation of numerous highly flexible tamoxifen analogues. The structures of these products were verified using the following; TLC analysis, melting points, ^{1}H NMR, ^{13}C NMR, F^{19} NMR, and Infra-red spectrometry and Mass spectrometry.

In the third section the prepared analogues were biochemically assayed for their estrogen receptor (ER) binding and their antiproliferative, cytotoxic, and apoptotic effects on MCF-7 cells (breast cancer cells). Their estrogenic and antiestrogenic properties were assessed in Ishikawa cells (uterine cells). In the final section the structure-activity relationship of these compounds was rationalised through the use of molecular modelling techniques including the flexible docking of the novel ligands in the crystal structure of the ER-α.

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Abbreviations

AF-1	Activation Function 1	LDH	Lactate dehydrogenase
AF-2	Activation Function 2	Lit.	Literature
cAMP	Cyclic adenosine monophosphate	LRMS	Low resolution mass spectroscopy
^{13}C NMR	Carbon-13 nuclear magnetic resonance	MCF-7	Human breast adenocarcinoma
DCM	Dichloromethane	MeOH	Methanol
DMEM	Dulbecco's modified eagles medium	MORE	Multiple outcomes of raloxifene
DMF	Dimethylformamide	m.p.	Melting point
DMSO	Dimethylsulfoxide	mRNA	Messenger ribonucleic acid
ELISA	Enzyme linked immunosorbent assay	MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide
ER	Estrogen receptor	*m/z*	Mass of an ion divided by its charge
ERE	Estrogen receptor element	PBS	Phosphate buffered saline
EMEM	Eagles modified essential medium	Pd/C	Palladium on charcoal
E_2	Estradiol	PI	Propidium Iodide
EtOH	Ethanol	ppm	Parts per million
FACS	Fluorescence-activated cell sorter	S.E.M.	Standard Error of the mean
FCS	Foetal calf serum	SERM	Selective estrogen receptor
^{19}F NMR	Fluorine-19 nuclear magnetic resonance	TAM	Tamoxifen
hrs	Hours	TAT	Tyrosine amino transferase
^{1}H NMR	Proton nuclear magnetic resonance	THF	Tetrahydrofuran
HRMS	High resolution mass spectroscopy	THP	Tetrahydropyran
IC_{50}	Concentration required to produce 50% growth inhibition	TLC	Thin layer chromatography
		TMEDA	Tetramethylethylenediamine
IR	Infra-red spectroscopy	UV	Ultra-violet
LBD	Ligand binding domain		

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1. Breast Cancer and Treatment

1.1. Introduction

The normal cell cycle in the body consists of cell division, cell growth and cell death. Under normal circumstances this cycle proceeds in an orderly fashion. But occasionally cells grow out of control and form a mass of cells or a lump called a tumour, which may be cancerous[1]. In medical terms this is called a "neoplasm". How does one know if this tumour is cancerous or not? Well, there are two different types of tumour: benign and malignant. Benign[1] tumours are not cancerous. They are simply a mass of cells that do not spread to other parts of the body, or invade surrounding tissue. Therefore they are not life threatening, but if left untreated may pose a health risk as they press on surrounding organs or tissue. When removed these tumours do not reappear. Malignant[1] tumours are cancerous. They are made up of abnormal cells that can spread to other parts of the body or invade surrounding tissue. Breast cancer is a malignant tumour that develops in the breast. If this cancer spreads to another part of the body via the blood or lymph vessels it is called a metastasis (a secondary growth).

1.2. Breast cancer

794,000 women[2] are diagnosed worldwide each year with breast cancer. It is the most common cancer in women aged between 35 and 54 years and the risk increases with age. It is suggested by the Irish Cancer Society[3] that one in thirteen Irish women will develop breast cancer at some stage during their lifetime. Based on statistics gathered by the Irish Cancer Society there are 20,000 incidences of cancer in Ireland each year. According to The Department of Health[4] 1,200 of these incidences are breast cancer and that more than half of these will be fatal as statistics show that six hundred and sixty six Irish women will die each year from breast cancer.

1.2.1. Who gets breast cancer?

Basically, being a woman[1, 5] is the number one risk factor for breast cancer. But how high or low the risk[1, 2, 5] is, can be established by looking at some factors that affect it; age, history, early beginning of menstrual cycle, late starting menopause, never having children, giving birth to first child after the age of 30, drinking more than one alcoholic drink per day, being overweight after menopause or gaining weight as an adult, taking

birth control pills for 5 years or longer (which can slightly increase your risk for breast cancer), currently or recently using hormone replacement therapy (HRT) for 10 years or longer (which may slightly increase risk)[1, 2, 5]. A woman can be considered at high risk[1, 2, 5, 6] if she possesses a single risk factor that greatly increases her risk such as lobular carcinoma *in situ*, personal history of ductal carcinoma *in situ*, mutation in Breast Cancer 1 or 2 genes (BRCA 1 or BRCA 2), family history of breast cancer, atypical hyperplasia or a previous breast cancer; and past exposure to high levels of ionising radiation in the childhood through young adult years or a combination of lesser factors (such as early age at menarche and late age at having a first child) that together increase risk. Age is a major factor[5, 6]. A woman's chance of getting breast cancer increases with age. As illustrated in the Table 1.1 below, the risk for a woman born today is:

Age	Risk
By age 30:	1 out of 2000
By age 40:	1 out of 233
By age 50:	1 out of 53
By age 60:	1 out of 22
By age 70:	1 out of 13
By age 80:	1 out of 9
Ever:	1 out of 8

Table 1.1 Risk of development of breast cancer according to age

Lobular carcinoma *in situ* (LCIS)[1, 5] develops when large numbers of abnormal cells grow in the lobules. It is not invasive cancer, as it doesn't invade into surrounding tissue or other areas of the body. However, patients who have LCIS are at higher risk of developing invasive cancer in either breast.

Ductal carcinoma *in situ* (DCIS)[1, 5] develops when the cells of the duct lining take on a malignant appearance but still stay within the breast duct itself. Although DCIS is not invasive cancer, it is considered to be a precancerous condition and is nearly always curable with surgery, radiation and tamoxifen.

Atypical hyperplasia[7] – "hyperplasia" means there are extra cells lining the inside of the milk ducts and lobules of the breast. When these extra cells appear normal, it is called hyperplasia. If the excess cells look abnormal, it is described as "atypical hyperplasia" or simply "atypical cells." Hyperplasia increases a woman's risk for developing breast cancer to twice that of a woman who does not have hyperplasia. A woman's risk of developing breast cancer is 4 times greater if she has atypical hyperplasia and 8 to 11 times greater if she has a family history of breast cancer and atypical hyperplasia. The atypical cells indicate an increase in breast cancer risk and predict for a 20-25% lifetime risk of developing breast cancer.

Mutations[1, 4, 7] may be inherited or spontaneous. It's not yet known exactly how, or if, these mutations are related to a woman's lifestyle, chemical changes inside the body or exposure to environmental toxins such as radiation or chemicals. Scientists[7] have identified two specific genes that are important in the development of breast cancer. They are called BRCA1 and BRCA2. Every woman has these genes, but some women have inherited a mutated form of one or both genes. Inheriting a mutated form of BRCA1 or BRCA2 greatly increases a woman's risk of breast and ovarian cancer. However, not all breast cancer is due to inherited mutations. The likelihood that you have mutations in the BRCA1 or BRCA2 genes is greater if one or more of the following statements are true for you:

- Your mother[1, 7, 8], sister or daughter has had breast cancer before age 50 or ovarian cancer at any age
- A woman in your family[1, 7] has had both breast cancer and ovarian cancer
- A woman in your family[1, 7] has had breast cancer in both breasts
- Your family is of Ashkenazi Jewish descent[5, 7]
- There is male breast cancer in your family[1, 7]

Inherited gene mutations[1, 7], including mutations in BRCA1 and BRCA2, account for only 5 to 10% of all cases of breast cancer, while most breast cancers are due to some kind of spontaneous gene mutations.

1.2.2. Symptoms

There are many different symptoms[1, 2, 5, 8] that may indicate the development of breast cancer. The most common sign of breast cancer is a new lump or mass. A mass that is painless, hard, and has irregular edges is more likely to be cancerous, but some rare cancers are tender, soft, and rounded. Other indications are; unusual swelling, warmth, redness or darkening that does not go away, change in size or shape of the breast, dimpling or puckering of the skin of the breast, an itchy, scaly sore or rash on the nipple, pulling in of the nipple or other parts of the breast, nipple discharge that starts suddenly or pain in one spot that does not vary with your monthly cycle. These symptoms may also be due to benign breast conditions, but medical examinations and tests may be the only way to determine their cause.

1.2.3. Treatment of breast cancer

Cancer treatment varies widely depending on the type and stage of cancer, as well as the age and medical history of the patient. Treatment[6, 7, 8] may include surgery (i.e. lumpectomy or mastectomy), radiation therapy, chemotherapy, and hormone therapy. Most women diagnosed with breast cancer today can be treated in a way that allows them to keep their breasts (i.e. lumpectomy).

1.3. Breast cancer, estrogen and the estrogen receptor (ER)

1.3.1. Estrogen – estradiol

Estrogen (estradiol)[4] [1] is secreted into the blood stream and transported throughout the body. It is a lipid soluble steroid hormone that diffuses freely across plasma cell membrane into a cell where it can then bind with the ER. This interaction between estrogen and the ER takes place in the nucleus. Human estrogens, as shown in Figure 1.1, are formed from androgenic precursors through an enzymatic process known as aromatisation[9]. The most prevalent of these are 17β-estradiol (estradiol) and estrone [2]. Both are biosynthesised by developing ovarian follicles although estrone is also produced in the adrenal glands and other organs. Another minor estrogen also present in the female body is estriol[10] [3]. Estrogen[4, 6] is the most active endogenous estrogen and

is vital to the development and maintenance of the female reproductive organs (mammary gland, uterus, ovary and vagina), the reproductive cycle, various neuroendocrine functions in centres (such as the anterior pituitary and hypothalamus) and secondary sex characteristics during puberty. It is also responsible for skeletal growth and development, and fat distribution in women.

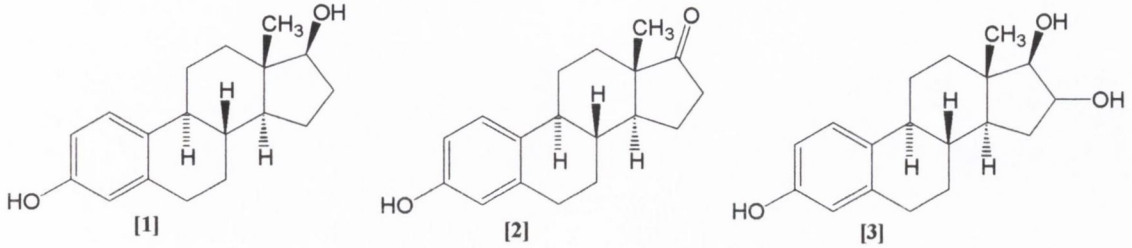


Figure 1.1 Estradiol [1], Estrone [2], Estriol [3]

1.3.2. Structure of estradiol

Estrogens[4] are steroid hormones. They possess a particular type of tetracyclic molecular skeleton as shown in Figure 1.2. Estradiol[10] is nonpolar and hydrophobic, except at its molecular termini. Its apolar surface area is 261 $Å^2$, and is a measure of the capacity of the ligand to interact hydrophobically with a binding site. Ring-A is planar. Skeletal flexibility of the estrogen exists mainly in the ring-B and it probably assumes a low-energy half-chair conformation for receptor binding. The higher energy conformer is described as boat-like and differs from the low energy form by 3.3 kcal/mol. The C-ring is in a chair conformation and the D-ring lies between an envelope and half-chair conformation.

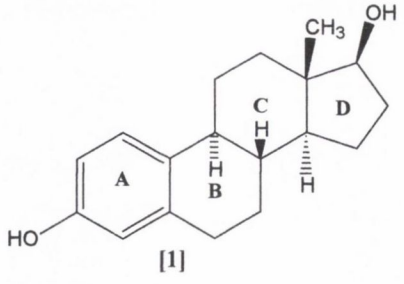


Figure 1.2 Estradiol structure

1.3.3. Synthesis and metabolism of estrogens[4]

Estrogens biosynthetically originate from cholesterol and are mainly synthesised in the

ovaries. They are also synthesised in the adrenal glands[4] and in male testes. Formation can also occur in other tissues in the body by the conversion of steroid precursors into estrogens. The principal path of metabolism of estradiol is reversible oxidation to estrone and then to estriol. Estrone can also be produced in peripheral tissues through the aromatisation of androstenedione[4], an androgen precursor produced in both the ovaries and the adrenal glands. After menopause estrone becomes the predominant estrogen as ovarian function declines and so instead of synthesis from estradiol (as it is in short supply) estrone is readily produced in adipose tissues by aromatisation of androstenedione. The metabolism of estradiol produces many metabolites with estrogenic activity. The combination of estrogens and their metabolites gives rise to an overall estrogenic effect in women.

1.3.4. Estrogen deficiency

Estrogens are known to beneficially affect lipid metabolism, skin and collagen tissue, neuronal function, and the cardiovascular system. When a deficiency in estrogen occurs an onset of hot flashes and night sweats can be triggered. In the cases of long-term deprivation, as in menopausal women or after an oophorectomy[5], more serious symptoms arise; urogenital atrophy, osteoporosis and tooth loss, atherosclerosis and coronary heart disease and a potential increase in the risk of dementias.

1.3.5. Estradiol and breast cancer

Estrogen dependent breast cancer[4] develops due to cell proliferation in the breast tissue, which is somehow caused by estrogen in an estrogen receptor interaction. It is referred to as estrogen receptor positive breast cancer. It has been long known that the menstrual cycle and breast tumour growth were connected (1836 Cooper). There are some indications in medical history that suggest this may well have been known as far back as the 14th century[12]. Breast cancer that is not estrogen related is called estrogen receptor negative cancer i.e. breast cancer caused by genetically mutated breast cancer genes (heritable) or mutations due to radiation. Its not fully understood why the estrogen-receptor interaction, which is a natural and necessary occurrence in the female body, suddenly leads to cell proliferation. Sirbasku[12] proposed in 1981 that all the stimulation of proliferation in estrogen receptor positive cancer is due to estradiol by inducing

synthesis and secretion of proteins with mitogenic activity. The ability of cancer cells to produce and secrete these proteins with growth factor activity had already been shown in 1978. These proteins[13, 9] stimulate tumour growth by binding to receptors on the same tumour cell (autocrine stimulation) or a neighbouring tumour cell (paracrine stimulation). They can also stimulate growth of stromal tissues i.e. fibroblasts and blood vessels, which release their own growth factors which can either stimulate the growth of the cancer cells or release proteolytic enzymes that promote invasion and metastasis. Although cancer cell growth is under the control of estrogen, cell proliferation is not instigated by estrogen. The following evidence though confirms its involvement in breast cancer development:

- When estrogens are added *in-vitro* to human breast cancer cell lines[4] rapid growth of the malignant clone is observed.
- After oophorectomy[5, 13] in premenopausal women with hormone sensitive breast carcinoma the tumour mass decreases.
- After 10 or more years [6] of estrogen replacement therapy the small but significant increase in breast cancer risk amongst postmenopausal women.

With this evidence its clear that estrogen plays a vital role in the continued growth of the cancer and therefore most be removed from the system by whatever means possible in order to treat many benign and malignant diseases of the breast and reproductive tract. It is also important to try and decrease production of these hormones from ovarian and extra ovarian sources by procedural or pharmacological methods as this can produce measurable reduction of tumour mass or delay disease progression. Around 60%[5] of all breast cancers are estrogen dependent. This means the cancer contains estrogen receptors and will continue to grow in the presence of estrogen. Therefore the removal of estrogen is necessary to prevent further cell proliferation.

The problem with this method of treatment is that estrogen is vital to many physiological processes[4] in the female body. Many problems can develop in consequence to the absence of estrogen. There may be an increased risk of uterine cancer. The lack of estrogen in postmenopausal women is linked to several health problems. For example, estrogen has positive effects on blood vessels and on bones. Women are at increased risk[4, 6] for heart disease and for osteoporosis, a weakening of the bones that causes them to become more vulnerable to fractures.

1.3.6. Estrogen and uterine cancer[4]

Estrogen triggers the proliferation of cells of the endometrial lining which subsequently leads to their death during menstruation. It also triggers the proliferation of cells lining the milk glands in preparation for pregnancy. Both of these are natural functions of estrogen that occur monthly. Over a span of 40 years hundreds of cycles of cell division and cell death occur. If a DNA mutation is present in any cell, estrogen will stimulate its proliferation, as normal. The repeated cycles of estrogen-induced cell division tends to increase the risk of developing cancer in the same way in the uterus as in the breast.

1.4. Estrogen and the estrogen receptor (ER)

1.4.1. Estrogen receptors

Figure 1.3 shows the distribution of the estrogen receptor and the interaction of estrogen with it in a cell. There are two types of estrogen receptor (ER)[9], ER-alpha (α) and ER-beta (β), which are distributed to different extents throughout the different target tissues. The β-isoform plays the most important role in the normal breast tissue. In contrast to previous theories, that estrogen and estrogen receptors were only found to influence reproductive tissues, and with the identification of the second ER it has been acknowledged that estrogen and ER's express their presence in many non-reproductive tissues. This discovery may explain the various symptoms of menopause where there is a deficiency in estrogen. The ER can induce or enhance the transcription of genes[9], which contain hormone responsive elements. It belongs to the nuclear hormone receptor family, which is a family of transcription factors. This ER has regions associated with DNA binding, hormone binding, receptor dimerisation and gene activation.

Figure 1.3 shows the cell with estrogen receptors, estrogen, and helper proteins: **A** estrogen receptor, **B** estrogen, **C** estrogen helper proteins, **D** cell nucleus, **E** DNA (genetic material) inside the cell nucleus.

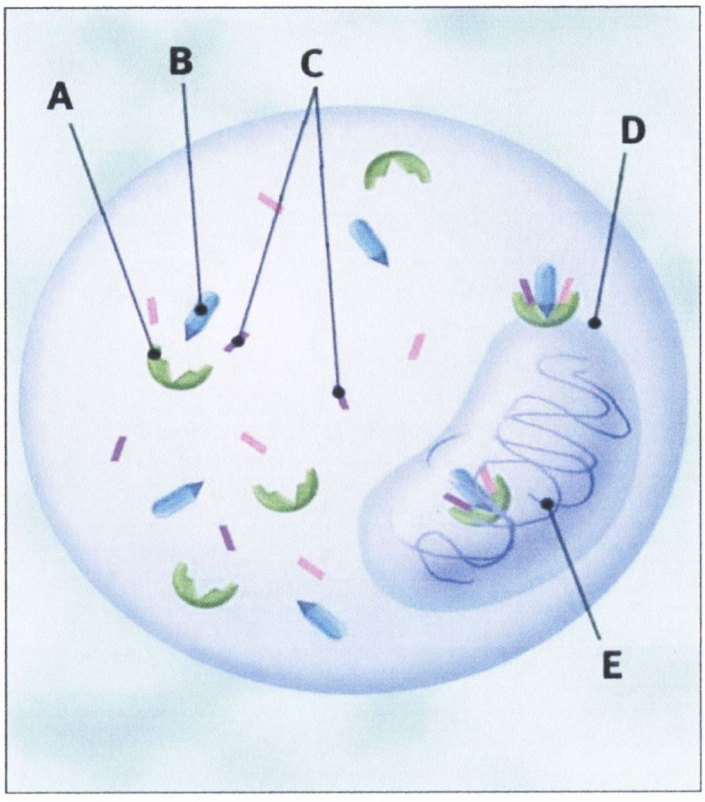


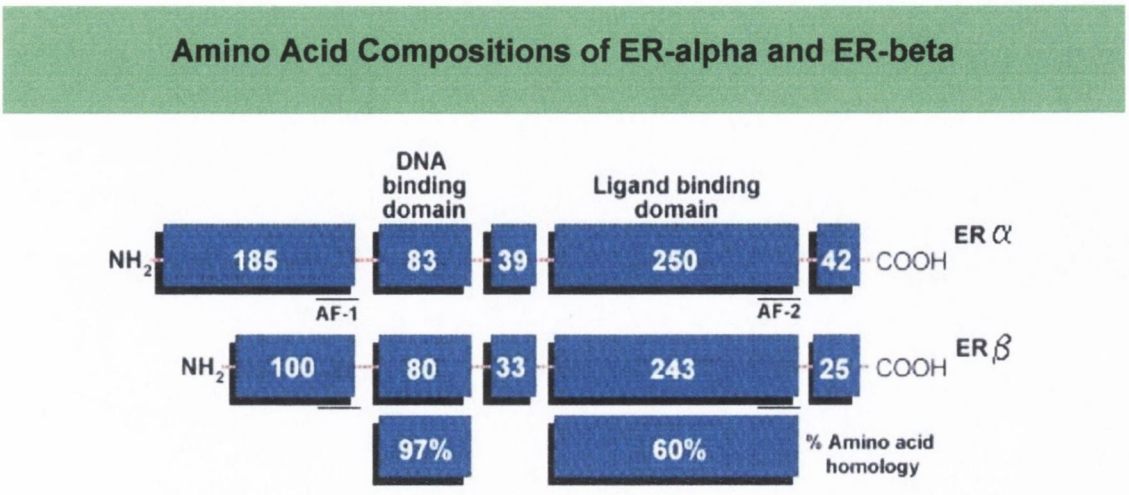
Figure 1.3 The estrogen receptor in the cell[14]

1.4.2. Structure of ER-α and ER-β

The estrogen receptor (ER-α)[9] consists of 595 amino acids with a molecular weight of 66kDa and is separated into six different functional domains. Its gene is located on chromosome 6. ER-β is located on chromosome 12. ER ligands bind to the C-terminal ligand-binding domain (LBD)[15]. This LBD recognises many different ligands, which vary in shape, size and chemical properties. These ligands can vary from endogenous estrogen estradiol to exogenous synthetic estrogen diethylstilbestrol (agonists). It also recognises tamoxifen and raloxifene, which function as antagonists. The estrogen receptor (ER-β) on the other hand has a molecular weight of 531kDa and has 530 amino acids.

Estrogen acts biologically through at least two receptors. ER-alpha and ER-beta are 97% similar in the amino acids of their DNA-binding domain as seen in Figure 1.4 below. In contrast, there is only approximately 60% similarity between their ligand-binding domains. This suggests that the two receptors interact with and activate the

same genes, and also that different estrogens or compounds may differentially bind to the two estrogen receptors.



* numbers in boxes indicate number of amino acids.

Figure 1.4 Estrogen receptors alpha and beta[9]

1.4.3. Action of the ER

When estradiol (ligand) binds to the LBD[9] of the ER in a specific tissue it causes receptor dissociation from its location in heat shock protein. The ligand/receptor complex undergoes dimerisation due to a change in conformation. Once it has dimerised the ligand-receptor complex binds to a protein (called an adaptor/promotor, specific for each tissue) that consists of specific DNA sequences, which are basically estrogen responsive elements on cell chromatin in the target genes that induce an alteration in the specific gene transcription and protein synthesis. A response[9] specific to that cell or tissue will then occur i.e. in bone tissue, where estradiol-ER dimer binds to an adaptor in the bone tissue, the tissue responds by growth and development. Additional levels of regulation are achieved through interactions with other systems such as molecular chaperones, cAMP-regulated kinases and AP1 activators. The genes with which the estrogen-ER complex interacts are called estrogen response elements (ERE). Included in this category of genes are those that encode the progesterone receptor and growth regulating proteins.

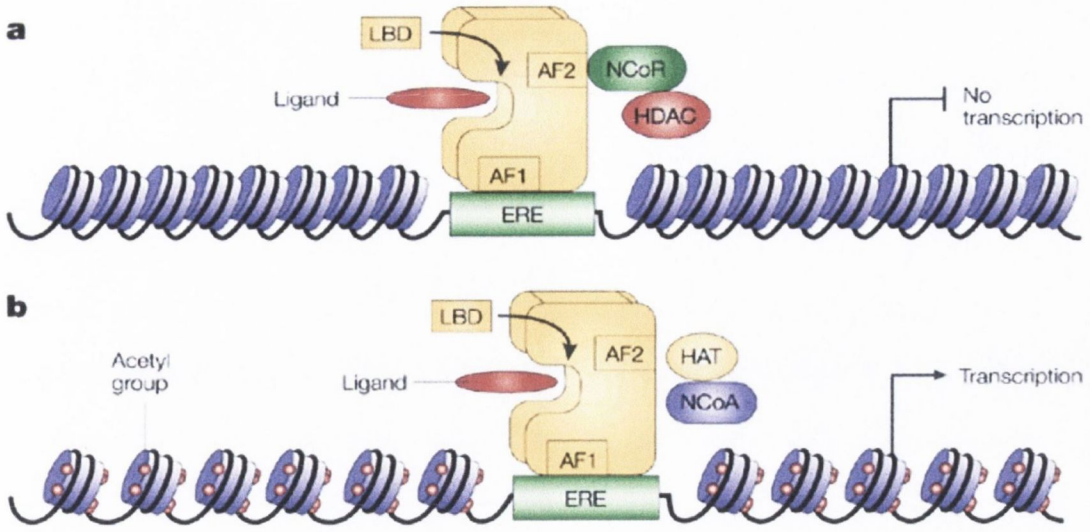


Figure 1.5 Structure of oestrogen receptor[16]

Ligand binding[17] to the oestrogen receptor causes it to bind to oestrogen-response elements (ERE) on DNA and recruit other proteins. **a** | Binding of corepressors (NCoRs) and histone deacetylases (HDACs) to the oestrogen receptor results in histone deacetylation and inhibition of transcription. **b** | Binding of co-activators (NCoAs) and histone acetyltransferases (HATs) results in histone acetylation and transcription activation. AF, activating function; LBD, ligand-binding domain.

1.4.4. ER structural domains

Both α and β estrogen receptors[18] are similar in their makeup to other members of the steroid hormone superfamily of nuclear receptors in that they are composed of independent but interacting functional domains. When a ligand binds to the receptor conformational changes occur which give rise to: dimerisation, dimer to DNA interaction, interaction with coactivators and transcription (which leads to a preinitiation complex). Each of these processes is achieved by contact with different sections of the estrogen receptor. The estrogen receptor is therefore divided into structural domains, as shown in Figure 1.5, so the functions of each domain of the receptor can be elucidated: N-terminal (AF-1) (A/B), a DNA binding domain (C), a hinge region (D), a ligand-binding domain (E) at the C-terminal end, which contains the activation function AF-2, and finally a terminal region (F). The D and F regions appear to modulate transcriptional activation by the estrogen receptor.

1.4.4.1. Structural domains A/B (AF-1) and E (AF-2)

ER contains two autonomous transcriptional activation[18] domains (see Figure 1.5). These are: AF-1 in the N terminus A/B domain and AF-2 in the LBD in the carboxy-terminal[19]. Independently but in most cases they synergise with one another in a promoter and cell context-specific manner. It is believed that ER activates gene expression by binding to estrogen response genes through the synergistic action of AF-1 and AF-2. AF-1[20] is highly variable in sequence and length and usually contains a transactivation function, which activates target genes by interacting with components of the core transcriptional machinery. The activity of AF-1 is regulated by growth factors. The amino terminus, and its activity, depends on the cellular and promoter environment, while AF-2 activity is responsive to ligand-binding. The binding of agonists triggers, as shown in Figure 1.7, AF-2 activity, whereas the binding of antagonists does not. Ligands regulate AF-2 activity by directly affecting the structure of the LBD. In contrast to this AF-1, the amino terminal region[19] is believed to be constitutively active and ligand-independent. AF-2 (E-terminal) [21, 18, 19] is multifunctional and mediates ligand-binding receptor dimerisation, nuclear translocation, and transactivation of target gene expression. Its also involved in binding to Hsp90 (D). A third putative independent function (AF-2a) is also located within the ligand-binding region. Biochemical and genetic analyses[17] have shown the conformational changes of the ligand-receptor complex to be implicated in dimerization, phosphorylation, chaperone interaction, and co-repressor inhibition.

1.4.4.2. Other domains C, D, F

The C-domain[20, 18] contains two type II zinc fingers, which are involved in DNA sequence-specific receptor binding and receptor dimerisation. It is a centrally located[19] and a well conserved DNA-binding domain. The D-domain is called the hinge region. The F-domain[19], the terminal region, plays a role in modulating transcriptional activation by ER-α (see Figure 1.5).

1.4.4.3. Dimerisation

The binding of a ligand to the receptor activates a change in the shape of the receptor and facilitates dimerisation[9]. The dimer axis coincides with the longest dimension of the LBD. Homodimerisation[22] occurs in cells containing either the ER-α or the ER-β whereas heterodimerisation[18, 22, 20] may occur in cells containing both receptors, depending on the ratio of the subtypes. Unique response elements within the target gene proteins interact preferentially with ER-β homodimers or ER-α/ER-β heterodimers.

1.4.4.4. Transactivation via phosphorylation

AF-1 is closely related to the phosphorylation[19] status of the receptor. Phosphorylation may be important for the activation of the receptor. In particular Ser-118 in the AB (AF-1) region of ER-α is important for the activation through the Ras-MAP kinase (MAPK) signalling cascade and Ser-106 and Ser-124 are two phosphorylation sites in the A/B region of ER-β, which are essential for ligand-independent activation of ER-β via the MAPK cascade.

1.4.4.5. Co activators and activation of transcription

Ligand-dependent activation[16, 17] of transcription by nuclear receptors (NRs) is mediated by interactions with coactivators (see Figure 1.5). Receptor agonists promote coactivator binding and antagonist's block coactivator binding. Coactivators[19] activate transcription of specific genes by enhancing formation of a stable pre-initiation complex. AF-2 is the most important site for coactivator recruitment. ER-α and ER-β may form heterodimers on DNA that can bind to the coactivator, SRC-1, and stimulate transcription of a target gene. The AF-1 and AF-2 domains have been shown to interact with coactivators such as: transcription factor IID (TFIID), TFIID-associated factor (TAF), $TAF_{II}30$. These coactivators do not mediate transcription they only facilitate a mechanism for exposing the required gene (see Figure 1.6). The process destabilises the DNA histone to allow RNA polymerase access to complete transcription. ERAP 160 and RIP 140 were also identified as coactivators. These coactivators have been identified as proteins that use the LBD of the nuclear receptor as a bridging function. However SRC family of coactivators are also involved in ligand-independent interaction with AF-1 in ER-α and

with ER-β through phosphorylation of AF-1 by the MAP-kinase signalling cascade. Another member of the SRC family is GRIP1[22, 18], a mouse p160 coactivator (human homolog TIF2), which when interacted with the ER-α LBD bound to diethylstilbestrol was found to bind most tightly by the NR box II (nuclear receptor box II) fragment of the protein.

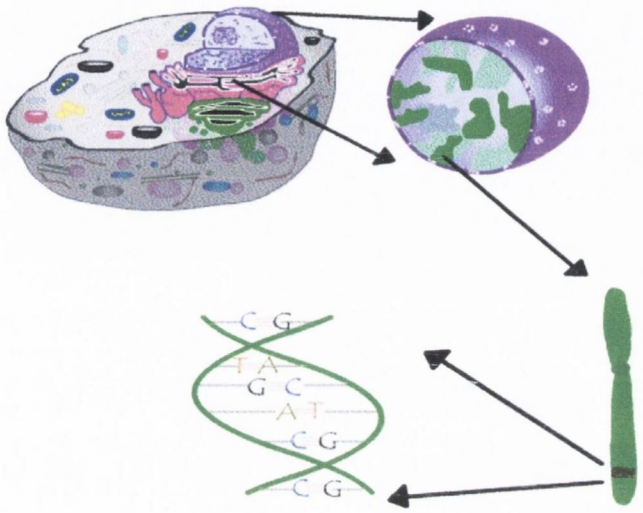


Figure 1.6 Transcription[23]

Using the mapping of mutagenesis[17], the existence of a coactivator-binding surface that includes the region around the 'signature sequence' (helices 3 and 4) as well as helix 12, where the ligand-dependent conformation of the AF-2 core is similar in all previously solved steroid/nuclear receptor LBDs. Residues in ER-α, such as Lys-362 and Val-364, in the highly conserved loop between the signature sequence have been implicated to a substructure responsible for coactivator interaction. The results suggested the existence of a coactivator-binding site encompassing the exposed surfaces of helices 3, 4 and 12 and the signature loop.

The goal of transcription, as seen in Figure 1.6, is to make an RNA copy of a gene. This RNA can direct the formation of a protein or be used directly in the cell. All cells with a nucleus contain the same exact genetic information. As discussed, only a small percentage of the genes are actually being used to make RNA at any given time in a particular cell.

1.4.4.6. Anti-estrogen-response elements through a protein[19]

It has been suggested that the shape of anti-estrogen-ER complexes dictates the silencing of AF domains but this does not explain the similarity between estradiol-like activity of tamoxifen and raloxifene in bone tissue. McDonnell and coworkers[19] suggested an alternative idea, where the anti-estrogen-ER-α complex interacts with another sequence of DNA (an anti-estrogen-response element), instead of the estrogen response element. They developed the idea by screening cDNA libraries. Yang and coworkers[19] proposed a raloxifene response element, which further developed into the suggestion that the interaction is receptor-protein and not receptor-DNA as previously thought. This receptor-protein interaction enhances the estrogen-like properties of raloxifene at a transforming growth factor promoter.

1.4.4.7. ER activation of target gene expression via AP-1 site

An alternative pathway for ER stimulation of transcription appears to be from promoters that contain an AP-1 site[20], rather than an estrogen response element. The mechanism by which this pathway works is unknown. It is believed to involve protein:protein interactions instead of DNA:protein.

1.4.5. Estrogen suitability for binding to the ER

Estradiol[10] assumes a low energy chair-like conformation when binding to the estrogen receptor through its ring-B. The hydroxy group of the phenolic ring-A contributes about 1.9 kcal/mol to the binding free energy and acts as both a hydrogen-bond donor and acceptor in the estrogen receptor-binding site. The ring-D hydroxy group contributes 0.6 kcal/mol to the binding and acts as a hydrogen acceptor. It has been suggested that the type of bonding[17] between the ligand-binding pocket of the ER are: hydrogen bonding at the two ends and Van der Waals along the body of the hormone

1.5. Agonistic and antagonistic ligand-binding to the ER

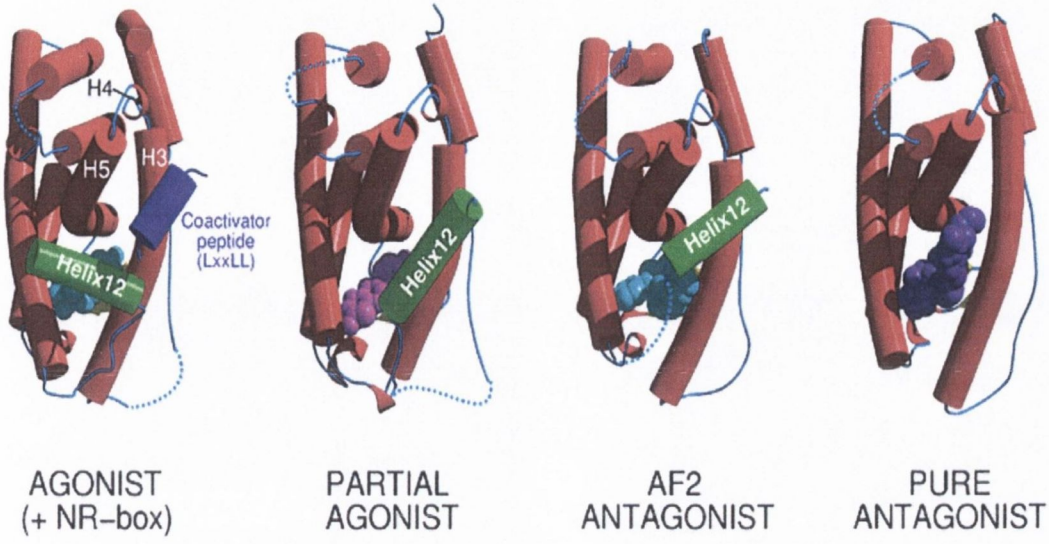


Figure 1.7: Structural basis of Estrogen receptor agonism/antagonism[24]

ER agonists and antagonists induce a different conformation in the ligand-dependent transactivation function (AF2) of ER-LBD. The crystal structures of four representative ER / ligand complexes are shown in Figure 1.7 above. The C-terminal helix (H12), which contains the AF2 core, is coloured green. In the presence of agonists (far left), H12 is aligned over the hormone binding cavity so that it contributes to the formation of a recruitment site for the LxxLL-containing NR-box motifs of coactivators (purple cylinder). Partial agonists and AF2 antagonists (middle) stabilise a LBD conformation that results in the occlusion of the LxxLL-binding site. In contrast, the pure antagonist ICI 164,384 (far right) completely abolishes interaction of H12 with the LBD. Both antagonised ER-LBDs are therefore unable to recruit coactivators essential for transcriptional activation.

The transcriptional response to hormones or antihormones is rooted in conformational changes induced by specifically bound ligands. Agonists and antagonists[24] bind at the same site within the core of the estrogen receptor LBD but they exhibit different binding modes. Also each class of ligand demonstrates a distinct conformation in the transactivation domain of the LBD. This provides evidence of the mechanism of antagonism. Estradiol [1] and diethylstilboestrol (DES) [4][24, 19] were investigated as agonists bound to the LBD of ER-α. The three dimensional structure was fully elucidated.

1.5.1.1. Agonist

X-ray studies[24, 25] of the crystal structure of the receptor bound to estradiol have provided detailed information on the tertiary structure of the ligand-binding domain of the ER-α in its agonist bound conformation. The architecture[24] of the ER LBD (helices H3-H12) is similar to that seen in the crystal structures of other nuclear receptor LBDs, and emphasizes the universal nature of this structure type within this receptor superfamily. The ER ligand-binding domain is folded into a three-layered antiparalleled α-helical sandwich with a central core layer formed from three helices (H5/6, H9 and H10). It lies between two other layers of helices (H1-4 and H7, H8, H11). This helical arrangement creates 'a wedge-shaped' molecular scaffold that maintains a sizeable ligand-binding cavity at the narrower end of the domain. The remaining secondary structural elements, a small two-stranded antiparalleled β-sheet (S1 and S2) and H12, are located at this ligand-binding portion of the molecule, and flank the main three-layered molecule.

The physical change in the receptor[25, 26] due to the binding of estradiol [1] (Figure 1.2) to it is that the helix 12 (H12) closes tightly over the ligand-binding cavity (see Figure 1.7) (an essential site for AF-2 activation) and is packed against helices 3,5/6, and 11. This folding arm forms a cavity on the protein surface called a nuclear receptor coactivator-binding site (AF-2) and facilitates nuclear transcription by the receptor by sealing the cavity and allowing coactivator binding. This presumably[27] allows AF-2 to activate gene transcription at the estrogen response element (ERE). The end of the ER, which holds the A-ring of estradiol, possesses a glutamate (Glu-353)[17] to accept the hydrogen bond donated by the estrogenic 3-hydroxyl group. Arginine (Arg-394) serves to correctly orient and position the discriminating glutamate side chain via highly polarized, water-mediated hydrogen bonds. The carbon of this arginine is itself held in place by a hydrogen bond by the carbonyl residue of the conserved phenylalanine, which is fixed by its Van der Waal bonds to ring-A of estradiol. At the other end of the ligand the 17-hydroxyl group is hydrogen bonded to the nitrogen of His-524. The variations in Van der Waals surface of the binding pocket of the ER appear designed to complement the estradiol structure with its flat aromatic A-ring and its Van der Waals surface. The binding of estradiol to the LBD of the ER by a specific network of hydrogen bonds and Van der Waals contacts retains stability in the hydrophobic interior

of the domain and allows the hormone to function as the structural core for the bottom half of the LBD, and is therefore the scaffold around which this region folds.

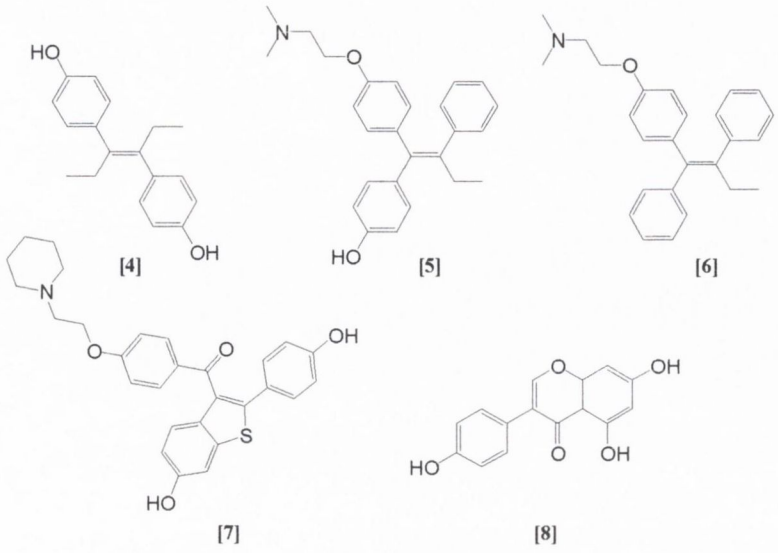


Figure 1.8 Diethylstilboestrol (DES) [4], 4-Hydroxytamoxifen (OHT) [5], Tamoxifen [6], Raloxifene [7], Geinstein [8]

The crystal structure[19, 18] of the ligand-binding domain (LBD) of ER-α was reported while bound to both the agonist diethylstilbestrol (DES) [4] (Figure 1.8) and a peptide derived from the NR box II region of the coactivator GRIP1. In the DES-LBD-peptide complex, the peptide binds as a short α-helix to a hydrophobic groove on the surface of the LBD. So except for orientation of helix 12[18] the structure of the peptide-binding groove is almost identical in the DES-LBD-NR box II peptide, and estradiol-LBD complexes.

1.5.1.2. Antagonist

When an antagonist[26] binds to the receptor this cleft is not formed as the H12 can no longer fold over the LBD (see Figure 1.7) and this prevents the receptor from fulfilling its role in transcription. Different anti-estrogens cause different conformational changes in the ER LBD and therefore affect the coactivator binding sites of the receptor, which influence the manner and degree to which the receptor will function in its transcriptional role.

The crystal structure[18, 26] of the hER-α LBD bound to the selective antagonist 4-hydroxytamoxifen (OHT) [5] (Figure 1.8) a metabolite of tamoxifen (Nolvadex; AstraZeneca plc) reported similarities between the binding of OHT and estradiol. Both ligands interact with Glu 353 and Arg 394 residues in the ER to locate the ligand correctly in the binding domain. Basically OHT is bound within the same pocket that recognises DES, estradiol and raloxifene. However like the bulky side chain of raloxifene the OHT side chain exits the binding pocket between helices 3 and 11. Interaction of the side chain with Asp 351[19] is essential for repositioning of the helix 12. In the OHT-LBD[28] complex helix 12 is prevented from being positioned over the ligand-binding pocket by the side chain. In addition the packing arrangement of the ligand-binding pocket residues permits helix 12 to reach the static region of the AF-2 surface and by doing so occludes the coactivator recognition groove by mimicking the interactions of the NR box peptide with the LBD. The differences in the interactions of raloxifene and 4-hydroxytamoxifen side chains with amino acids in helix 3 ultimately cause minor but significant differences in the positioning of the helix 12. In addition to competing for the estrogen receptor binding site[20], the anti-estrogen 4-hydroxytamoxifen reacts with a second binding site in the ER, which is not recognised by estrogen. It silences AF-2, while AF-1 remains constitutively activated.

A solution to the crystalline structure[25] of the ligand-binding domain of ER-α (after binding to raloxifene) was reported. Raloxifene [7] (Figure 1.8) binds at the same site as estradiol within the LBD with the hydroxyl group of the benzothiophene moiety mimicking the A-ring phenolic hydroxyl of estradiol by binding in the polar pocket between H3 and H6. Although the phenolic hydroxyl (O11) occupies the same position as estradiol by bonding with His-524 it is displaced by 5.1 Å. Despite similar coordination of raloxifene in the ER ligand-binding pocket to estradiol binding, the alkyl aminoethoxy side chain of raloxifene protrudes into the confines of the binding cavity between H3 and H11 displacing helix 12 via direct interaction with aspartate 351 of ER-α, and so the helix[24] lies in a groove formed by H5 and the carboxyterminal end of H3. The positioning of H12 clearly disrupts the overall surface topography of AF-2 and presumably renders it incapable of activating ERE-driven gene transcription. This observation physically accounts for the well-known ability of ER antagonists, such as raloxifene, to block estradiol activation of ERE-driven genes by blocking activity.

The X-ray crystal structures[21] of the estrogen receptor (ER-β) ligand-binding domain (LBD), in the presence of the phyto-estrogen geinstein [8] (Figure 1.8) and the antagonist raloxifene [7], were reported by Pike *et al.* The overall structure of the ER-β-LBD is very similar to that previously reported for ER-α. Genistein is an isoflavanoid phytoestrogen found in soya products and in contrast to raloxifene genistein is completely buried within the hydrophobic core of the receptor and binds in a similar way to that observed for estradiol. However, in the ER-β-genistein complex, H12 does not adopt the distinctive 'agonist' position but, instead, lies in a similar orientation to that induced by raloxifene. The difference in their orientations relative to their hydroxy-hydroxy axis is around 20°, so that the respective hydroxyls that interact with His-475 are separated by 3.9 Å. Such an alignment of the transactivation helix is consistent with genistein's partial agonist character in ER-β and demonstrates how ER's transcriptional response to certain bound ligands is attenuated.

1.5.1.3. The estrogen receptor role in tumour growth

ER-β is the more dominant receptor[27, 29, 18] in normal breast tissue and where there is very little ER-α present. It appears to exhibit a protective function over the normal breast tissue by playing the opposite role to the ER-α. It acts negatively towards estrogen and therefore does not encourage cell proliferation in normal cells or mutated potentially cancerous cells. Heterodimerisation[22] is thought to be another means by which ER-β exhibits its negative regulatory role over estrogen. In breast cancer tissue ER-α is the more dominant receptor and because it acts positively towards estradiol it somehow causes the growth of the tumour. Despite the role of estrogen in the proliferation of the breast neither of the ERs are present in the epithelial cells, which divide in response to estrogen. The mechanism[18] through which estrogen induces epithelial growth is not clear. A concept is that estrogen stimulates growth factor secretion from breast stroma and this in turn stimulates epithelial cell growth. The analysis of estrogen receptor status in breast cell carcinomas was performed via an enzyme linked immunosorbent assay (ELISA)[27, 29, 18]. Results of the assays showed in primary breast cancers ratio of the presence of ER-α to ER-β is 65:40 percentage and in fibroandenomas the ratio of the α to β receptor presence was 55:68%.

1.6. Selective estrogen receptor modulators (SERMs)

SERMs are designer estrogens, which are estrogen-like in some tissues and block the action of estrogen in others. Researchers predict that these SERMs will demonstrate all the beneficial characteristics of estrogen but will prevent the negative cancerous effects.
SERMs are classified in many different groups:

1. Hydroxy tamoxifen analogues
2. Halogenated analogues of tamoxifen
3. Tamoxifen structure related analogues
4. Structurally diverse estrogen receptor modulators
5. Steroidal antiestrogens

1.6.1. Tamoxifen[30]

1.6.1.1. Introduction

Tamoxifen (see Figure 1.8)(trade name Nolvadex)[4, 5, 6, 26, 31, 32] is an orally active drug that acts as an antagonist of estrogen. Due to the pioneering work of Lerner[33] and co-workers the biological properties for the first non-steroidal antiestrogen, Ethamoxytriphetal (MER 25), were discovered. Subsequently, in the years to follow, ranges of antiestrogens were synthesised and tested for their effectiveness in treating breast cancer. Following this, tamoxifen was developed in 1969 by ICI pharmaceuticals[34] (now Zeneca) as a potential oral contraceptive but was found to stimulate ovulation and so was used as a fertility drug. Eventually tamoxifen's efficacy in treating breast cancer (and relative lack of toxicity) was realised. Tamoxifen has been used to treat both advanced and early stage breast cancer for nearly 20 years. More recently, it also is being used as adjuvant, or additional, therapy following primary treatment for early stage breast cancer. More importantly it meant tumour suppression and the same hormonal effects could be achieved without performing surgeries that would remove the body's supply of estrogen (ovariectomy, adrenalectomy, and hypophysectomy). Tamoxifen is clinically used as the Citrate derivative[35] under a variety of trade names, with Nolvadex still the market leader, with sales over $500 million world wide, per annum.According to a recent study published in The Lancet[36] (May 16, 1998) it was found that taking tamoxifen for five years significantly reduces both breast cancer recurrence (42%) and mortality (22%) for all women. Results found premenopausal

women, not just postmenopausal women, and those whose breast cancer has spread to the local lymph glands benefit substantially from tamoxifen therapy. Although tamoxifen blocks the effect of estrogen on breast tissue, it acts like a weak estrogen in other body systems. This means that women who take tamoxifen may share some of the beneficial effects of taking estrogen replacement therapy, such as a decreased risk of osteoporosis[4, 6, 9] and a decreased risk of heart disease[4, 9]. Although there are side effects to taking tamoxifen they are mild compared to the alternative treatments available and the rate of survival is greater.

1.6.1.2. Structural elucidation of Tamoxifen

Tamoxifen (see Figure 1.8), triphenylethylene, [1-[4-(2-dimethylaminoethoxy) phenyl]-1,2-diphenyl-but-1-ene][26] consists of two isomeric forms with the *trans* (Z) isomer exhibiting the antagonist properties and the *cis* (E) form, the agonist, exhibiting partial estrogen-like properties. Both isomers are metabolised to 4-hydroxytamoxifens in the body and it is in this form that they express highest affinity for the estrogen receptor. *Trans*-4-hydroxytamoxifen[37] has a binding ability equal to or greater than estradiol. The *cis* form on the other hand starts as an agonist but in its hydroxy form it acquires antagonist properties. The *cis* and *trans* isomers were distinguished between by proton magnetic resonance spectroscopy (^{1}H NMR) and dipole moment measurement. The protons of the *trans*-isomer were shifted slightly further up field than those of the *cis*-isomer. This effect was even more pronounced in the aromatic protons it was also observed that the OCH_2 protons of the side chain have a chemical shift of less than 4.0ppm because of the double shielding effect it experiences due to being sandwiched between two other rings. X-ray crystallography demonstrates the conformation of the triphenylethylene structure to be propeller like, as more than 50° twists the rings out of the plane of the double bond[37].

1.6.1.3. Other pharmacological actions of tamoxifen

Other ways in which tamoxifen is thought to inhibit the growth of tumour cells are outlined below:

- Insulin-like growth factor I (IGF-I)[38] was shown to be a potent mitogen for breast cancer cells *in-vitro*, and IGF-I receptors have been demonstrated on human

primary breast neoplasms. In a randomised, placebo-controlled study, it has been found that administration of the anti-estrogen tamoxifen to patients with breast cancer was associated with a statistically significant (P=0.002) reduction in the serum level of IGF-I and this reduction may contribute to the therapeutic effect of the drug.

❖ Calmodulin[27, 39, 30], a calcium-dependent regulatory protein of numerous cellular processes, including proliferation, interacts directly with tamoxifen.

❖ Inhibition of CAMP phosphodiesterase activation[30]. Since this is a key component of the second messenger system and regulates the metabolism of cyclic nucleotides, the inhibition of breast cancer cell growth may be due to the antagonism of calmodulin by tamoxifen.

❖ Production of the enzyme protein kinase C[40] that plays a key role in tumour promotion, was reported to have being inhibited by tamoxifen, this is thought to contribute to the anti-tumour action of the drug.

❖ The negative growth factor TGF – β[41] production is induced by tamoxifen, a factor which has been proven effective in decreasing the growth of estrogen (+) and (-) tumours.

❖ It is apparent that at least one other anti-tumour effect of tamoxifen is mediated through induction of polyamine depletion[42]. This action has been recorded both *in-vitro* and *in-vivo* and may be produced by tamoxifen inhibiting the rise of ornithine decarboxylase, which leads, in MCF-7 tumours, to a dose related decrease of putrescine and spermidine.

❖ In Ishikawa (endometrial adenocarcinoma) cells[32], 4-hydroxytamoxifen inhibits significantly cell proliferation and simultaneously decreased both the TGF-alpha mRNA and TGF-alpha secretion.

❖ There are other widely documented estrogenic and non-estrogenic-related methods by which tamoxifen may exert its action in inhibiting tumour cell growth. Yet while many theoretical proposals have been put forward to explain the mode of action of tamoxifen, the exact pharmacological actions of it remain difficult to define. There is some new evidence that suggests a positive clinical response to the drug by estrogen independent breast cancer patients.

1.6.1.4. Beneficial aspects of tamoxifen

The fact that tamoxifen blocks the action of estrogen in breast tissue while mimicking the action of estrogen in the uterus means that it functions as a SERM[15], selectively blocking or stimulating the estrogen receptors of different target tissues. In addition to acting like estrogen in the uterus, tamoxifen resembles estrogen in its ability to lower LDL cholesterol levels[43], improves cardiovascular profile and cognitive function, preventing against colon cancer. In postmenopausal women, tamoxifen also resembles estrogen in its ability to preserve or increase bone density[26] it therefore maintains the skeleton integrity also protecting against tooth loss and macular degeneration. Thus, aside from its tendency to increase the risk of uterine cancer, tamoxifen has a number of potentially beneficial properties. Tamoxifen is not only used to treat cancer but also to prevent it, especially in high breast cancer risk women. High-risk women are advised to undergo closer surveillance and use antiestrogen drug therapy. Without tamoxifen prophylactic mastectomy would have to be preformed maybe unnecessarily if no cancer was ever to develop. In a recent study conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP)[44], the antiestrogen therapy tamoxifen was found to reduce the risk of breast cancer development by 50% in the group of high-risk women studied. In addition, five years of tamoxifen treatment decreased breast cancer risk by 86% in women who had a history of atypical hyperplasia.

Deciding whether the potential benefits of risk reduction antiestrogen therapy outweigh the potential downsides can be a difficult decision for high-risk women and their physicians. Recent research also compared five years of adjuvant tamoxifen therapy with more than five years of tamoxifen. This indicated no additional benefit is obtained from extending the duration of tamoxifen therapy past five years. Additionally, the medical literature on adjuvant therapy shows benefits as long as 10 to 15 years after starting tamoxifen or 5 to 10 years after stopping it. Considering all the current research data, five years of tamoxifen therapy provides the optimum benefit for those at high risk of developing breast cancer.

1.6.1.5. Disadvantages to using tamoxifen (carcinogenicity and toxicity)

It is believed that tamoxifen may increase the risk of uterine cancer[45] and there have been some reports of uterine cancer in women taking tamoxifen. However, the incidence

is less than 1% in women taking doses of 20 mg daily. Most cancer specialists believe that the benefits of tamoxifen outweigh the risk of developing uterine cancer. But despite the benefits of tamoxifen the risks and other side effects must be acknowledged and considered in the case of each patient:

- Tamoxifen[46] mimics the action of estrogen on other tissues i.e. the uterus
- It can stimulate proliferation of the uterine endometrium and therefore increase the risk of uterine cancer[47].
- On a less serious level some women will experience hot flushes[47], irregular menstrual periods, vaginal discharge or bleeding, and irritation of the skin around the vagina. These symptoms are similar to those of the menopause[48].
- Increases blood clot risk[48].

1.6.2. Tamoxifen analogues

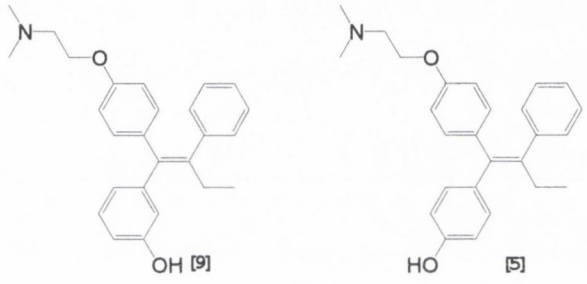


Figure 1.9 Droloxifene [9], 4-Hydroxytamoxifen [5]

1.6.2.1. Droloxifene (3-hydroxytamoxifen)[49, 50]

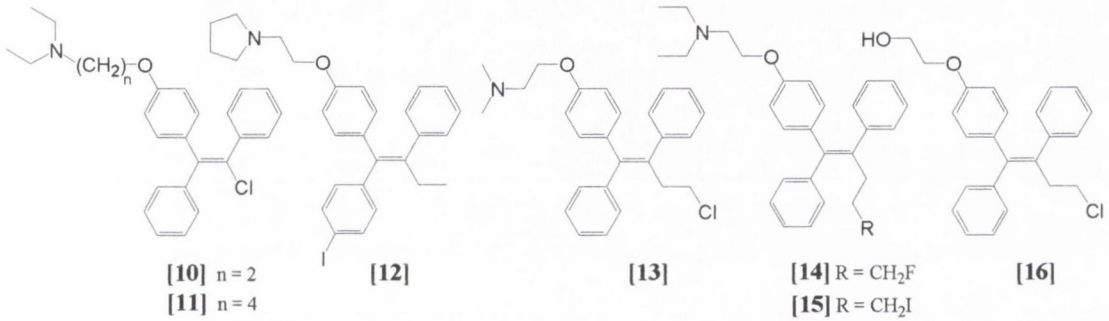
Droloxifene[49] [9], as seen in Figure 1.9, or 3-hydroxytamoxifen was initially developed to treat atherosclerosis. It possesses the triphenylethylene core structure and is a full antagonist in breast tissue, having a possible role in treating metastic breast cancer. Multiple phase II trials results from women with advanced breast cancer receiving 20, 40, and 100 mg/day droloxifene, showed response rates of 17-31%, 30-44%, and 31-42%, respectively. When droloxifene was compared with tamoxifen, it was found that droloxifene has less of an effect on rat uterus, indicating a higher therapeutic index. Droloxifene also more potently inhibits the growth of breast cancer cell lines than tamoxifen. Droloxifene has a beneficial effect on BMD, preventing a decrease in femoral BMD and trabecular bone volume in estrogen deficient ovariectomised rats.

Droloxifene[50] has a 10-60-fold higher binding affinity to the ER compared to the related compound tamoxifen and showed a greater inhibition of ER positive human breast cancer cells proliferation than tamoxifen.

1.6.2.2. 4-Hydroxytamoxifen[32]

4-Hydroxytamoxifen[32] (4OHT), as seen in Figure 1.9 [5], the active metabolite of tamoxifen with the triphenylethylene core structure, possesses an 8 times higher relative binding affinity (RBA) than TAM, and about 100 times higher antiestrogenic potency in hormone independent MCF-7-2a ER negative breast cancer cells, stably transfected with the plasmid $ERE_{wtc}luc$.

1.6.2.3. Halogenated analogues of tamoxifen[51]



[10] n = 2 **[11]** n = 4 **[12]** **[13]** **[14]** R = CH_2F **[15]** R = CH_2I **[16]**

Figure 1.10 Clomiphene [10], MDL 103,323 [11] Idoxifene [12], Toremifene [13], N,N-Diethylfluoromethyltamoxifen [14], N,N-Diethyliodomethyltamoxifen [15], Ospemifene [16]

1.6.2.4. Clomiphene[52]

Clomiphene [10][52] is an antiestrogen used for ovulation inducement in fertility treatment and estrogen dependent breast cancer treatment. The highly active 4-hydroxy metabolite formed *in-vivo* isomerises readily. In infertility treatment it produces an increase in estrogen and gondotrophin secretion, which induces ovulation. The clomiophene then binds to the estrogen receptor causing a blockade to the feedback inhibition exhibited by estrogens.

1.6.2.5. MDL 103,323[49]

MDL 103,323 is a derivative of clomiphene [11][49], which is used clinically as an infertility drug to induce ovulation in anovulatory women. It was found to be an inhibitor of MCF-7 human breast cancer cell proliferation. It also exhibited effects on bone and is currently being studied as an osteoporosis treatment. In rats, MDL 103,323 increased BMD in the lumbar spine, tibia and femoral neck, with the greatest effect on the lumbar spine.

1.6.2.6. Idoxifene[49]

Idoxifene [12][49] is a novel SERM with a triphenylethylene core structure. It is metabolically more stable than tamoxifen with a higher binding affinity for the estrogen receptor and reduced agonist activity in breast and uterine cells. Like tamoxifen, idoxifene has been shown to decrease tumour size, prevent bone loss, and decrease LDL-cholesterol levels. It has a 2.5 fold higher binding affinity for the ER than tamoxifen, causes no proliferative changes in the endometrium. Idoxifene also has effects on bone serum lipid metabolism. Adverse effects in the phase I/II trials were nonhaemorrhagic vaginal discharge (second only to headache).

1.6.2.7. Toremifene[5, 25, 30, 49]

Toremifene [13][5,30,49], a triphenylethylene, is also know as chlorotamoxifen (fareston), is the first antiestrogen to be approved for the treatment of stage IV postmenopausal breast cancer since the introduction of tamoxifen. This is a chlorinated version of tamoxifen that exhibits similar that exhibits similar clinical efficacy to tamoxifen. It doesn't produce DNA adducts in rat liver and therefore has a lower carcinogen potential than tamoxifen. Its estrogenic activity involves altering lipid levels, which can reduce the risk of coronary heart disease and help prevent bone loss.

1.6.2.8.N,N-Diethylfluoroethyltamoxifen and N,N-Diethyliodomethyltamoxifen[51]

N,N-diethyl halogenated analogues[51] of tamoxifen such as [14] and [15], as seen in

Figure 1.10, have been evaluated for imaging estrogen receptors using positron emission tomography (PET) or single photon emission computed tomography (SPECT). These analogues have been found to have a greater binding affinity for the estrogen receptor than then N,N-dimethyl tamoxifen analogue. N,N-Diethylfluoroethyltamoxifen [14][51] exists as two isomers just like tamoxifen with the Z isomer having the greater affinity for the estrogen receptor. However the E-isomer has been found to be more potent. This iodo-analogue[51] [15] shown in Figure 1.10 is useful for predicting the response of estrogen-receptor-positive breast cancer to tamoxifen analogues used in chemotherapy. It binds to the receptor with fifteen times greater affinity than tamoxifen but only half that of the fluoro analogue. This compound is more potent though and the E-isomer exerts a greater cytostatic effect than tamoxifen or the fluoro analogue

1.6.2.9. Ospemifene[49]

Ospemifene [16][49] (formerly named FC-1271a) is a novel triphenylethylene being developed for the treatment and prevention of osteoporosis. Since ospemifene is very similar to tamoxifen, it is also being studied for its effects on breast tissue. In a recent study, MCF-7 cells were treated with ospemifene and growth inhibition was seen, indicating that it is antiestrogenic in breast cancer cells. Its ability to inhibit cell growth was compared with toremifene and raloxifene. In an *in-vitro* study using MCF-7 ER-dependent human breast cancer cells and ZR 75-1 breast cancer cells, ospemifene did not cause an increase in cell growth at concentrations of 0.1 nM and 10 μM, as compared with control. In an *in-vivo* study, rats with DMBA-induced tumours were treated with ospemifene or placebo. Rats treated with ospemifene had fewer numbers of breast tumours than control rats. Ospemifene has an estrogenic effect on bone and is currently being developed to treat osteoporosis. It was able to prevent bone loss in the ovariectomized rat by suppressing bone turn over and reducing the number of osteoclasts.

1.6.3. Tamoxifen structure related analogues

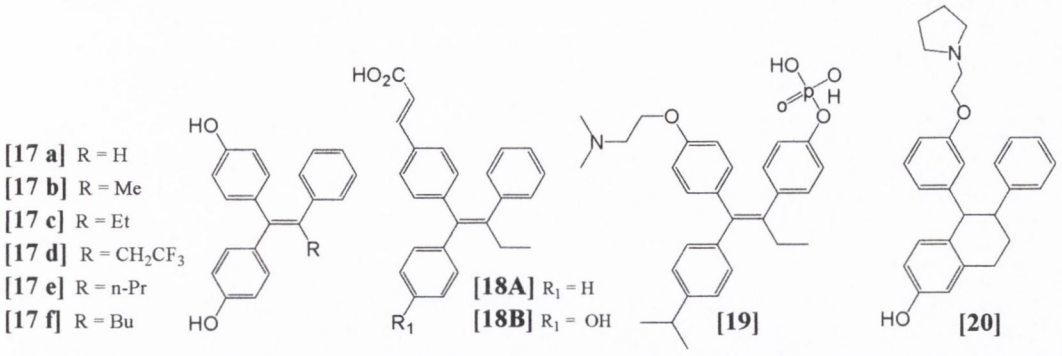


Figure 1.11 1,1-Bis(4-hydroxyphenyl)-2-phenylethenes [17a-f], GW5683 [18A], GW7604 [18B], Tat-59 [19], Lasofoxifene [20]

1.6.3.1. 1,1-Bis(4-hydroxyphenyl)-2-phenylethenes[53]

In a study by Lubczyk *et al* on estrogen receptor antagonists, a series of 1,1-bis(4-hydroxyphenyl)-2-phenylethenes [17a-f][53] were produced by varying the R group by size and in one case introducing a trifluoromethyl group. All the compounds exhibited antiestrogenic activity but the most active of these [10d] and showed the same antagonistic potency as 4OHT and almost 50 times more active than tamoxifen (see Figure 1.11).

1.6.3.2. GW5683[54]

This non-steroidal tamoxifen derivative possesses an acrylic acid side chain and not a tertiary nitrogen group. GW5683 [18A] (see Figure 1.11) is a prodrug that is converted to its active metabolite, GW7604 [18B][54], similar to the way tamoxifen is converted to 4-hydroxytamoxifen. It is an antiestrogen with less estrogenic activity than tamoxifen and yet maintains its agonistic activity in bone. Also it was found that this drug had low potential to stimulate the proliferation of endometrial cells and therefore might not increase the incidence of endometrial cancer in patients. It is now being tested on animal models of human drug resistance to tamoxifen.

1.6.3.3. Tat-59 (miproxifene)[25]

Tat-59 [19][25] is a prodrug that is being developed for the treatment of breast cancer. It

contains a hydroxyphosphate group and is activated metabolically to a dephosphorylated form that binds to the estrogen receptor. This compound shows potential for use in the treatment of breast cancer as it has been shown to inhibit cancer cell growth in mice.

1.6.3.4. Lasofoxifene[49]

Lasofoxifene [20][49] also a novel SERM, has been shown to be a very promising new agent having positive effects on bone and lipid metabolism, without having an effect on uterine growth. It was also shown to decrease serum cholesterol levels in both male and female rats. Preclinical studies show lasofoxifene binds to oestrogen receptors with an affinity comparable to that of 17β-estradiol and, in bone, duplicates many of the effects obtained following administration of oestrogen. These properties suggest efficacy in osteoporosis and this is indeed confirmed in phase II trials. Compared with raloxifene, lasofoxifene appeared more effective in improving spinal bone density in post-menopausal women. More recent phase II data show that the improvements in bone density are sustained after two-years dosing with lasofoxifene

1.6.4. Structurally diverse estrogen receptor modulators

1.6.4.1. Triarylpyrazoles[55]

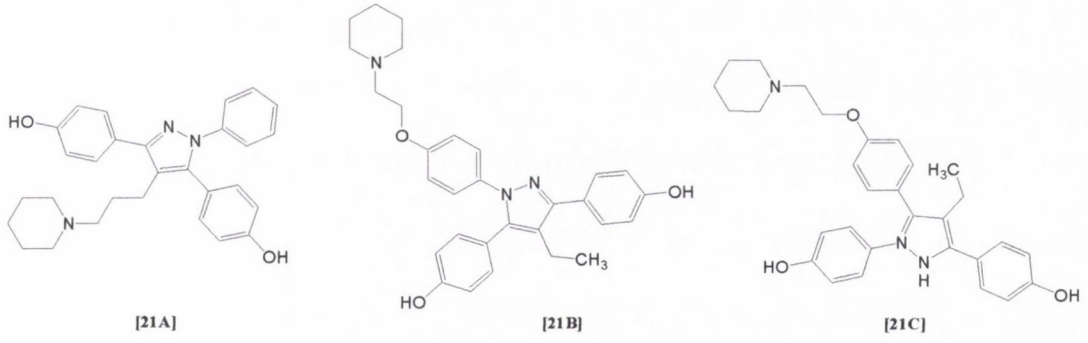


Figure 1.12 Triarylpyrazoles [21A-C]

The triaryl-substituted pyrazole ligand system[55], has been identified as a high affinity ER ligand. A series of ER ligands [21A-C] were developed using this ligand as the core structure with the addition of a basic side chain *N*-piperidinyl-ethyl as seen in Figure 1.12 above. The compound with the highest binding affinity was the C-5 piperidinyl-

ethoxy-substituted pyrazole [21C]. Also it bound to the ER-α with 20-fold higher affinity than to the ER-β.

1.6.4.2. Diazenes (pyrazines, pyrimidines, pyridazines)[56]

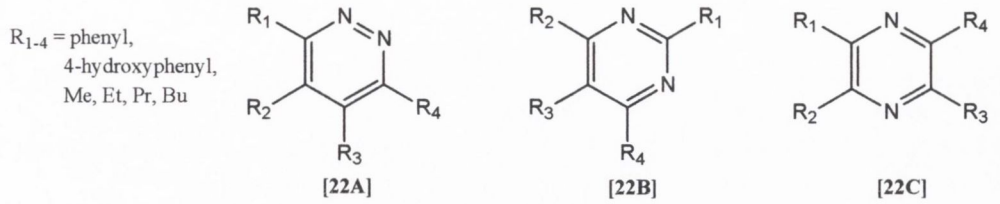


Figure 1.13 Pyridazines [22A], Pyrimidines [22B], Pyrazines [22C]

The diazene cores are common features of natural products and drugs. A series of diazenes[56] [22A-C], seen in Figure 1.13 above, bearing aromatic and aliphatic substituents at the R positions that were patterned after the structures of the high affinity pyrazoles were produced and tested for ER binding ability and selectivity. These compounds were not better at ER-α binding than the pyrazole and furan series. The ER ligands based on five-membered heterocycles, namely pyrazoles and furans, were found to be better at ER binding. A pyrimidine analogue [22B] with R_1 as 4-hydroxyphenyl and aryl groups at positions R_2, R_3 and R_4 expressed high ER-α binding affinity. A pyrazine derivative [22C] with a similar substitution pattern also exhibited high affinity for the ER. These analogues may have potential for the development of novel estrogen pharmaceuticals.

1.6.4.3. Indoles

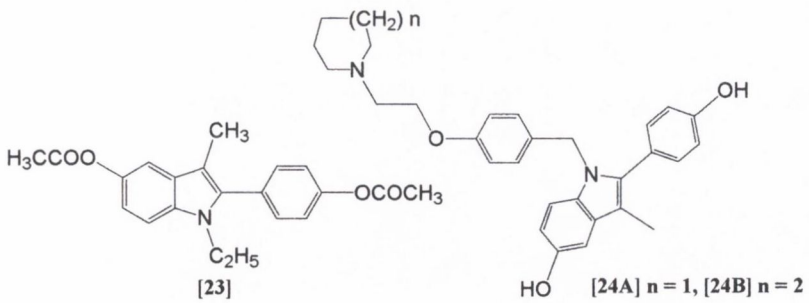


Figure 1.14 Zindoxifene [23], ERA-923 [24A], TSE-424 [24B]

1.6.4.3.1. Zindoxifene[57]

Zindoxifene [23][57] is an acetylated indole, which is hydrolytically cleaved to produce a dihydroxy-indole (D15414), displayed in Figure 1.14, with a high affinity for the estrogen receptor. It has mixed agonist and antagonist properties and inhibits growth of DMBA-induced rat mammary carcinoma. Clinical results of the drug though have been disappointing failing to produce any objective responses.

1.6.4.3.2. ERA-923 [24A], TSE-424 [24B][58]

Using the stilbene-like indole core structure[58] as a template, a series of compounds [24A, 24B], as seen in Figure 1.14, were produced as potent SERM's. After various explorations of rigidifying the core structure, by placing linkers between the amine terminus and the indole nitrogen, ERA-923 and TSE-424 were produced. Both compounds displayed very attractive selective estrogen profiles. ERA-923 is currently in phase II clinical trials for the treatment of hormone-dependent metastatic breast cancer, while TSE-424 has completed phase II clinical trials for the prevention and treatment of postmenopausal osteoporosis.

1.6.4.4. Benzothiophenes

1.6.4.4.1. Raloxifene[5,19, 15, 26, 31, 49]

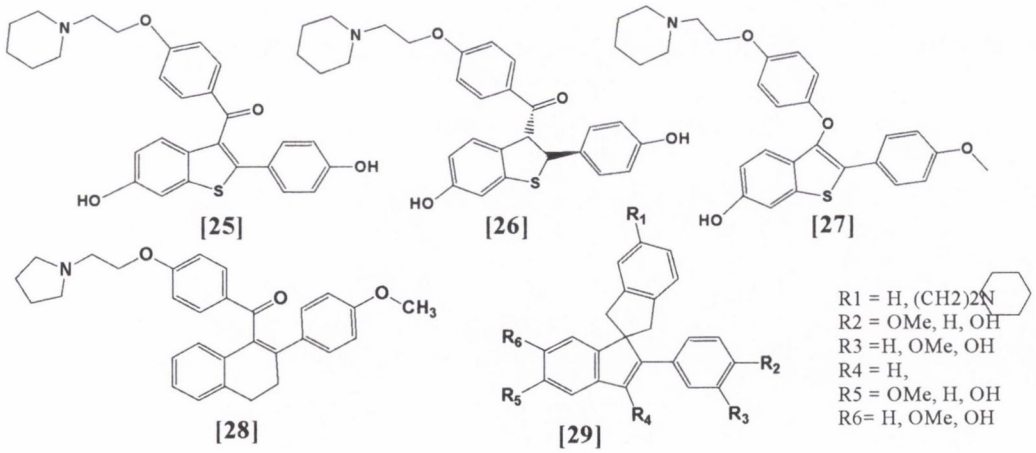


Figure 1.15 Raloxifene [25], dihydroraloxifene [26], arzoxifene [27], trioxifene [28], 2-phenylspiroindines [29]

contains a hydroxyphosphate group and is activated metabolically to a dephosphorylated form that binds to the estrogen receptor. This compound shows potential for use in the treatment of breast cancer as it has been shown to inhibit cancer cell growth in mice.

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1.6.4. Structurally diverse estrogen receptor modulators

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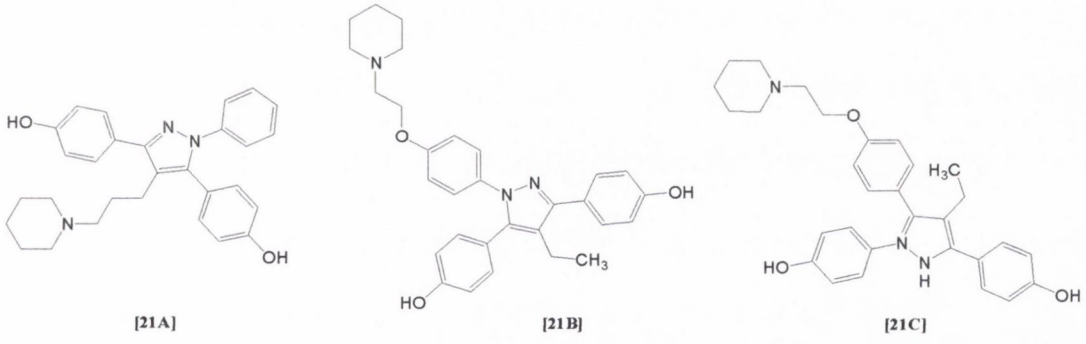

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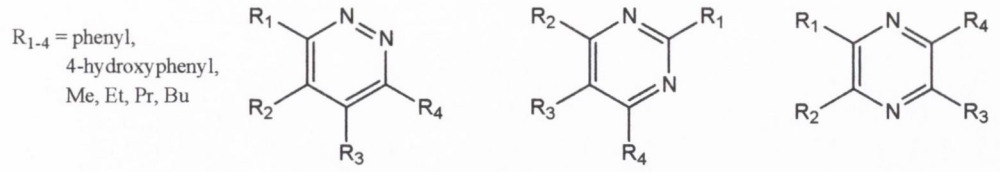

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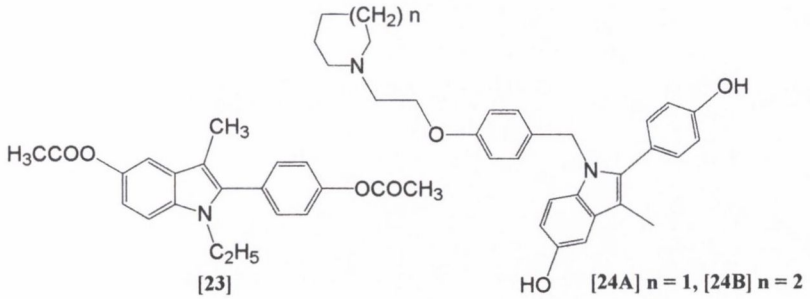

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1.6.4.4. Benzothiophenes

1.6.4.4.1. Raloxifene[5,19, 15, 26, 31, 49]

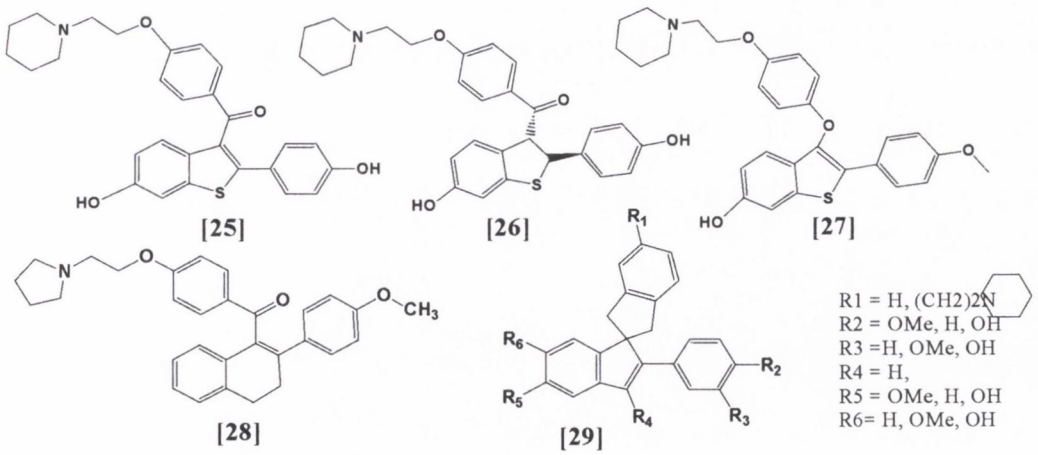


Figure 1.15 Raloxifene [25], dihydroraloxifene [26], arzoxifene [27], trioxifene [28], 2-phenylspiroindines [29]

2-Arylbenzothiophene raloxifene [25][49], a benzothiophene displayed in Figure 1.15, is a selective estrogen receptor modulator currently used to prevent and treat postmenopausal osteoporosis by competitively blocking[31] the estrogen-induced DNA transcription in the breast and endometrium. It also reduces levels of fibrinogen and cholesterol[31], which is of great benefit to postmenopausal women who are at increased risk of heart disease. It also possesses a higher binding affinity for the estrogen receptor[26] than tamoxifen (most likely due to the presence of appropriate hydroxy substituents) and therefore prevents the development of breast and endometrial cancer in women. The Multiple Outcomes of Raloxifene Evaluation (MORE)[15, 49] study found that raloxifene decreased the risk for ER-negative breast cancer by 90%. Further studies[5] are required as preliminary results from clinical trials suggest that raloxifene has a short biological half-life, which may impair its ability to maintain a complete blockade of the estrogen receptor at relevant sites.

1.6.4.4.2. Dihydroraloxifene[59]

Structure activity relationship studies were undertaken employing the benzothiophene nucleus as the core structure for synthetic elaboration. One of the most potent raloxifene analogues showed strong *in-vitro* estrogen antagonism and moderate relative binding affinity suggests its potential as a potent SERM and indicates the use of the dihydrobenzothiophene [26] (Figure 1.15) scaffold for exploration as an antiestrogen.

1.6.4.4.3. Arzoxifene[49, 60]

Arzoxifene[49, 60] (formerly known as LY353381) [27] (Figure 1.15) is a new benzothiopene analogue similar to raloxifene. Preclinical trials have shown arzoxifene to have antiestrogenic effects on breast and uterine tissue while having estrogenic effects on bone and lipid metabolism, similar to raloxifene. Furthermore it has been shown to be a more potent inhibitor of tumour growth than both tamoxifen and raloxifene, given at an equivalent dose. Preliminary data indicates that when multiple doses of arzoxifene were given to healthy postmenopausal women, subjects showed a decrease in bone turnover and LDL-cholesterol at the lowest dose, with no adverse effect on the uterus. Serious effects included one incident of deep vein thrombosis and

one incident of pulmonary embolus. Less mild effects were headache, constipation, stomatitis, and rash.

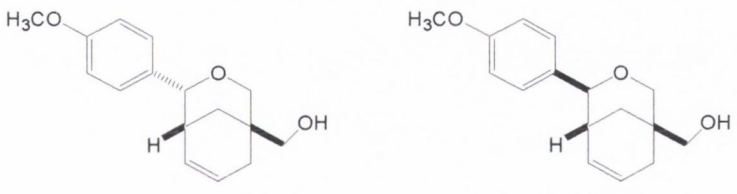
1.6.4.4.4. Trioxifene[61, 62]

Trioxifene [28][61] (Figure 1.15) is derived from the triphenylethylene structure by the introduction of a ketone-bridging group that links the phenyl ring to the rest of the molecule. It is a dihydronaphthalene and it possesses potent antiestrogenic activity in the rat. It appears to exhibit more estrogenic activity than tamoxifen but further trials are necessary to evaluate it as a breast cancer treatment.

1.6.4.5. Isoteres (2-Phenylspiroindines)[63]

Its known from X-ray crystal studies that the active conformation of raloxifene was one in which the phenylketone at C-3 of the benzothiophene [29] (Figure 1.15) was orthogonal to the benzothiophene ring rather then coplanar. It was hypothesised that locking the aromatic rings in this relative configuration might result in an especially active compound. Constructing a spiroindene system resulted in the discovery of the 2-phenylspirodene [20] and related compounds with potent SERM activity.

1.6.4.6. Bicylco-[3.3.1]-nonene[64]



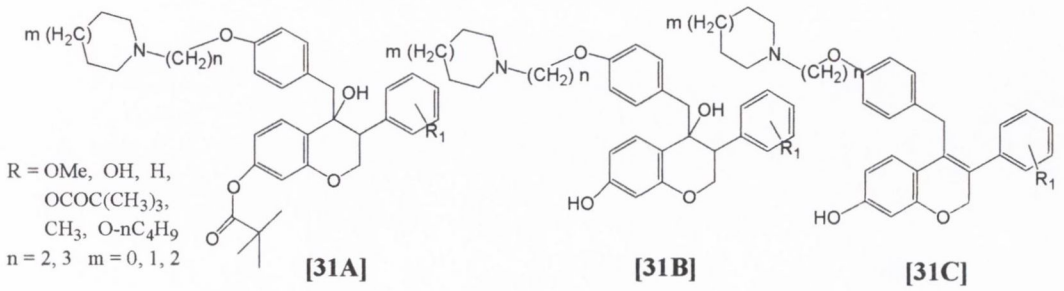
[30A] **[30B]**

Figure 1.16 Bicylco-[3.3.1]-nonene [30A-B]

As part of an effort to find a novel estrogen receptor modulator template, high throughput screening was carried out. A bicyclic ether [30A][64] was discovered but was acid sensitive and therefore not suitable for an orally administered drug. So from this compound a related product was developed, bicylco-[3.3.1]-nonene [30B][64], as seen in

Figure 1.16. It showed improved binding to ER-β when compared with the first analogue and also showed a reversal in selectivity of the ERs when compared with raloxifene. Both analogues showed full agonistic activity in MCF-7 cells.

1.6.4.7. Benzopyrans[65]



[31A] [31B] [31C]

Figure 1.17 Benzopyran derivatives [31A-C]

This range of ER ligands [31A-C], as seen in Figure 1.17, were developed using 7-hydroxyisoflavone, a metabolite of ipriflavone, as the starting point. The structure is stilbene like and the hydroxyl group at position 7 may mimic the phenolic hydroxyl of 17β-estradiol and raloxifene, which is known to be strongly involved in electrostatic interaction with a carboxyl group at both the receptor subtypes. The carbonyl group at position 4 of the benzopyranone nucleus was easily modified through a C-C forming reaction, which allowed the addition of a side group. By making the connection through a methylene hinge the side chain was allowed to take an orthogonal position with respect to the plane of the stilbene scaffold. The side chain was piperidinyl-alkoxy-phenyl and the resulting compound was named CHF4056. As predicted the compound behaved like a new nonsteroidal estrogen agonist/antagonist binding with high affinity to human ER-α and ER-β. The compound and its derivatives had the added advantage over other benzopyran derivatives of being devoid of chiral centres, thus avoiding the problems related to racemization and optical purity.

1.6.4.8. Benzopyranone[66]

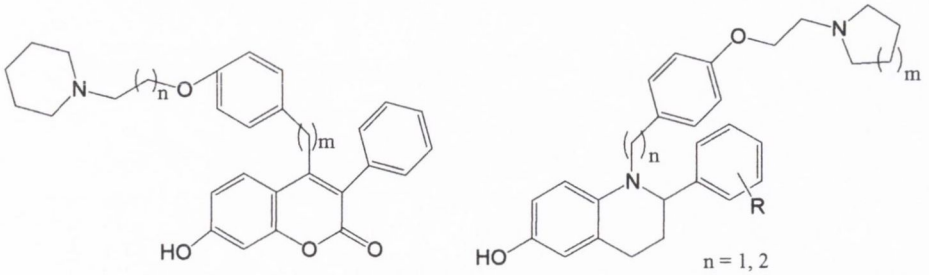


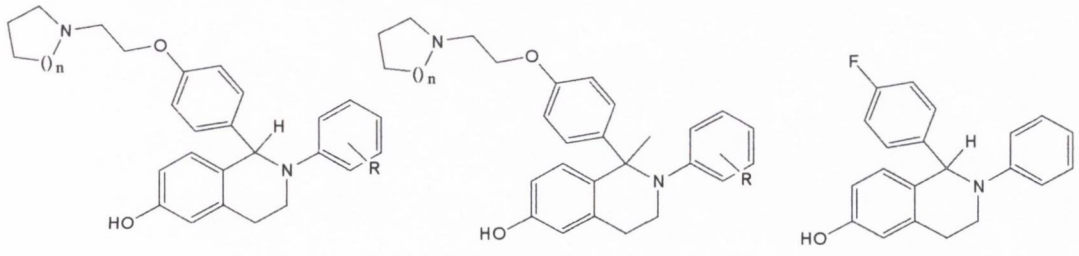
Figure 1.18 Benzopyranone analogue [32], tetrahydroquinolines [33]

The screening of commercially available phenolic compounds[66], for their ability to inhibit the release of the cytokine, interleukin-6 (IL-6) from an estrogen receptor negative osteosarcoma cell line transfected with ER-α, was performed. The benzopyranone [32] analogues proved to be of particular interest. They displayed desirable estrogen receptor agonist activity by blocking cytokine release but also some undesirable agonist properties that manifest in an increase in cellular proliferation. The most active [32] of these compounds contained an extra CH_2 group in length of the side chain (n=3, m=0) added to the hydroxy of the phenolic ring as indicated.

1.6.4.9. Tetrahydroquinolines[67]

N-Aryltetrahydroquinolines [33][67], which lacked the A-phenol ring, were reported as anti-fertility agents in rats and an investigation followed to assess their use as ER ligands. The first series of analogues had the side chain connected to the tetrahydroquinoline core via an amide linker. Then flexibility was introduced between the tetrahydroquinoline core and the basic side chain. These compounds had weak binding affinity and somewhat weaker functional activity. Finally the linker between the side chain and the core was removed [28c] as seen in Figure 1.18. Both the N-benzyl and the N-aryl derivatives displayed high binding affinity with modest ER-α selectivity.

1.6.4.10. Tetrahydroisoquinolines[68]



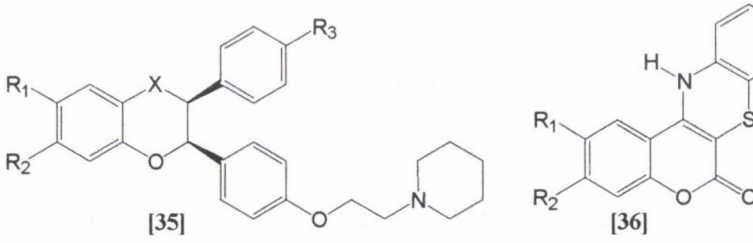
[34A] n = 1, 2 [34B] n = 1, 2, 3 [34C]

R = H, Me, OH, F, Cl, NMe_2, iPr

Figure 1.19 Tetrahydroisoquinolines [34]

Tetrahydroisoquinoline [34][68] was identified by high throughput screening using a binding assay as an ER ligand. Varying the R_1 substituent and the size of the side chain a series of these compounds were produced as seen in Figure 1.19. The best of these at ER binding contained no substituents. A number of these compounds were potent estrogen antagonists and displayed only weak estrogenic activity in the absence of estrogen and therefore have potential for breast cancer treatment and prevention.

1.6.4.11. Benzoxathiins[69]



[35] [36]

A X = CO, R_1 = H, R_2 = OH, R_3 = OH
B X = CH_2, R_1 = H, R_2 = OH, R_3 = OH
C X = S, R_1 = H, R_2 = OH, R_3 = OH
D X = S, R_1 = H, R_2 = H, R_3 = OH
E X = SO, R_1 = OH, R_2 = H, R_3 = OH
F X = SO_2, R_1 = OH, R_2 = OH, R_3 = OH

A R_1 = H, R_2 = H
B R_1 = CH_3, R_2 = H
C R_1 = Cl, R_2 = H
D R_1 = Br, R_2 = H
E R_1 = H, R_2 = OCH_3
F R_1 = H, R_2 = OH

Figure 1.20 Benzoxathiins [35], benzopyranbenzothiainones [36]

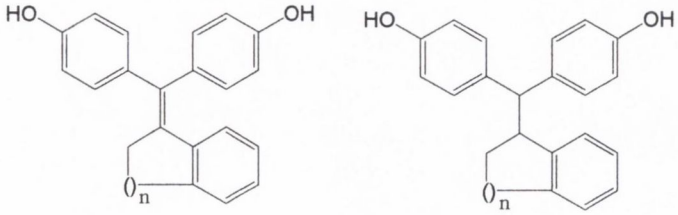
Using the lead structure benzoxathiin[69] a series of compounds [35A-F] (Figure 1.20) were produced by replacement of the carbonyl moiety of the isoflavone structure with CH_2. This improved binding to the ER-β but decreased binding to the ER-α. The CH_2 was then replaced with a sulphur atom, as seen in Figure 1.20 [35B], which regained ER-α selectivity and also increased binding affinity to the ER-α. The presence or absence of the

hydroxyl groups at R_2 and R_3 positions had little effect on the binding affinity or selectivity of the compound for either ER. The removal of the hydroxyl group at R_1 induced a detrimental effect on the binding affinity, as did the replacement of the sulphur atom with a sulphone moiety in one compound and a sulphoxide group in another case. The compound with most potential as an antiestrogen had the basic benzoxathiin structure with a sulphur group replacing the CH_2 of the isoflavanone structure and R_1-hydrogen, R_2-hydroxyl and R_3-hydroxyl.

1.6.4.12. Benzopyranbenzothiainones[70]

Coumarins[70] (natural products) constitute a very relevant family of pharmacological active compounds. Derivatives of this family are used as anticoagulant drugs, photosensitive drugs, potent and selective human dopamine D4 antagonists, non-peptidic HIV protease inhibitors or as antibiotic agents, such as novobiocin. Some estrogenic-like activities have been discovered leading to the development of the natural potent non-steroidal estrogenic compound coumestrol. A series of 2/3-substituted benzopyranobenzothiazin-6-ones [36] was synthesised, using a coumestrol-like core structure, and their estrogenic activity assessed. The compounds differ from coumestrol in that they possess a sulphur atom and a nitrogen atom in place of the oxygen in ring B, which is a six-membered ring instead of a five-membered ring. Two of the compounds; one that possessed a *p*-hydroxy substitutent [36F] and another that had a *p*-methoxy substituent [36E] on ring D as seen in Figure 1.20 were regarded as possible candidates that could lead to the development of a large chemical library of related compounds by a combinatorial synthesis approach.

1.6.4.13. Triarylethylene and triarylethane bisphenols[71]



[37A] n = 1, 2, 3 **[37B]**

Figure 1.21 Bisphenols [37A-B]

The novel tetracyclic system[71] formed by this alternative ring closure, as seen in Figure 1.21, can be considered to be an analogue of cyclofenil, a non-steroidal bisphenol with high affinity for ER that has partial antagonist activity. A series of triarylethylene and triarylethane bisphenol [37] analogues were produced. Synthesis was achieved by closing of the distal ring cycle (cyclofenil analog) and varying the size of the cyclohexylidine ring (cyclofenil). The binding affinities of these compounds were comparable to those of related acyclic and cyclic triarylethylene or cyclofenil parental ligands with some moderate selectivity between the two ERs.

1.6.4.14. Aldoximes[72, 73]

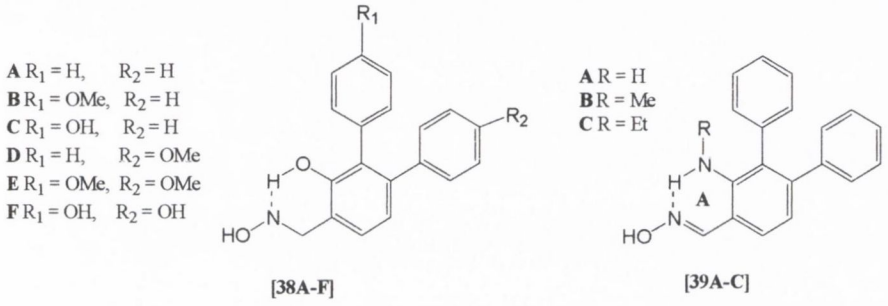


Figure 1.22 Aldoximes [38A-F], anthranylaldoximes [39A-C]

3,4-Diphenylsaliclaldoxime [38A][73], a non-steroidal estrogen of unique structure, has good binding affinity with both ERs. It was assumed that the reason behind its binding affinity was the presence of *pseudo*-ring A', an effective replacement of the phenolic A-ring typically present in ER ligands. Using this compound as the core structure a series of compounds were then produced by introducing *p*-OMe and *p*-OH [38B-F], as shown in Figure 1.22, into the various rings of the core structure. The *p*-OMe had no effect on the binding affinity but the *p*-OH improved the binding significantly. In an attempt to improve the ER binding affinity of these compounds anthranylaldoximes [39A-C][72] were developed where the pseudocycle was absent. The oxygen atom was replaced with an aniline type nitrogen onto which varying size and type alkyl groups could be attached. This series compounds exhibited better binding affinities than their predecessors.

1.6.5. Steroidal antiestrogens[74]

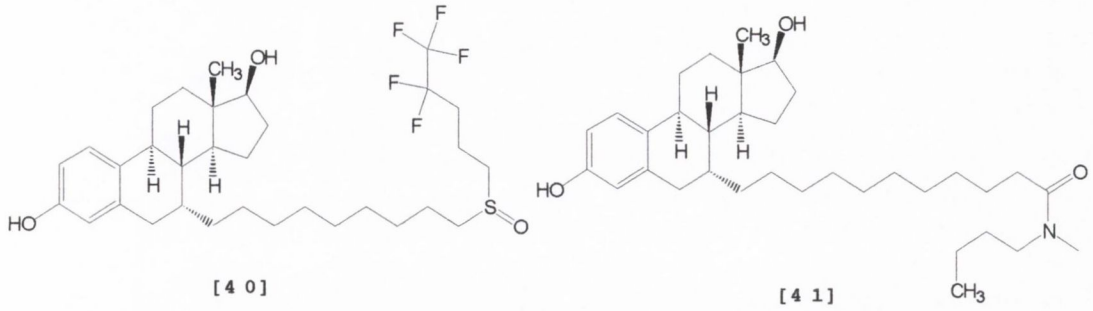


Figure 1.23 Fulvestrant [40] and ICI 164,384 [41]

The first class of specific ''pure''antiestrogens[74] obtained were 7α-substituted estradiol derivatives especially ICI 164,384, and ICI 182,780. The development of these compounds as drugs is problematic due to their limited oral bioavailability. These compounds exhibited no stimulatory effect in human breast and uterine cancer cell lines and therefore are classified as ''pure'' antiestrogens.

1.6.5.1. Estradiol derived pure antiestrogens (ICI 164,384)[75, 30]

ICI 164,384 [40][75, 30], the alkylamine derivative of 17β-estradiol shown in Figure 1.23 is a steroidal antiestrogen. It binds to the estrogen receptor with high affinity and has all the characteristics of a pure antiestrogen and yet does not demonstrate the partial agonist properties of tamoxifen. It competes with estrogen for the ER's of tamoxifen-induced uterine tumors in rats and mice.

1.6.5.2. Fulvestrant (ICI 182780)[5, 76]

The aromatase enzyme[5] converts androgen substrates to estrogen the primary source of estrogen in women. However aromatase inhibitors cannot abrogate the tropic actions of dietary or environmental estrogens. So a third class of agents called estrogen receptor down-regulators, which act by completely suppressing the effects of estrogens. The first of these to be identified was Fulvestrant (ICI 182,780) [41][76] (see Figure 1.23). It acts as a pure antiestrogen and lacks any partial agonist activity. It is an analogue of estradiol with an alkylsulphinyl side chain at position 7α. It was identified in the late 1980s as an agent that completely blocked estrogen-stimulated uterine proliferation in the rat without stimulating proliferation in the absence of estrogen. In contrast to tamoxifen,

while ICI 182,780[30] is capable of binding to the ER receptor dimerization is impaired, receptor degradation is accelerated by increase of receptor protein turnover, and AF-1 and AF-2 remain inactive. This results in disrupted nuclear transcriptional coactivators and leads to complete suppression of the expression of estrogen-dependent genes and thus ICI 182,780 is described as a pure antiestrogen. Currently in clinical trials.

1.7. Aromatase inhibitors[77]

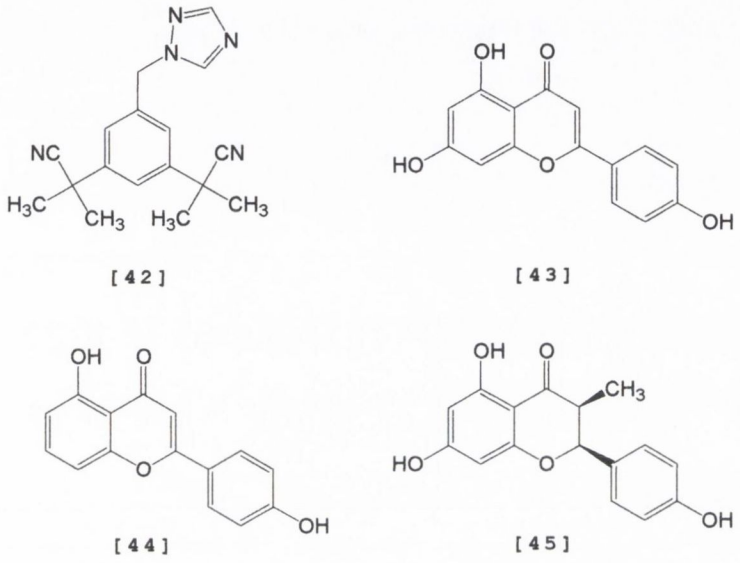


Figure 1.24 Arimidex [42], Apigenin [43], 6,4'-Dihydroxy-flavone [44], BE-14348B [45]

1.7.1. Aromatase inhibitors[77, 78, 79, 80, 81]

Anastrozole [42][77] (Arimidex)(see Figure 1.24) is a new orally active nonsteroidal selective aromatase inhibitor used in the treatment of hormone-responsive metastic breast cancer. In the postmenopausal woman it is believed that aromatase (a cytochrome P450-dependent enzyme)[78] is responsible for the conversion of adrenal androgen substrates to estrogens, therefore the blocking of aromatisation would significantly impact the total circulating estrogens and subsequently inhibit tumour growth. Anastrozole is the first aromatase inhibitor to be licensed for first-line treatment in advanced beast cancer. A study[79] carried out with oral doses daily of 1mg and 10 mg anastrozole demonstrated a lower risk of death for patients treated. Treatment with anastrozole[80] for up to three months led to a marked reduction in mastectomy rates. In

the area of prevention[81] of breast cancer in high risk or postmenopausal women aromatase inhibitors could be a feasible option.

1.7.2. Flavonoids and phytoestrogens [26, 82]

Besides known steroidal and nonsteroidal inhibitors, the anti-aromatase effects of flavonoids, some natural and synthetic flavonoids including isoflavonic phytoestrogens or dietary flavonoids found in our daily environment[82] were found to inhibit aromatase activity. Aromatase affinity for flavonoids is generally lower than it is for steroidal and imidazole-derivatives. Flavonoids[26] are known to exhibit various biological activities. A large number of them are known to have anti-proliferative effects against breast cancer cells. Apigenin [43] is reported to possess anti proliferative activity against the human breast cancer cell line ZR-75-1, 6,4'-dihydroxy-flavone [44] has a binding affinity for the estrogen receptor and the flavone derivative BE-14348B [45] exhibits both estrogenic and antiproliferative activity (see Figure 1.24). The consumption of flavonoid rich plant foods may contribute to a reduction of estrogen-dependent diseases, such as breast cancer.

1.8. An immunotherapeutic agent - Herceptin (trastuzumab) [83, 84, 85]

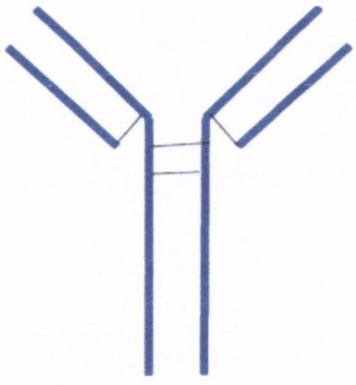


Figure 1.25 Herceptin antibody structure[46] [83]

The human epidermal growth factor receptor-2 (HER2) [46][83], is an accessible target for novel and specific anticancer treatment, e.g. monoclonal antibody (MAb) therapy. Herceptin[84] is a recombinant, humanized, monoclonal antibody whose antigen is the HER2/neu protein, which possesses intrinsic tyrosine kinase activity and partial

homology with the epidermal growth factor receptor. The HER/neu protein is overexpressed on the surface of 20-30% of breast cancer cells in 10-35% of patients with breast cancer. The discovery of this overexpression of the HER2 receptor led to the development of Herceptin immunotherapy (see Figure 1.25), in which monoclonal antibodies (Herceptin) specific for the receptor inhibited the growth of HER2 resulting in the tumour growth inhibition. Studies[85] are underway to assess the trastuzumabs use as a single agent first line therapy for metastatic breast cancer.

1.9. Objectives of this thesis

In previous work[86] in this area a number of tamoxifen derivatives were identified as possible lead structures for development as treatments for breast cancer. In this project my objectives were

1) The design and synthesis of a library of flexible triphenylarylpropenes and related compounds using the McMurry reaction as the key step in the synthesis.

2) Their investigation as estrogen receptor modulators by performing biochemical studies on the compounds synthesised by investigating their receptor binding ability, cytotoxicity and inhibition of proliferation of the estrogen receptor on MCF-7 breast cancer cells together with estrogenicity and cell cycle profile analysis.

3) Structural activity relationship studies (SARS) via molecular modelling will be performed to elucidate the active components of the flexible triphenylarylpropenes involved in estrogen receptor binding.

2. Synthesis of Tamoxifen Analogues Series 1, 2 and 3

2.1. Introduction

Due to the fact that tamoxifen is such an effective antiestrogen[87] it is logical to assume modifications to the tamoxifen structure may produce a more efficient antiestrogen or at least reduce the side effects of tamoxifen. 4-Hydroxytamoxifen[88], a metabolite of tamoxifen, binds to the estrogen receptor with 100 times the affinity of tamoxifen and it has being suggested that this metabolite plays an important role in mediating tamoxifens' overall activity. However research[89] carried out on dimethylbenzanthracene-induced mammary tumours in rats has reported it to be a weaker agent than tamoxifen and this has been attributed to the rapid clearance of 4-hydroxytamoxifen by way of deactivating metabolic conjugation of the hydroxyl group. Despite this, on examination of the structures of tamoxifen and 4-hydroxytamoxifen in correlation with their activities in breast cancer cells and their binding abilities to the estrogen receptor it was suggested that a free hydroxy was very important for increasing the activity of analogues in the treatment of breast cancer. This lead to the suggestion of developing analogues which possess other polar substitutents, in place of the hydroxy group of 4-hydroxytamoxifen in the hope of developing a drug that binds with great affinity to the estrogen receptor and yet is not cleared as quickly as 4-hydroxytamoxifen due to the decrease in polarity. Also substituent position variation from *para* to *ortho* and *meta* was examined for increasing the efficacy of the analogues. These changes in the tamoxifen structure combined with the introduction of flexibility, may improve the binding ability of the analogues to the estrogen receptor. The development of flexible tamoxifen analogues[86] had previously being examined by the use of methylene spacing groups between the aryl and vinylic systems. Biochemical studies confirm that this area has good potential in the search for a SERM at least as potent as tamoxifen and therefore will be pursued in this thesis in the development of a new series of flexible tamoxifen analogues. The effects of the replacement of the hydroxy-substituent of 4-hydroxytamoxifen with halogen groups has also previously being investigated[90] and showed some positive results but the analogues researched did not also possess the required structural flexibility and so a new series of compounds have now developed for this thesis in order to investigate this area further.

The object of this thesis was to first produce tamoxifen analogues series 1, 2, 3, and 4 which would contain various basic side chains and substituents; methoxy, pivaloyloxy, hydroxy, flouro, bromo introduced in *orth*, *meta* or *para* positions. These compounds are

novel non-steroidal flexible compounds based on the triphenylethylene structure of tamoxifen. Flexibility has being introduced in some cases, as this has been shown to enhance binding affinity to the estrogen receptor. These tamoxifen analogues are shown below in Figure 2.1.

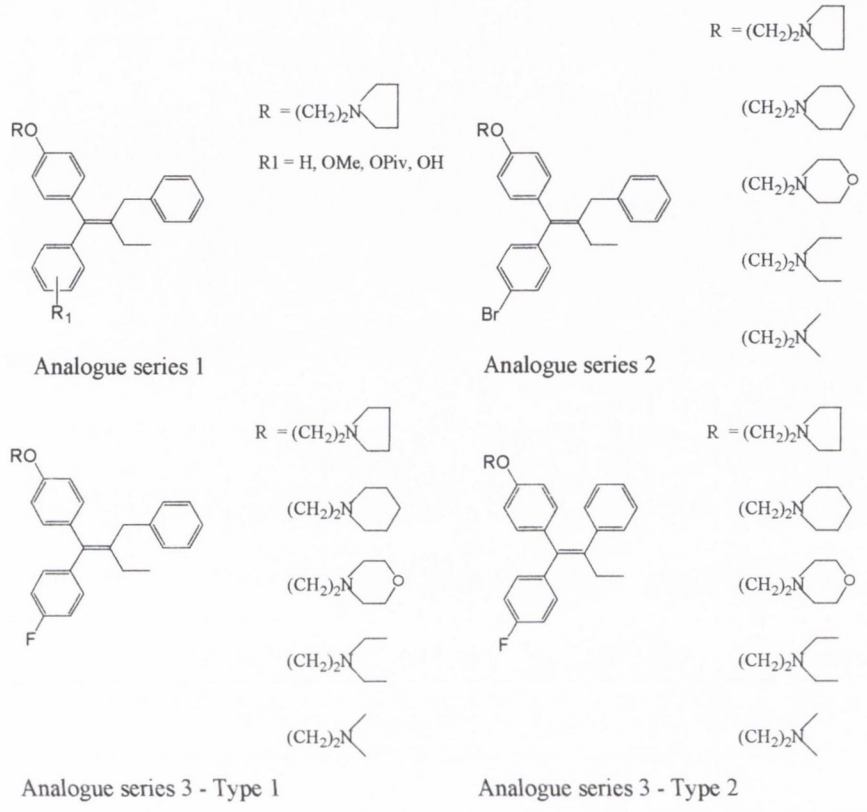


Figure 2.1 Tamoxifen analogues

The synthetic route chosen will require the McMurry reaction, but initially the various tamoxifen syntheses will be reviewed. Then, concentrating on the synthesis of tamoxifen, the McMurry reaction and its mechanism will be examined. The synthesis of analogues series 1 to 4 via the McMurry reaction will then be discussed.

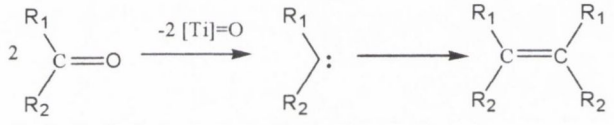
2.1.1. McMurry coupling

The McMurry reaction[91] involves the reductive coupling of two carbonyl compounds using low valent transition metals (e.g. low valent titanium salts) to produce alkenes. The original low valent-titanium[92] reagent system that appeared best for the widest variety of substrates was prepared by reaction of $TiCl_3$ with $LiAlH_4$ in the ratio of 2:1. The McMurry reaction is one of the most versatile reactions and a huge interest in the McMurry reaction[92] has

developed an extraordinary variety of synthetic uses of the titanium-induced carbonyl coupling reaction. The ability to couple all manner of ketones and aldehydes to give olefins in high yield is unique to titanium. Three different groups discovered the McMurry reaction[93] independently, almost at the same time. In 1972[94], Sharpless *et al.* reported that ketones and aldehydes could be coupled by reduction into alkenes by reaction with WCl_6 and RLi reagents. A year later, two groups, Tyrlik and Wolochowicz, discovered that low-valent titanium complexes were also efficient in this coupling process.

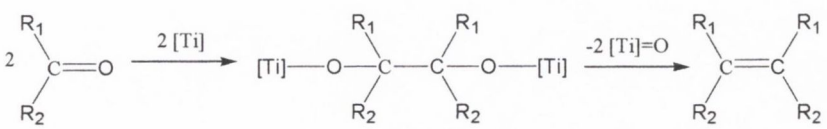
2.1.2. Proposed mechanisms for the McMurry reaction

Tyrlik and Wolochowicz[94] (Scheme 2.1), who used the $TiCl_3$-Mg system, suggested that tetramethylene was obtained *via* the carbene species Me_2C: resulting itself from deoxygenation of acetone.



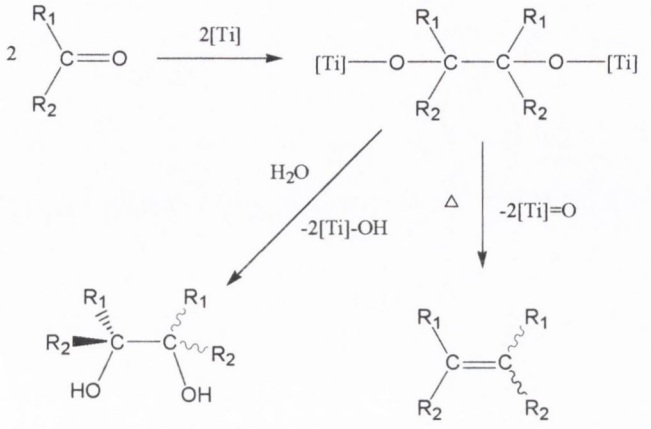
Scheme 2.1 Wolochowicz mechanism

On the other hand, it was proposed by Mukaiyama *et al.* (Scheme 2.2) in describing the low valent titanium-mediated coupling[95] that metallopinacols were intermediates in the reductive coupling of aromatic ketones by means of the $TiCl_4$-Zn system. Although Mukaiyama's $TiCl_4$ reagent[96, 97] successfully couples aromatic ketones and aldehydes, it was found to be less effective in aliphatic systems. In 1973 Mukaiyama[98] reported the synthesis of vicinal diols by pinacol coupling with zinc as well as the synthesis of olefins via the above diols using titanium (IV) chloride and lithium aluminum hydride.



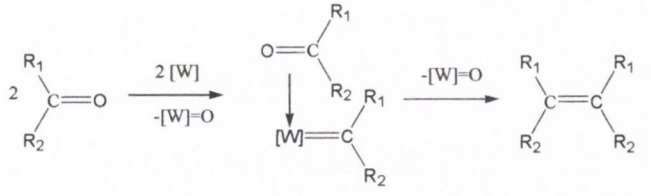
Scheme 2.2 Mukaiyama mechanism

In 1974 McMurry and Fleming (Scheme 2.3) described a new method for the reductive coupling of carbonyls to olefins with $TiCl_3$ and $LiAlH_4$; they also proposed the presence of pinacol intermediates in the reaction.



Scheme 2.3 McMurry mechanism – via a metallopinacol intermediate

In 1978 Fujiwara *et al.* (Scheme 2.4) detected carbene species resulting from the reductive cleavage of ketonic C=O bond. These compounds were found to give the olefin at room temperature. However this proposed mechanism still does not provide a satisfactory explanation for the selective formation of heterocoupled products when one of the ketones is a diaryl ketone.



Scheme 2.4 Fujiwara mechanism via carbenoid species

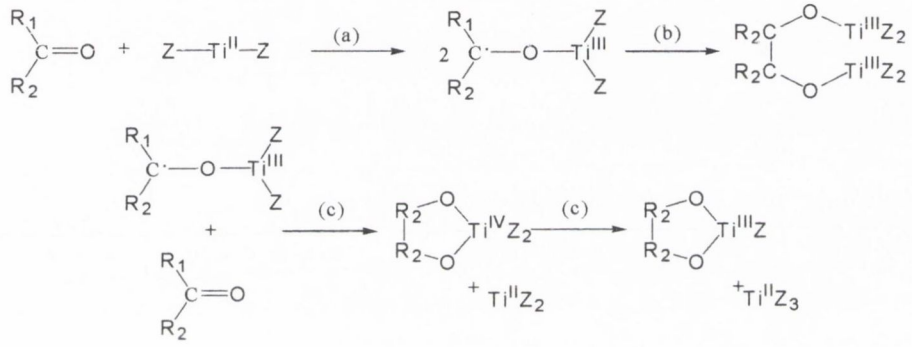
Mechanistic studies of the coupling provide insight into the actual process of the reaction. They suggest that it takes place on the surface of the titanium particle. The ketone attaches to the active coupling species and picks up an electron from the titanium to yield the organic anion radical. This binds to the titanium particle. Sequential homolytic cleavage occurs liberating the alkene and the titanium dioxide.

2.1.3. Mechanism of the McMurry reaction

McMurry considered four different possibilities to explain the coupling reaction

2.1.3.1. Corey[99]

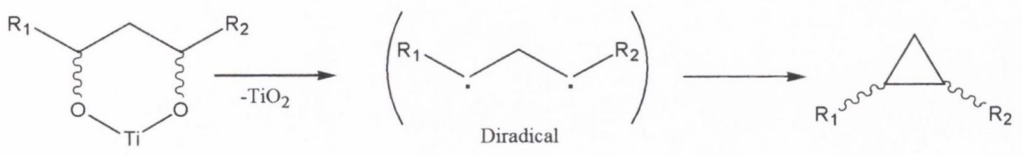
Corey considered Ti^{II} (Scheme 2.5(a)) as the reactive species in the reductive coupling followed by the formation of an intermediate with a Ti^{III} species. This allowed for two possibilities: a) the formation of the pinacol intermediate Ti^{III} species (Scheme 2.5(b)) by coupling with another Ti^{III} species, b) cycloaddition process to form a cyclic Ti^{IV} complex (Scheme 2.5(c)) and successive reduction by Ti^{II} to two Ti^{III} species.



Scheme 2.5 Corey mechanism

2.1.3.2. Baumstark[99]

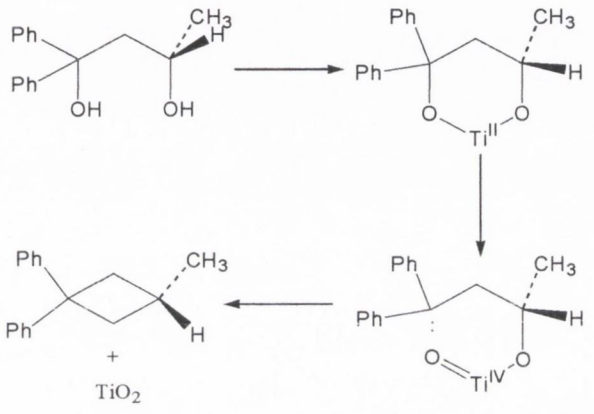
Baumstark (Scheme 2.6) reports the coupling of 1,3-diphenyl-1,3-propanediol using titanium by assuming the intermediate formed were a diradical.



Scheme 2.6 Baumstark mechanism

2.1.3.3. Walborsky[99]

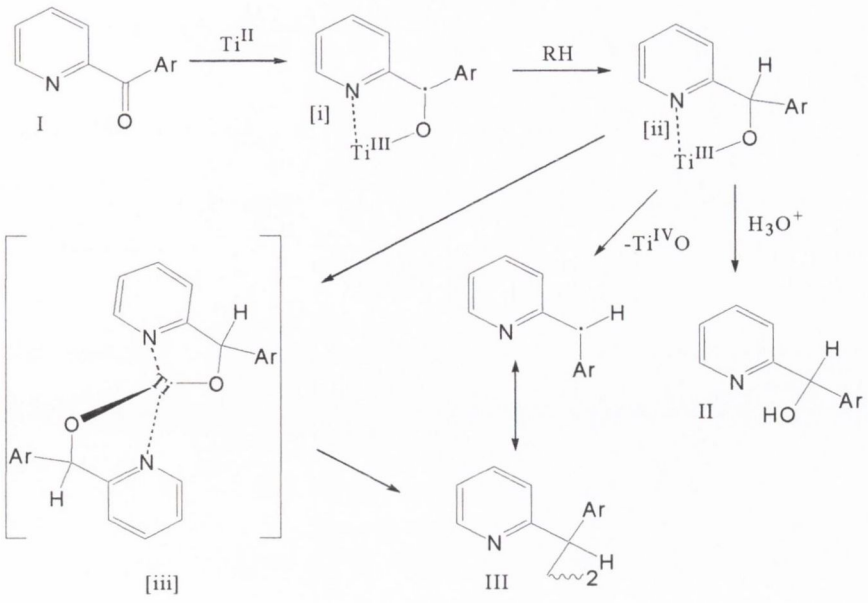
Walborsky questioned Baumstark's theory by suggesting the presence of a diradical intermediate should imply the retention of configuration and the reductive coupling should proceed with inversion of configuration. On the basis of this theory he proposed the mechanism, as seen in Scheme 2.7 using 1,1-diphenyl-1,3-butaneglycols.



Scheme 2.7 Walborsky mechanism

2.1.3.4. Newkome[99]

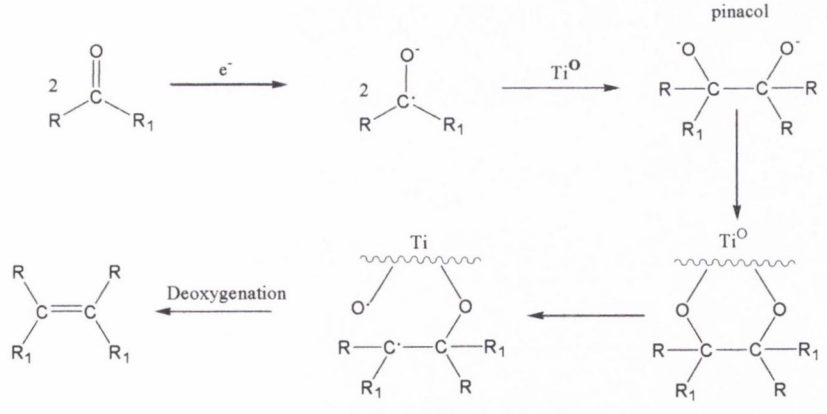
Newkome proposed the initial formation of an intramolecular complex, which abstracts hydrogen from the solvent giving a Ti^{III} intermediate. This could take place in two different ways: a) hydrolysis of such a Ti^{III} intermediate to give [II] as seen in Scheme 2.8 or b) reaction of the Ti^{III} intermediate with Ti^{II} to generate a radical which dimerizes to give the tetrarylethane.



Scheme 2.8 Newkome mechanism

2.1.3.5. McMurry reaction *via* pinacol formation[99]

According to the McMurry reaction[93, 92], the carbonyl coupling takes place in two steps. McMurry considered the proposed mechanisms listed above and then proposed a more probable mechanism (Scheme 2.9) involving the initial formation of a pinacolic compound followed by deoxygenation on the surface of the titanium to yield the alkene.



Scheme 2.9 McMurry mechanism

2.1.3.5.1. Pinacol formation

The first step is the reductive dimerization[100] of the aldehyde (or ketone), forming a carbon-carbon bond. The product of this step is a 1,2-diolate or pinacolate intermediate. In this step the titanium reagent donates one electron to the ketone to generate a ketyl[101], which dimerizes, yielding the intermediate as seen in Scheme 2.9. The first step is essentially the pinacol reaction known since 1859[93] to occur with reducing metals. Evidence of these pinacol intermediates in the reductive coupling of pinacols with low-valent titanium species includes the isolation of pinacols in high yield when the reaction is performed at 0°C[100] and quenched after a brief period of time, as well as the observation that deoxygenation of the pinacols to olefins occurs with the same reagent at 60°C.

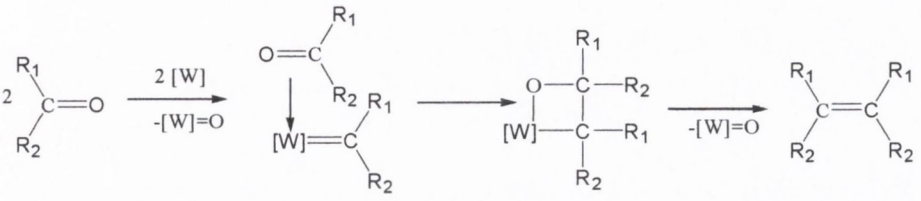
It is generally accepted[102] that this reaction involves the intermediacy of a titanium pinacolate but when concerned with mixed couplings of diaryl ketones something was absent in the mechanism. It was first suggested by McMurry[103], that the pinacol formation occurred via the dimerization of anion radicals. On examination of the mixed coupling of acetone and benzophenone it was considered unlikely that the mixed pinacol formed via a radical coupling process. It was then proposed that a dianion forms from one component of the reaction and that this adds by nucleophilic addition to the other component to give the mixed coupling product. More evidence[104] was later produced to support this theory where the initial step in the pinacol formation was described as a two-electron transfer from the low-valent titanium to the diaryl ketone to afford the dianion.

So it was accepted as part of the McMurry mechanism theory[102] that the pinacol could be formed by dimerization of two ketyl radicals or nucleophilic attack of the carbonyl dianion on a free ketone.

2.1.3.5.2. Deoxygenation to olefin mechanism[100]

The second step is the deoxygenation (Scheme 2.9) of the 1,2-diolate to form the olefin, which may occur on the surface of the titanium *via* a heterogeneous process. It occurs readily by a *cis*-type concerted mechanism[104] leading to the alkenes, due to the large affinity of titanium for oxygen. It is the more interesting step because it was

species[108] comes from the unsuccessful McMurry coupling reactions, which have no logical explanations as to why they were unsuccessful.



Scheme 2.10 McMurry coupling via carbenoid species

2.1.4. Optimisation of the reaction conditions[109]

The difference in behaviour of different low valent titanium preparations[92] is probably due to the particle size, surface area, physical nature of the surface and solvent.

a) Choice of the reducing agent[109]

Three classes of reducing agents are commonly used to reduce $TiCl_3$ to the active coupling species: alkali metals e.g., Li or K, group II metals, e.g., Mg or Zn-Cu couple, and metal hydrides, e.g., $LiAlH_4$. Studies showed that once in the (0) state necessary for an active coupling species all systems worked equally efficiently in performing the coupling.

b) Choice of solvent[109]

The reactivity of the active coupling species restricts the choice of solvents to hydrocarbons and ethers. The combination of the agent-solvent turned out to be critical. Solvent[92] is very important during the preparation of the reagent because it appears to stabilize the zero-valent particles during their formation. A strongly coordinating solvent like THF or DMF is recommended. THF performed in all instances.

c) Molar ratio of $TiCl_3$ to reducing agent[109]

Tyrlik *et al.*[110] showed that the yield of olefin using the $TiCl_3$/Mg system depends upon the ratio of $TiCl_3$ to Mg, while for the $TiCl_3$/K system with $TiCl_3$/K ratios larger than 1:3, yields of olefin were reported to be inferior. For the $TiCl_3/LiAlH_4$ system various ratios have been used.

d) Molar ratio of $TiCl_3$ to Ketone[109]

It was found after investigating different ratios of $TiCl_3$/K, $TiCl_3$/Mg, $TiCl_3$/$LiAlH_4$ involved in the coupling of ketones that the most efficient ratio was $TiCl_3$ to ketone 1:1.

2.1.5. Valency of the catalyst (titanium) involved in the McMurry reaction

Kahn and Reike[93], pointed out that the coupling of ketones and aldehydes in the liquid phase could be carried out homogeneously with organometallic titanium complexes, or heterogeneously with "slurries" of low valent titanium derived from the reduction of $TiCl_3$ or $TiCl_4$. Their work provided some insight into the identity of the active species by observing the differences between the homogeneous and heterogeneous coupling reactions especially the oxidation states. The nature of the titanium site has not yet been resolved for the heterogeneous reaction. These studies[93], performed to determine the valency of the catalyst (titanium) involved in the McMurry reaction, considered batch, stoichiometric, liquid-solid, carbonyl coupling reaction. It was originally proposed that Ti^{2+} is the active site[93] but ESR studies[92] have shown that Ti(0) particles in finely defined form are almost certainly the active low valent coupling species. Examination of the system $TiCl_3$/Mg[109] using ESR showed the existence of a Ti(III) signal on the addition of Mg to $TiCl^3$ which persisted until the completion of the reduction after which only an extremely weak signal was observed that was identified as contact of the active coupling species with air. Other than that signal no other signal existed and the fact that the active coupling species has no signal is consistent with a Ti(0) species. The fact that Ti(III) exists up to the completion of the reaction indicates the slow conversion of Ti(III) to Ti(II) immediately followed by Ti(0) suggesting also that Ti(III) and Ti(0) can coexist. The ESR phenomena show that the active coupling species must be polymeric (dimeric) before coupling and that sometime during the coupling the titanium is broken down into Ti monomers. This process is accompanied by H_2 evolution. The breakdown into monomers is brought about by the bonding of the oxygen of a carbonyl species to the titanium surface, which produces the oxygen bonded to the active coupling species as a monomeric Ti(III) species. Because the ketone requires two electrons for the reductive coupling process the titanium species must initially be in the Ti(I) state.

Furstner *et al.*[93] summarized some time ago a number of inconsistencies regarding the nature of the low-valent titanium species required for the McMurry reaction and questioned

the need for Ti(0) in the McMurry reaction though it is still regarded as the most probable means by which the McMurry reaction proceeds.

2.2. Reductive coupling of carbonyl compounds by the McMurry reaction

The McMurry[94] reaction continues to find new important applications but suffers from problems of reproducibility. The McMurry coupling[99] can be used for aliphatic, aromatic, and heterocyclic carbonyl compounds and involves intramolecular reductive coupling.

a) Aliphatic carbonyl compounds

The reductive coupling reactions of the aliphatic carbonyls[99] occur readily, but with increasing complexity of the alkyl groups causing steric hindrance the products are produced in lower yields. For example, methyl tert-butyl ketone couples easily to give the olefin but the ethyl tert-butyl ketone did not couple under any circumstances due to the extra CH_2 present.

b) Aromatic and α, β-unsaturated carbonyl compounds

The reductive coupling[99] here occurs readily but with increasing steric hindrance, especially around the carbonyl, the yield decreases.

c) Intramolecular reductive dicarbonyl compounds

Dialdehydes, ketoaldehydes and diketones[99] can also be coupled using the McMurry reaction *via* an intramolecular route to produce rings of all sizes from three through to twenty. The yields[92] though can vary according to the reducing agent used but overall are good. The reducing agent copper-zinc couple is recommended.

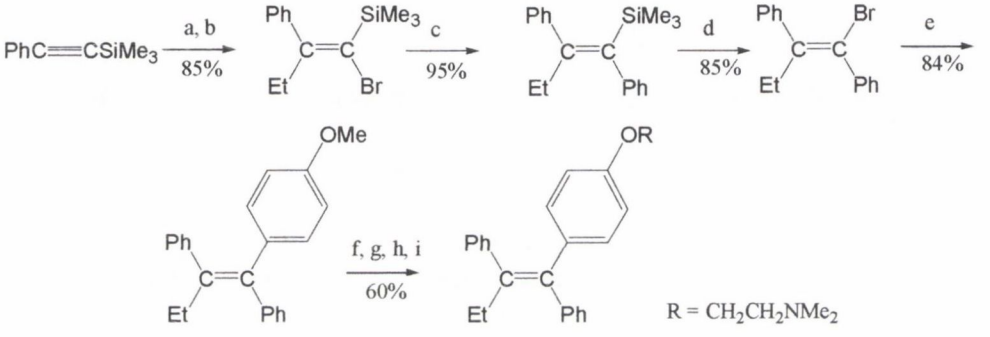
d) Heterocyclic compounds

With heterocyclic ketones[99], the coupling can only be performed when the carbonyl group is distal or far enough away from the heteroatom. Tetraphenylfuran, starting from carbonyl derviatives, is one example of heterocyclic coupling.

2.3. Tamoxifen synthesis by various methods

2.3.1. Tamoxifen synthesis by the carbometalation of an alkynylsilane

Many newer coupling reactions have been explored for the synthesis of tamoxifen like products[111, 112]. Synthesis of tamoxifen (Scheme 2.11) was performed using the carbometalation of an alkynylsilane as the key step. This is a stereospecific synthesis of tamoxifen involving carbometallation of a substituted alkyne to the vinyl bromide but the procedure is somewhat lengthy and therefore simpler stereoselective approaches are preferable.



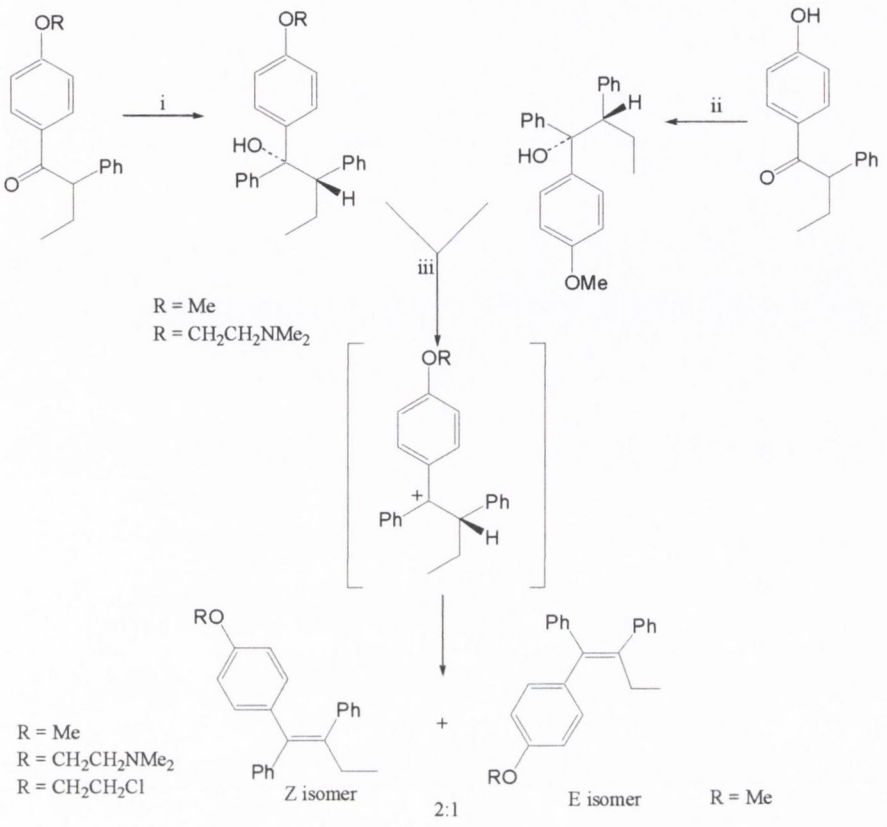
Scheme 2.11 Carbometalation synthesis of tamoxifen

Scheme reagents: (a) Et_2AlCl, Cp_2TiCl_2, CH_2Cl_2 ; (b) NBS, -78°C ; (c) PhZnCl, $Pd(PPh_3)_4$ (catalyst), THF, reflux ; (d) Br_2, CH_2Cl_2, NaOMe/MeOH, -78°C to room temperature ; (e) *p*-$MeOC_6H_4ZnCl$, $Pd(PPh_3)_4$ (catalyst), THF, reflux ; (f) NaSEt, DMF, reflux ; (g) $ClCH_2CH_2NMe_2$·HCL, NaOEt, EtOH, reflux ; (h) HCl (g), Et_2O ; (i) 0.5 N NaOH,.

2.3.2. Tamoxifen synthesis *via* Perfluoroarenes

Perfluoroarenes[113] were used in the synthesis of versatile intermediates of both Z and E tamoxifen. A convenient precursor for the synthesis of tamoxifen (Scheme 2.12), 1,2-diphenyl-1-(4-hydroxyphenyl)-1-butene, was developed. Reaction of a mixture of isomeric phenols, *cis* and *trans* 1,2-diphenyl-1-(4-hydroxyphenyl)-1-butenes, with octafluorotoluene under phase transfer conditions afforded a high yield of a corresponding mixture of perfluorotolyl derivatives, *cis* and *trans* isomers in a 1:1 ratio. These isomers could be easily separated by column chromatography. The Z isomer was then converted to the

alcohol with dilute hydrochloric acid solution as a 2.1:1 mixture of isomers Z/E in a 91% yield.

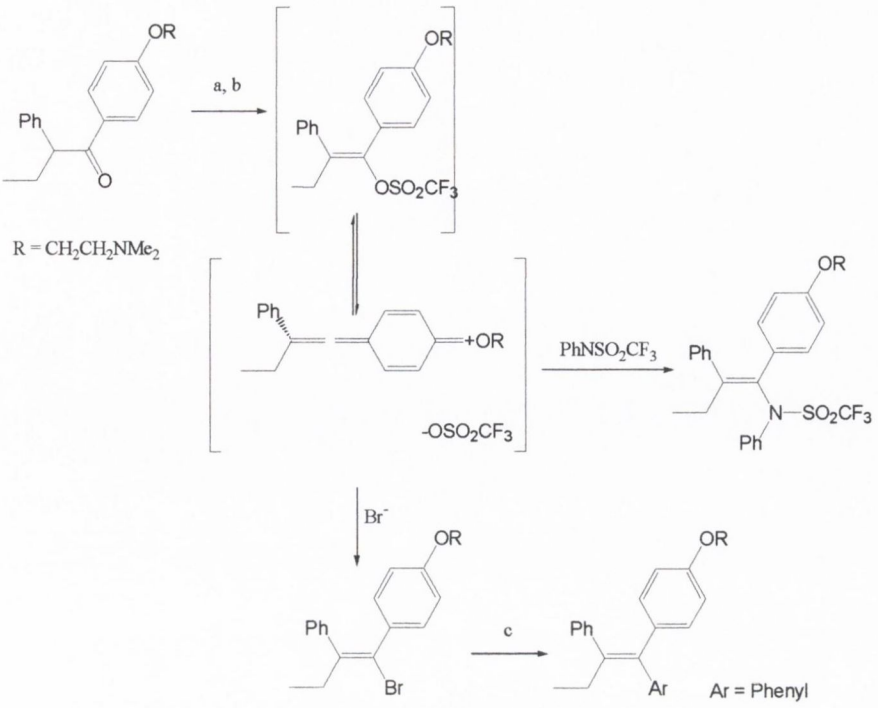


Scheme 2.13 Tamoxifen synthesis by selective dehydration

Scheme reagents: (i) PhMgBr, ether ; (ii) *p*-$MeOC_6H_4MgBr$, ether ; (iii) HCl (aq), EtOH, 80°C.

2.3.4. Tamoxifen synthesis *via* a vinyl bromide intermediate or a triflate intermediate

The synthesis of tamoxifen[88], as seen in Scheme 2.14, is achieved *via* the vinyl bromide intermediate or the triflate intermediate since the bromide or triflate function should be replaceable by an aryl group with retention of configuration in a palladium complex catalysed coupling reaction. The synthesis of a vinyl bromide has been used previously for the synthesis of tamoxifen as shown in section 2.3.1. In this case the ketone was converted with a high degree of stereoselectivity into the (E)-vinyl bromide, which is a versatile precursor to tamoxifen and analogues. A 93% yield of Z-tamoxifen was obtained.

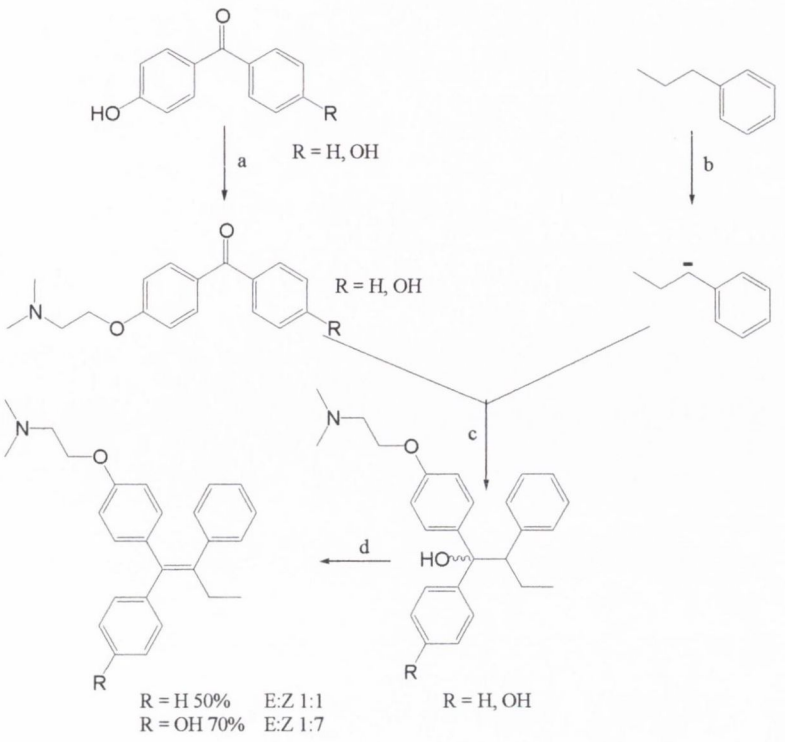


Scheme 2.14 Tamoxifen synthesis *via* a vinyl bromide intermediate or a triflate intermediate

Scheme reagents: (a) KH, THF, 25°C ; (b) Tf_2Ph (+LiBr), 25°C ; (c) ArMX, Pd catalyst ; (d) Me_2NH, EtOH, 100°C.

2.3.5. Tamoxifen synthesis *via* carbanion

This is a direct synthesis[115] of tamoxifen in which the key step is the condensation of a fully functionalised benzophenone (Scheme 2.15) with the previously unreported anion of propylbenzene, generated from the arene by three-component super-basic medium. This reaction allows facile synthesis of a stable, deep red carbanion from propylbenzene in hexane at room temperature under argon. This is followed by addition of an ethereal solution of ketone to an excess of this anion at –70°C to give the corresponding carbinols. Mineral acid was used to effect dehydration of the carbinols and produced a mixture of tamoxifen, *cis* and *trans* isomers in ratios of 1:1 in a good overall yield of 50% for tamoxifen.



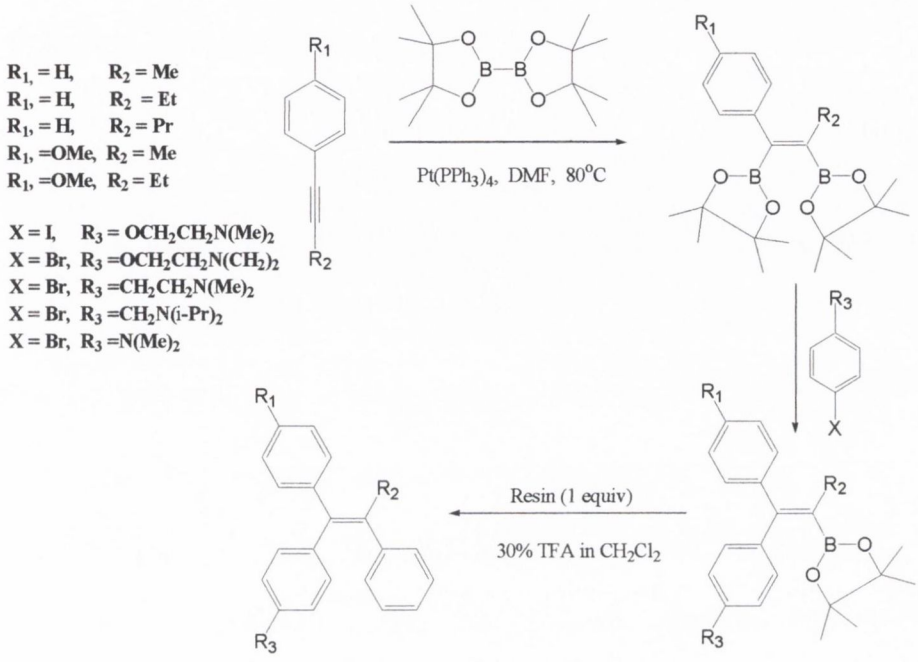
Scheme 2.15 synthesis of tamoxifen *via* carbanion route

Scheme reagents: (a) $ClCH_2NMe{\cdot}HCl$, 2 equiv K_2CO_3, DMF, 5h, 110°C ; (b) *n*-BuLi, *t*-BuOK, TMEDA, hexane, room temperature, argon atmosphere ; (c) EtO_2, added to 6 equiv of anion at -70°C ; then 0°C, 5 h ; (d) 32% H_2SO_4, 16 h, 50°C.

2.3.6. Tamoxifen synthesis on solid support *via* resin

Recently the synthesis of substituted ethylenes on solid support *via* resin capture[116] has been reported. This chemistry can be used in the parallel synthesis (Scheme 2.16) of triphenylethylene derivatives based on tamoxifen. A 25-member library was synthesised using five alkynes, five aryl halides and a polymer bound aryl iodide as inputs. Conversion of the alkynes into bis(boryl)alkenes (in solution) followed via a platinum-catalysed reaction developed by Ishikayama and co-workers. The crude intermediates were used in solution Suzuki reactions with an excess of aryl halide to generate to produce a second mixture of intermediates, which were then introduced to the resin, and the reaction was continued on solid support. The products were cleaved from the polymer as amine salts of

trifluoroacetic acid. Finally the products were filtered through a plug of basic alumina to give free amines one of which was tamoxifen.



Scheme 2.16 Synthesis of tamoxifen on solid support *via* resin

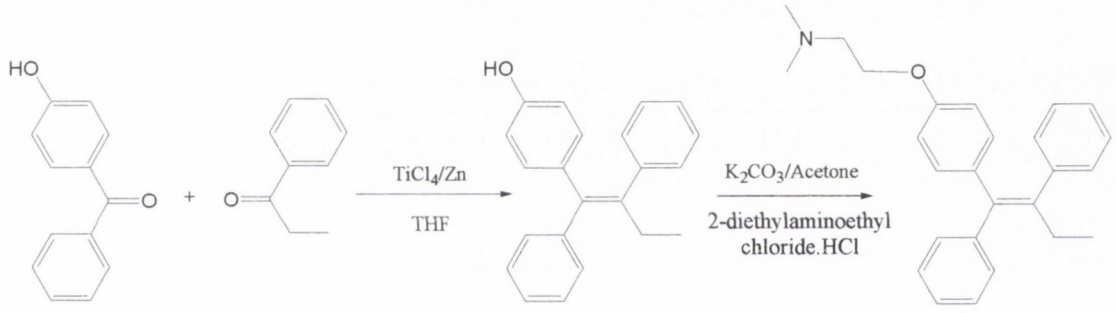
Scheme reagents: (a) bis(boryl)alkene (10equiv), aryl halide (15 equiv), $Pd(dppf)Cl_2$ (0.5 equiv), 3,5-dimethoxyphenol (50 equiv), 6M KOH (50 equiv), DME, 25°C, 18 h , (b) 1 equiv, 6M KOH, 25°C, 18 h.

2.4. Synthesis of tamoxifen and related analogues *via* McMurry reaction

Coe and Scriven performed synthesis of tamoxifen[117] using the McMurry route. They attempted to introduce some selectivity for either the E or Z isomer into the synthesis. (Scheme 2.17). They first attempted a direct one step synthesis of tamoxifen, based on a successful coupling between 4-methoxybenzophenone and propiophenone [18], by coupling 4-[2-(N,N-dimethylamino)ethoxy]benzophenone and propiophenone [18] but none of the desired product was recovered. They then attempted the coupling of 4-hydroxybenzophenone and propiophenone [18] taking great care in the preparation of the catalyst by ensuring the addition of $TiCl_4$ to an oxygen free dry suspension of Zn powder in dry THF and refluxing for a short period. To this the reagents were added and refluxed. They reacted readily to produce 4-(1,2-diphenylbut-1-enyl)-phenol in a 98% yield as a 7:1

mixture of Z:E isomers. Using this same method the coupling of 4-[2-(N,N-dimethylamino)ethoxy]benzophenone and propiophenone [18] was attempted again and this time was successful in that it produced an 88% yield with a ratio of 3:1 Z:E isomers.

Due to the selectivity[117] for the Z-isomer in the coupling of hydroxybenzophenone to propiophenone [18], over the direct coupling of 4-[2-(N,N-dimethylamino)ethoxy]benzophenone and propiophenone [18] that produced tamoxifen in one step, it was suggested that there could be a preferred orientation of the two ketones, one of which may be bound to the metal surface brought about by a weak interaction between the substituted phenyl group of the benzophenone and the phenyl group of the propiophenone [18]. Its therefore believed that the benzophenone reacts first with the metal, since some reactions investigated by Coe and Scriven[117] found traces of self coupled products of propiophenones but not of the benzophenones.

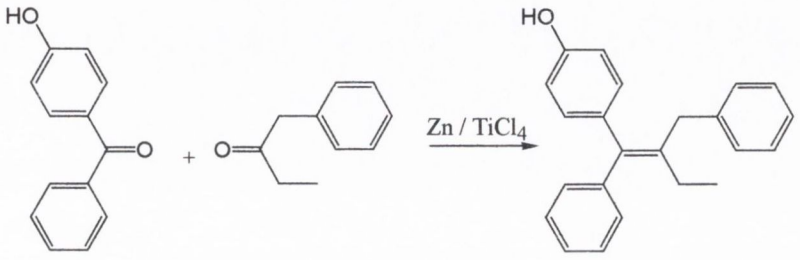


Scheme 2.17 Synthesis of tamoxifen *via* McMurry reaction

4-Hydroxytamoxifen and 4-hydroxytoremifene were also synthesised *via* the McMurry coupling. Firstly one hydroxy group of 4,4'-dihydroxybenzophenone [1] was protected using pivaloyl chloride and the McMurry coupling reaction was then performed between the monopivaloylated benzophenone and propiophenone [18]. A deprotection was finally performed to reveal the hydroxy group. After alkylation it was clear the reaction favoured the synthesis of the Z isomer with a 99:1 Z:E isomeric ratio.

2.5. Prototype synthesis

In the present work, for synthesis of the required products outlined in Figure 2.1 initial McMurry coupling of 4-hydroxybenzophenone (1 equivalent) and 1-phenyl-2-butanone [3] (3 equivalents) was performed to synthesise 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (BRI030) using the McMurry coupling reaction.



[3] [BRI030]

Scheme 2.18 Synthesis of 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (BRI030)

Following the protocol developed by Coe and Scriven[117] (see section 2.4) the McMurry coupling reaction was performed in order to synthesise 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (BRI030). Some experimentation with the reaction conditions was necessary as the order of addition of reagents can be crucial in obtaining a successful coupling outcome. The optimum conditions for the coupling reaction in Scheme 2.18 were elucidated as initial catalyst formation via the refluxing of titanium tetrachloride (4 equivalents) with zinc dust (8 equivalents), in dry THF (tetrahydrofuran) at 100°C for 2 hours in the dark. The reactants were added in solution in THF to the catalyst and refluxed for a further 5 hours. The reaction was performed under nitrogen in a reaction carousel, which allowed many of these couplings to be carried out simultaneously. An 80% yield of light yellow oil 5:1 ratio of Z:E isomers was obtained in this case and so the reaction conditions were maintained for all coupling reactions that followed. Positive identification of the products was obtained from spectroscopic data. The infrared spectrum positively identified a C=C double bond at ν1608 cm^{-1}.

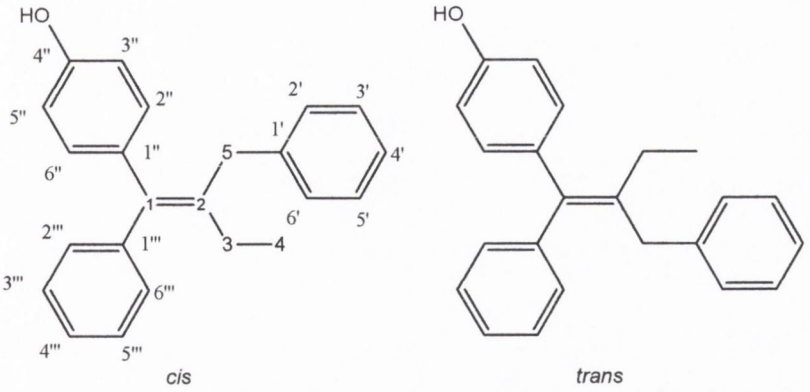
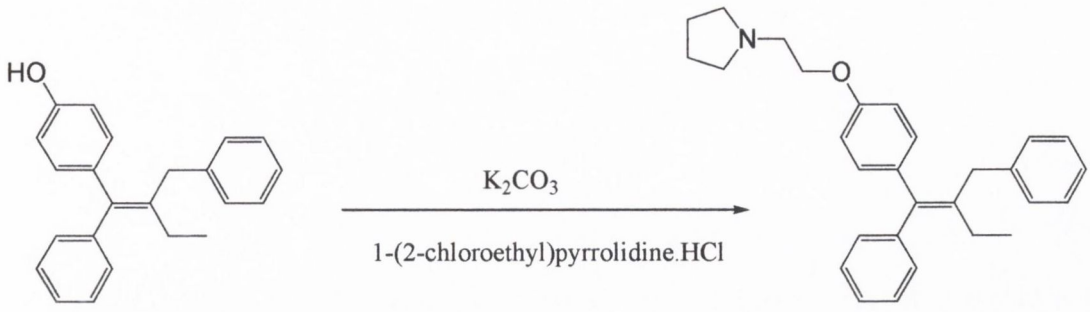


Figure 2.2 Lead structure *cis* and *trans* isomers and NMR assignment

The isomeric ratios (E:Z) (Figure 2.2) of the compound were determined by simply observing the signal heights of the benzylic CH_2 groups that appear around 4.00ppm in the H^1NMR spectrum. The downfield signal to the left represents the Z-isomer and the more upfield signal to the right the E-isomer[118]. The Z-isomer produces a distinct A_2B_2 pattern in the ^1H NMR spectrum, from the phenolic ring of the triaryl-structure, which is shifted to a higher field by approximately 0.40ppm, relative to the E-isomer. This double shielding effect experienced by the phenolic ring and the basic side chain is due to its positioning between the two other rings and their ring currents. In the ^1H NMR spectrum the H-4 protons are observed as a triplet at δ1.04 (J=7.5Hz) being split by and the methylene H-3 protons, which occur as a quartet at δ2.11 (J=7.5Hz). The benzyl CH_2 group H-5 exists as two singlets and these appear at δ3.62 and δ3.66. The partially shielded H-3'', H-5'' protons are found as a doublet in the region δ6.76-6.78. The remaining aromatic protons are observed as a multiplet in the range δ7.13-7.3.

In the ^{13}C NMR spectrum, the methyl C-4 is characteristically observed upfield at 12.80ppm. The C-3 and the C-5, which appear at 36.86ppm and 24.31 ppm, are also in positions characteristic of these type structures. Although the somewhat deshielded benzyl C-5 CH_2 has double signals at 36.73 and 36.86ppm, illustrating the presence of the geometric *E* and *Z*-isomers. Aromatic carbons C-3'' and C-5'' are the most shielded of the aromatic carbons due to their location beside a hydroxy group and therefore appear at 114.50ppm and 114.57ppm. The C=C bond carbons appear at 128.97ppm (C-1) and 138.52ppm (C-2). The C-1' signal occurs at 143.02ppm. The remaining aromatic carbons generally appear in the region 125.00ppm to 130.00ppm.

2.5.1. 2-Benzyl-1-(4-pyrrolidinyl ethoxy)-1-phenylbut-1-ene (BRI039)



BRI030 BRI039

Scheme 2.19 2-Benzyl-1-(4-pyrrolidinyl ethoxy)-1-phenylbut-1-ene (BRI039)

Alkylation[119] was performed on the previous compound (BRI030) with 1-(2-chloroethyl)pyrrolidine hydrochloride using a procedure outlined by Mittal *et al.* [120]. This method consisted of refluxing 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (BRI030) for 5 hours with anhydrous K_2CO_3, in acetone : water (19:1), with the corresponding alkylamino derivative, which in this case is 1-(2-chloroethyl)pyrrolidine.HCl (Scheme 2.19). This basic side group addition was performed to ensure the reaction was feasible for similar types of compounds. The yield of the reaction was 22% of 2.2:1 Z:E isomeric ratio, but due to the simplicity of the reaction and the purity of the product produced the reaction appeared to be suitable for alkylation of other phenolic analogues despite its low yield. The infrared spectrum confirmed the presence of a basic side chains by the signal at $\nu 1507 cm^{-1}$ (NCH_2).

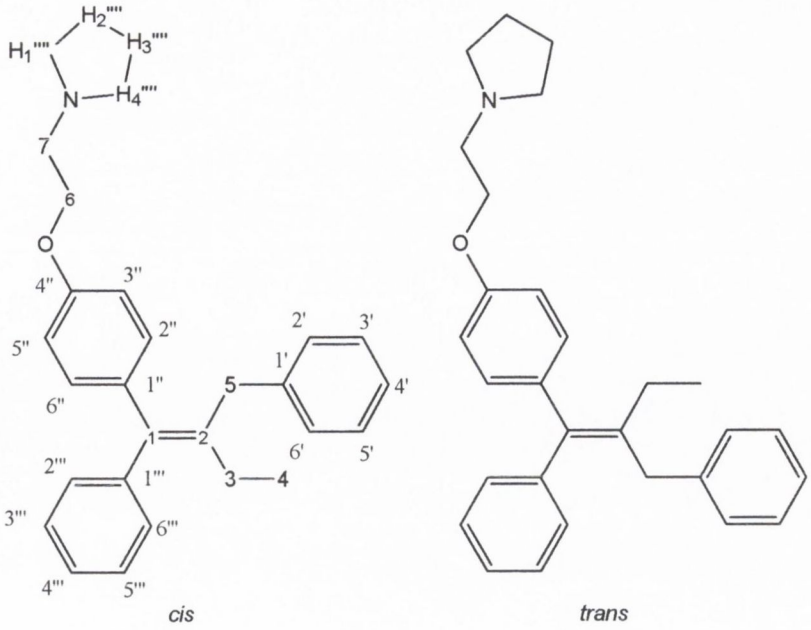


Figure 2.3 *Cis* and *trans* isomers with side chain added NMR assignment

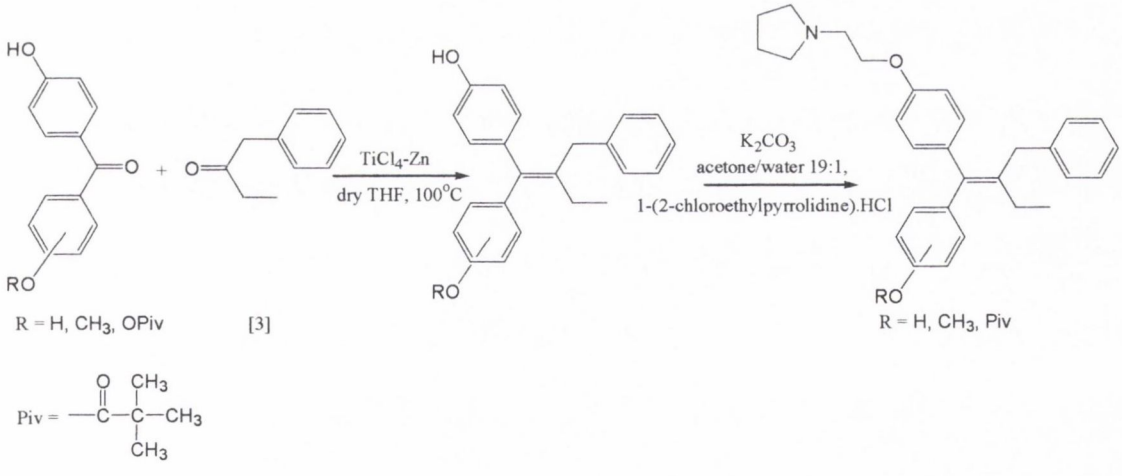
In the ^{1}H NMR spectrum of 2-benzyl-1-(4-pyrrolidinyl ethoxy)-1-phenylbut-1-ene (BRI039) (Figure 2.3) the signals at δ0.98 (J=7.5Hz) and δ2.09 (J=7.5Hz) represent H-4 protons and the methylene H-3 protons and occur as a triplet and a quartet. Two singlets that appear at δ3.62 and δ3.66 represent the benzyl CH_2 group. The hydroxy group signal has disappeared as the basic side chain has replaced the hydroxy signal. Signals representing the basic side chain (H-1'''' and H-4'''', H-2'''' and H-3'''') appear at δ1.90 and δ2.88 each integrating for four hydrogens. The signals at δ3.03 and δ4.22 (H-6, H-7) integrate for two hydrogens each and represent the ethylene chain of the side group. They are more deshielded due to the oxygen to which they are linked. The partially shielded H-3'', H-5'' protons are found as a doublet in the region δ6.80 to δ6.9. The remaining aromatic protons are observed as a multiplet in the range δ7.26-7.36.

On examination, the ^{13}C NMR spectrum displayed similar signals for C-4, C-3 and C-5 as for its phenolic version. New signals representing the basic side chain carbons such as C-2'''' and C-3'''' are observed and are most shielded carbons of the basic side chain appearing at 22.90ppm. Next in line due their positions next to the nitrogen and its deshielding effect are C-1'''', C-4'''' and C-5 which appear downfield at 54.11ppm and 54.30ppm respectively. Finally the most deshielded and therefore most downfield of the basic side chain carbons is C-7, which appears at 65.44ppm. Aromatic carbons C-3'' and C-5'' are the most shielded of the aromatic carbons due to their location beside the basic

side chain oxygen and therefore appear at 113.63ppm and 113.71ppm. The C=C bond carbons, C-1', and all remaining aromatic carbons generally appear as in the ^{13}C NMR spectrum for the phenolic version of this compound BRI039. High resolution mass spectrometry for this compound affords the molecular ion $M^{+}+1$ 412.2640 ($C_{29}H_{36}N_1O$) in 100% abundance, calculated for 411.2562

2.6. Analogue series 1

The synthesis of the analogue series I (Figure 2.1) was performed *via* two main synthetic steps; benzophenone synthesis (*via* a Friedel-Crafts reaction, which was followed by various protection and deprotection reactions), and the two-step McMurry coupling reaction. Firstly the synthesis of the methoxybenzophenones will be discussed and then their subsequent coupling to 1-phenyl-2-butanone [3] (Scheme 2.20). This will be followed by the synthesis of pivaloyloxybenzophenones and their couplings to 1-phenyl-2-butanone [3]. And finally the synthesis of hydroxy substituted analogues will be discussed. The pivaloyloxy substitutent was employed for three reasons a) as a lipophilic ester substitutent for evaluation and b) as a protecting group, which could be removed to reveal the free hydroxy group c) it is also relatively stable to hydrolysis *in-vivo*.



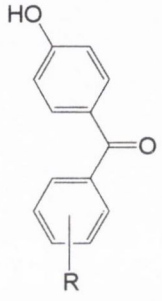
Scheme 2.20 Synthesis of analogue series 1

2.6.1. Synthesis of methoxy-substituted triarylbutene analogues

The yields of the substituted benzophenones varied according to the difficulty with which the different positions could be substituted. The desired *para* substituted benzophenones, as

expected, were the least difficult to obtain and were produced in high yields. Whereas the *ortho* and *meta* substituted benzophenones were more difficult to obtain. They required a series of protecting and deprotecting steps using different reagents for different protecting groups to produce the desired benzophenone. Methoxy and pivaloyl groups were used as protecting groups and sodium ethanethiolate, pyridine and boron trifluoro-dimethyl sulphide were the reagents used for deprotecting.

2.6.1.1. Methoxy substituted benzophenone synthesis



R = *para*-OMe BRI004

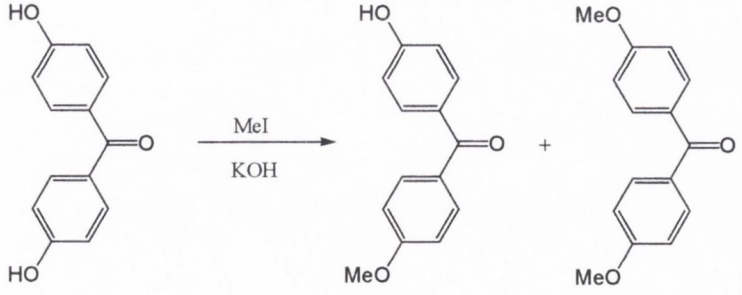
R = *meta*-OMe BRI015

R = *ortho*-OMe BRI019

Figure 2.4 *Ortho*, *meta* and *para*-methoxy-4'-hydroxybenzophenones

In order to synthesise the methoxy-substituted triarylbutenes the initial synthesis of the methoxy-substituted benzophenones (Figure 2.4) was necessary. These benzophenones were synthesised using various different Friedel-Crafts, methylation and demethylation reactions.

2.6.1.1.1. Synthesis of 4-hydroxy-4'-methoxybenzophenone (BRI004) from 4,4'-dihydroxybenzophenone [1]



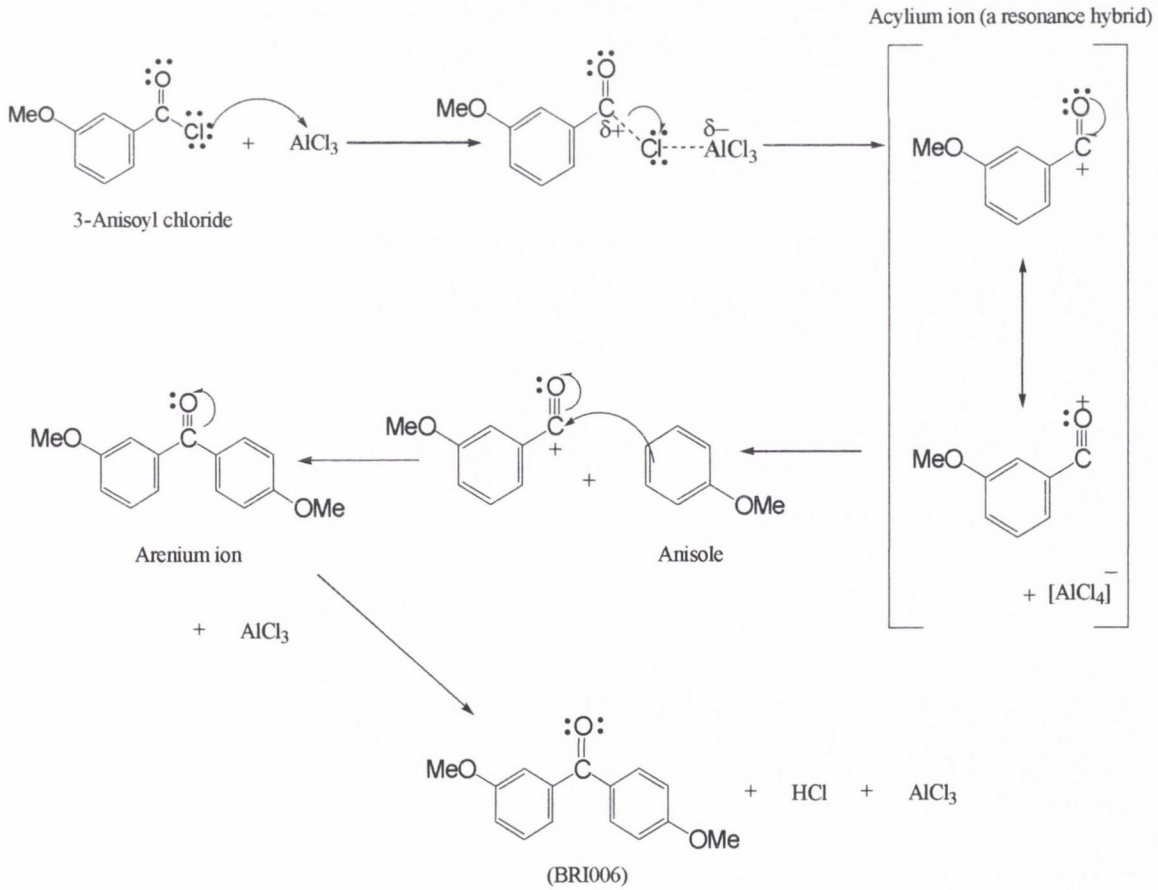
[6] (BRI004) (BRI004B)

Scheme 2.21 4-Hydroxy-4'-methoxybenzophenone (BRI004)

Synthesis of 4-hydroxy-4'-methoxybenzophenone (BRI004)[121] is performed by the methylation of 4,4'-dihydroxybenzophenone [1] as seen in Scheme 2.21. The reaction involves the production of an anion from 4,4'-dihydroxybenzophenone [1] by treatment in DMSO with KOH for 10 minutes then methylation[122] by the addition of methyl iodide (MeI). The reaction produced a mixture 2.6:1 ratio of mono-methoxy and dimethoxybenzophenone with the mono-methoxy in higher yield (73%). The products were separated by chromatography.

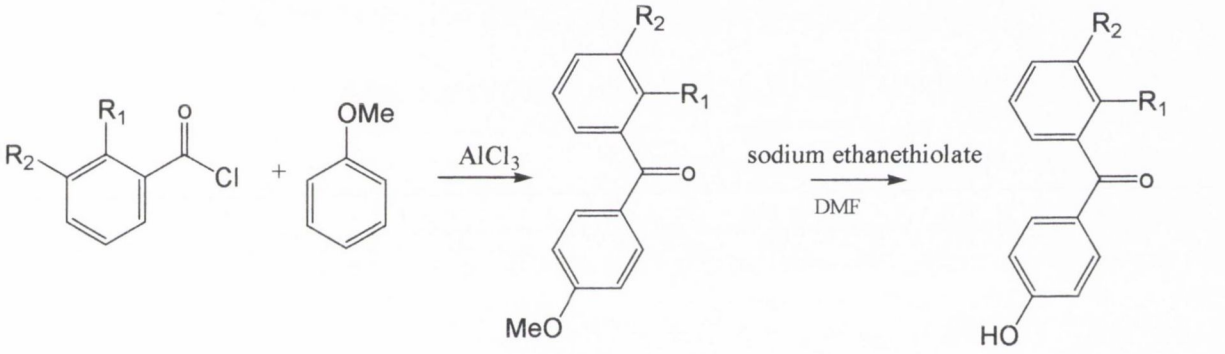
The monomethylated product BRI004 was identified from the ^{1}H NMR spectrum: the signal at δ3.90 is a singlet integrating for three protons and represents the methoxy group. A singlet at δ6.02 represents the hydroxy proton.

2.6.1.1.2. Synthesis of 4-hydroxy-3'-methoxybenzophenone (BRI015)[123] from 3,4'-Dimethoxybenzophenone (BRI006)[123, 124, 125]



Scheme 2.22 Mechanism for Friedel-Crafts reaction

In the Friedel-Crafts reaction[126] shown above in Scheme 2.22 the electrophile is an acylium ion formed from an acyl halide, which in this case is *meta*-anisoyl chloride. The reaction first involves the generation of the electrophile species or acylium ion by an interaction between the catalyst and the halide. The catalyst used here is aluminium chloride and is a Lewis acid. Lewis acids are electron deficient and generally attack electron rich atoms such as a chlorine atom. Once the acylium ion has formed it then attacks the aromatic nucleus of anisole forming an arenium ion and 3,4'-dimethoxybenzophenone (BRI006)[127] is formed with the release of HCl and the recovery of the catalyst. Another major factor that determines the success of a Friedel-Crafts reaction is the increasing reactivity of the aromatic compounds with acyl chloride when the electron donating capability of substitutents increase i.e. hydrogen>methyl>methoxy>dimethoxy.



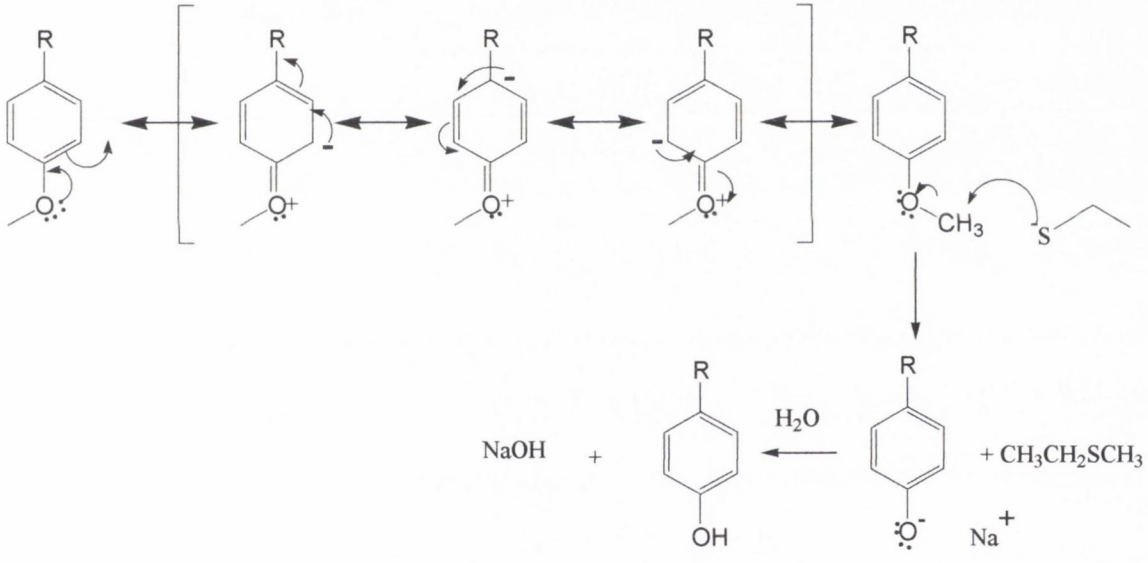
R_1 = OMe R_2 = H	[9]	(BRI008) R_1 = OMe R_2 = H	(BRI019) R_1 = OMe R_2 = H
R_1 = H R_2 = OMe		(BRI006) R_1 = H R_2 = OMe	(BRI015) R_1 = H R_2 = OMe

Scheme 2.23 Synthesis of 4-hydroxy-3'-methoxybenzophenone (BRI015) and 4-hydroxy-2'-methoxybenzophenone (BRI019)

3,4'-Dimethoxybenzophenone (BRI006) was prepared via the Friedel-Crafts reaction of *neta*-anisoyl chloride and anisole [2] using $AlCl_3$ and then selectively deprotected using sodium ethanethiolate (EtSNa) in dimethylformamide (DMF) to yield 4-hydroxy-3'-nethoxybenzophenone (BRI015)[123] as seen in Scheme 2.23. The Friedel-Crafts reaction produced little product on the first attempt and so the reaction conditions were varied to optimise the yield. Firstly the reagents were all placed in a round bottom flask in 30ml of etrachloroethane and left stirring on ice for two days. Little product was observed to be

present in comparison to starting materials when observed by TLC. So the reaction was repeated by placing everything in the flask except $AlCl_3$ and stirring for 45 minutes. The $AlCl_3$ was then added and the reaction left for another 45 minutes. But the yield remained low. In the next attempt, the *m*-anisoyl chloride was added after 45 minutes to the mixture and with this the yield increased somewhat. In a final attempt the reagent quantities were increased 10-fold and only 20 ml tetrachloroethane was used. The reaction was stirred on ice while the *m*-anisoyl chloride was added to the solution and the reaction was left stirring for three days. A 90% yield of 3,4'-dimethoxybenzophenone (BRI006) was obtained.

In the ^1H NMR spectrum of 3,4'-dimethoxybenzophenone (BRI006) the singlet signals which appear at δ3.87 and δ3.90 represent the two methoxy groups. Following this a multiplet represents the aromatic protons in the region δ6.97-7.87 with the *para*-substituted ring forming two distinct doublets that integrate for two protons each.



Scheme 2.24 Deprotection mechanism

To synthesise 4-hydroxy-3'-methoxybenzophenone (BRI015), 3,4'-dimethoxybenzophenone (BRI006) was demethylated in N,N-dimethylformamide (DMF) with sodium ethanethiolate. Deprotection was achieved by the mechanism as seen in Scheme 2.24 *via* the nucleophilic displacement of the ether function with sodium thioethoxide. Sulphur compounds form better nucleophiles than their oxygen analogues and are therefore more reactive. Resonance stabilisation of the phenolate anion, which

weakens the O-Me bond considerably, drives the nucleophilic attack of the thiolate anion on the methyl group. The reaction produced a good yield of the mono methoxy product and a little of the dihydroxy product.

This deprotection method[123] of sodium ethanethiolate in DMF provides a convenient and regioselective method for the demethylation of aryl methyl ethers-substituted electron-withdrawing groups. Electronic factors appear to control the observed selectivity i.e., methyl ethers *para* to the electron-withdrawing functionality react preferentially with the thiol anion. Another reason for the demethylation reaction favouring the deprotection of the *para*-methoxy over the *ortho*-methoxy may simply lie in the fact that at a molecular level, due to its position, the para-methoxy is more accessible and therefore less hindered by other atoms on the molecule. In other words it is more available to attack and substitute.

The deprotection was confirmed by the presence of a characteristic OH signal at $\nu 3334 cm^{-1}$ in the IR spectrum. In the ^1H NMR spectrum, a singlet signal occurs at δ3.87 and represents the protons of a methoxy group. The next signal is the hydroxy proton signal, which appears at δ6.39. The presence of this hydroxy signal plus the disappearance of a methoxy signal also confirms the deprotection of 3,4'-dimethoxybenzophenone (BRI006). The doublets that represent the *para*-substituted ring are shifted slightly upfield from their position in the ^1H NMR spectrum of 3,4'-dimethoxybenzophenone (BRI006) at δ6.99 and δ7.87 to δ6.93 and δ7.80 suggesting that the deprotection has being performed on the *para*-substituted ring. The remaining aromatic protons for the *meta*-substituted ring are unchanged and occur in the region δ7.12-7.33.

2.6.1.1.3. Synthesis of 4-hydroxy-2'-methoxybenzophenone (BRI019) and 2-hydroxy-4'-methoxybenzophenone (BRI018) and 2,4'-dihydroxybenzophenone (BRI188) from 2,4'-dimethoxybenzophenone (BRI008)[128, 123, 124, 125]

The synthesis of 2,4'-dimethoxybenzophenone (BRI008)[129, 130, 131] was attempted as for 3,4'-dimethoxybenzophenone (BRI006) in section 2.6.1.1.2 (see Scheme 2.23) but the reaction required some optimisation as very little product was initially obtained. O-anisoyl chloride was dissolved in tetrachloroethane and added dropwise to the reaction mixture (also in tetrachloroethane) as diluting of the *o*-anisoyl chloride was expected to slow down the addition of the *o*-anisoyl chloride and therefore allow the reagents time to react and not

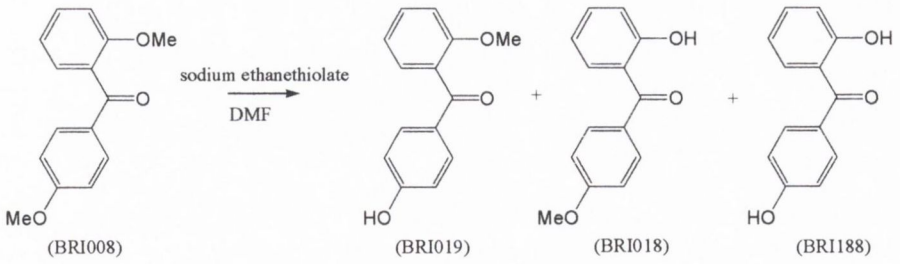
bombard the anisole with reactant. This procedure did not increase the yield as expected. $AlCl_3$ was replaced with $SnCl_4$ as the Lewis acid for the next attempt. O-anisoyl chloride was added dropwise to the reaction mixture and once again the reaction (Scheme 2.23) was left stirring for two days. This time an 81% yield of product was obtained.

The failure of the acylation[126] to proceed in this case with aluminium chloride can be assigned to the fact that the *ortho*-position of the methoxy group may in some way have hindered the formation of the acylium ion. The reaction was successful when the Lewis acid was replaced with tin chloride. The reason for this may simply be that tin is more reactive than aluminium as reactivity increases on descent of the periodic table of elements, as does the size of the individual atoms.

In the ^{1}H NMR spectrum of BRI008 the first singlet signal appearing at δ3.76 integrates for three protons and therefore represents the methoxy group. The second signal appears slightly downfield to it at δ3.89 and it represents a second methoxy group. A series of doublets represent the aromatic protons in the region δ6.92-7.84. This ^{1}H NMR spectrum confirms the synthesis of 2,4'-dimethoxybenzophenone (BRI008).

The synthesis of 2-hydroxy-4'-methoxybenzophenone (BRI018) was attempted using the methylation method discussed in section 2.6.1.1.1 and varying the reaction conditions. Only the completely protected 2,4'-dimethoxybenzophenone (BRI008) was synthesised each time as yellow crystals (92%).

The selective deprotection of 2,4'-dimethoxybenzophenone (BRI008) (Scheme 2.25) was performed using sodium ethanethiolate as in section 2.6.1.1.2 in order to obtain 4-hydroxy-2'-methoxybenzophenone (BRI019) [132, 124]. The yield was very low (4%) and so optimisation of the synthesis was necessary. A new method was attempted involving the heating of 2,4'-dimethoxybenzophenone (BRI008), phenol and polyphosphoric acid[133] at 100°C for 20 minutes. This reaction was unsuccessful so optimisation of the deprotection was pursued. The desired product was obtained as yellow crystals (26.3%) Some useful by-products from this reaction were; 2-hydroxy-4'-methoxybenzophenone (BRI018)[134] (50%) and 2,4'-dihydroxybenzophenone (BRI188) [135] (20%).



(BRI008) (BRI019) (BRI018) (BRI188)

Scheme 2.25 Synthesis of 4-hydroxy-2'-methoxybenzophenone (BRI019), 2'-hydroxy-4-methoxybenzophenone (BRI019) and 4,2'-dihydroxybenzophenone (BRI118) from 4,2'-dimethoxybenzophenone (BRI008)

The deprotection was confirmed by the presence of a characteristic OH signal at $\nu 3358 cm^{-1}$ in the IR spectrum of 4-hydroxy-2'-methoxybenzophenone (BRI019).

The ^1H NMR spectrum of 4-hydroxy-2'-methoxybenzophenone (BRI019) displays a singlet at δ3.76, which represents one methoxy group. A hydroxy signal occurs at δ5.58. This confirms the demethylation process, but whether it was the *para*-methoxy or *ortho*-methoxy that had being removed was determined by examining a COSY spectrum. It was obvious from the COSY spectrum that the methoxy group directly affects the aromatic signals that represent the *ortho* substituted ring whereas the distinct set of doublets that represent the *para* substituted ring were unaffected confirming that the remaining methoxy group in the molecule is on the *ortho* substituted ring as required. It was also observed that a small singlet integrating for one appeared around δ6.20 and it was suggested that this represented the hydroxy group.

One of the side-products of the deprotection was 2-hydroxy-4'-methoxybenzophenone (BRI018) where the incorrect methoxy group had being removed. The ^1H NMR spectrum for 2-hydroxy-4'-methoxybenzophenone (BRI018) was similar to that of 4-hydroxy-2'-methoxybenzophenone (BRI019) except for the signal at δ11.94. The assigning of the *para*-positioned hydroxy group in the ^1H NMR spectrum of 4-hydroxy-2'-methoxybenzophenone (BRI019) to the signal at δ5.58 allowed the assumption to be made that the signal at δ11.94 represented the hydroxy group in the *ortho*-position. The great difference in the positioning of the *ortho*-hydroxy group compared with that of the *para*-hydroxy may be due to the deshielding effect of the carbonyl group to which it is closer.

The other side-product of the reaction was the completely deprotected product 2,4'-hydroxybenzophenone (BRI188) which was easily identifiable by ^{1}H NMR spectrum as both methoxy signals had disappeared and both distinct hydroxy signals are present at δ5.39 and δ11.95 as discussed above appeared in the same spectrum.

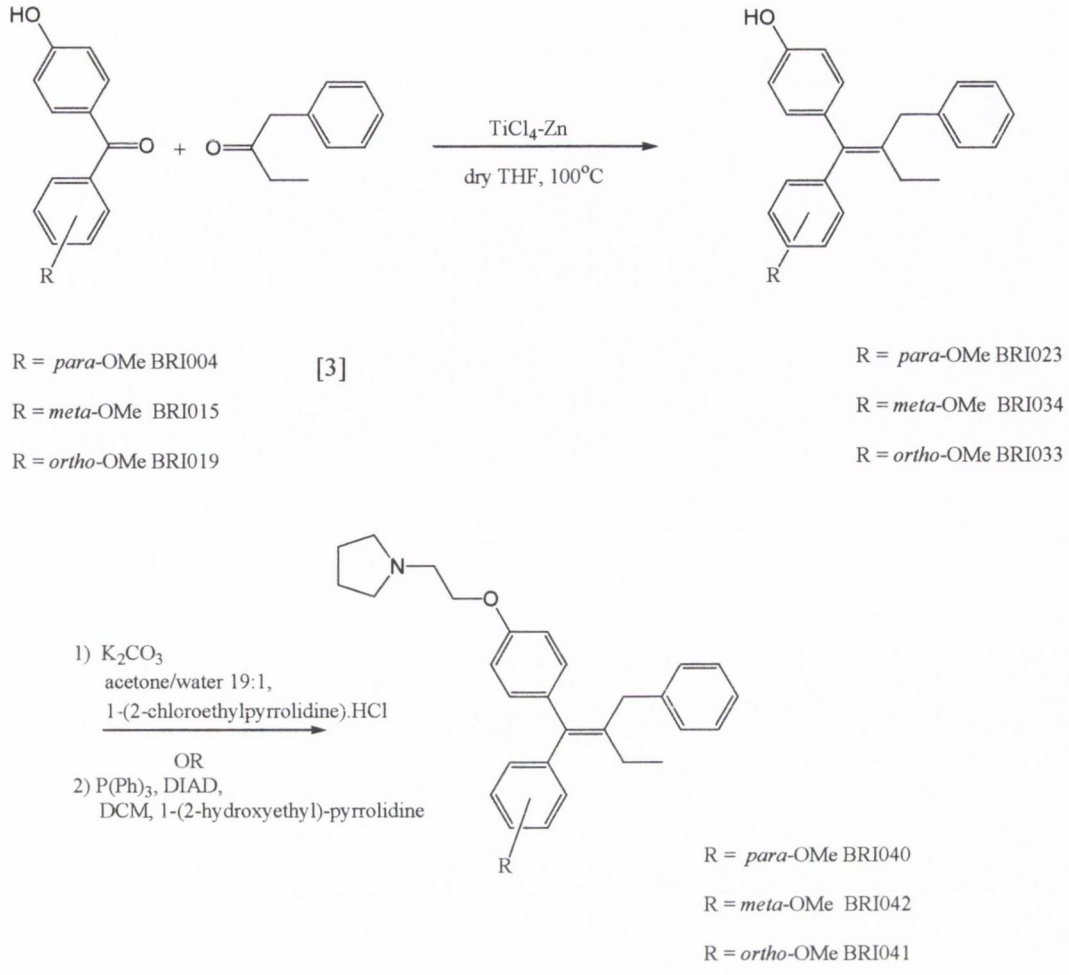
The infrared spectrum, for each of the benzophenones produced above, shows the presence of the carbonyl (C=O) stretch in the region υ1651-1658cm^{-1} and the aromatic (C=C) stretch in the region υ1601-1605cm^{-1}.

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI004	**40**	**1617 (C=O)**	**1605 (C=C)**
BRI015	**67**	**1647 (C=O)**	**1597 (C=C)**
BRI019	**26**	**1643 (C=O)**	**1576 (C=C)**

Table 2.1: Yield and infrared data for compounds BRI1004, BRI015 and BRI019

2.6.1.2. McMurry coupling of methoxy substituted benzophenones to 1-phenyl-2-butanone [3]

The general synthetic route followed for these products is illustrated in Scheme 2.26. Positive identification of the products was obtained from spectroscopic data.



Scheme 2.26 Synthesis of methoxy-substituted analogues series 1

2.6.1.2.1. Synthesis of 2-benzyl-1-(4-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI040).

Synthesis of 2-benzyl-1-(4-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI023) was performed *via* the McMurry coupling reaction. 4-Hydroxy-4'-methoxybenzophenone (BRI004) was reacted with 1-phenyl-2-butanone [3] and an 88% yield of 2-benzyl-1-(4-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI023) was achieved.

In the ^{1}H NMR spectrum of BRI023 the H-4 protons are observed as a triplet at δ1.04 (J=7.5Hz) as in the prototype spectrum, and the methylene H-3 protons occur as a quartet at δ2.16 (J=7.5Hz). Two singlets appear at δ3.66 and δ3.62. These represent the benzyl CH_2 group as Z and E isomers. A signal not present in the prototype spectrum appears at δ3.82 and integrates for three therefore representing the *para*-methoxy group. The *para*-

hydroxy group appears as a singlet at δ6.18. The partially shielded H-3'', H-5'' aromatic protons are found as a doublet between δ6.80-6.85 (J=8.56). The remaining aromatic protons are observed as a multiplet in the range δ6.90-7.32, while the Z-isomer aromatics are found at a slightly lower field.

The ^{13}C NMR spectrum for 2-benzyl-1-(4-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI023) is very similar to that for 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (BRI030). The signals for C-4, C-3 and C-5 are almost identical as are the aromatic carbon signals. An additional signal at 53.82ppm represents the methoxy group.

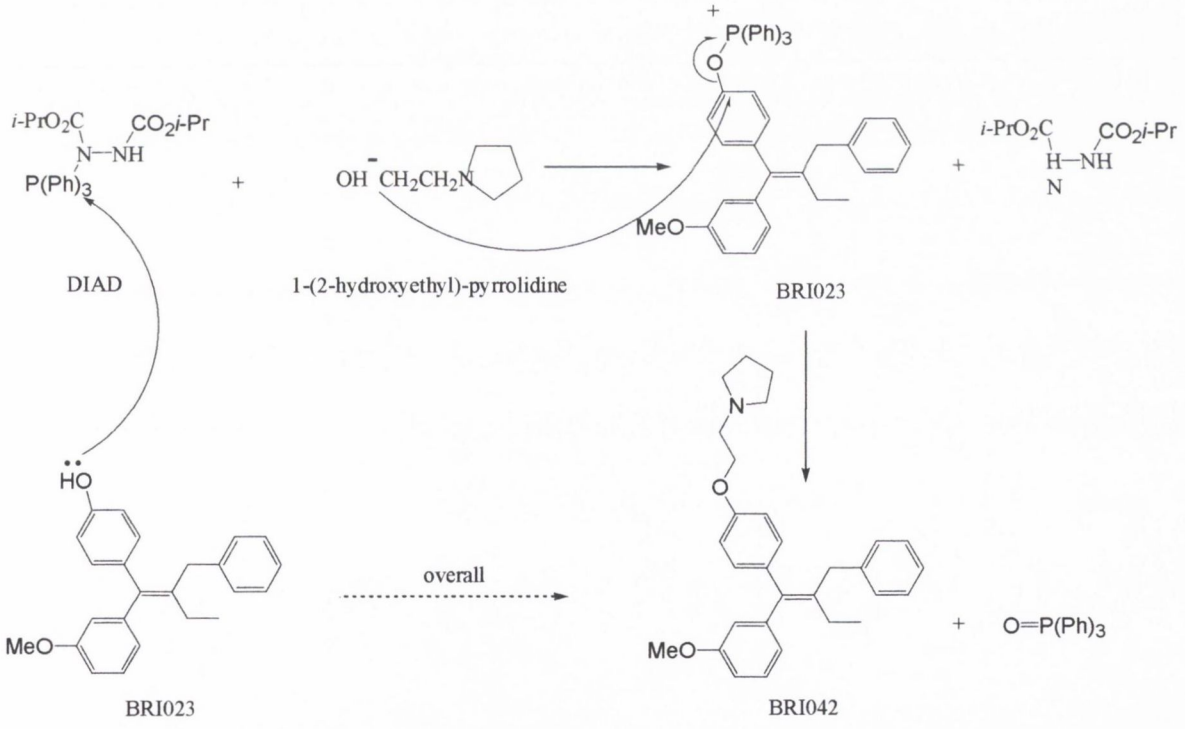
To complete the synthesis, a basic side chain was added by alkylation to 2-benzyl-1-(4-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI023) to produce 2-benzyl-1-(4-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI040) using the usual alkylation[119] reaction conditions as previously outlined.

In the ^{1}H NMR spectrum of BRI040 the signals for H-4, H-3 and H-5 protons are observed as a triplet, a quartet and a singlet at δ0.95 (J=7.5Hz), δ2.03 (J=7.5Hz), δ3.40. The hydroxy group signal is absent as the basic alkyl group is now present. New signals representing the basic side chain (H-1'''' and H-4'''', H-2'''' and H-3'''') appear at δ1.77 and δ2.61 each integrating for four protons. At δ3.03 and δ4.26 are signals that integrate for two hydrogens each and represent the remaining protons of the sidechain H-6 and H-7, which are more deshielded due to the adjacent oxygen. The aromatic protons are observed as a multiplet in the range δ6.78-7.28.

The ^{13}C NMR spectrum confirms the presence of the basic alkyl side chain. New signals that are not present in the ^{13}C NMR spectrum of 2-benzyl-1-(4-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI023) represent the newly added basic side chain. C-2'''' and C-3'''' appear as a signal at 23.11ppm while C-1'''' and C-4'''' appear more downfield at 50.03ppm. C-6 and C-7 appear at 53.82ppm and 63.15ppm and the methoxy group is characteristically represented by a signal at 54.73ppm. C-3'' and C-5'' aromatic carbons appear at 113.01ppm and 113.06ppm due to the deshielding effect of the basic side chain. High resolution mass spectrometry for this compound confirms the molecular ion (M^{+}+1), 442.2746 ($C_{30}H_{36}NO_2$) in 100% abundance, calculated for 441.2668.

2.6.1.2.2. Synthesis of 2-benzyl-1-(3-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI042)[136]

The coupling of 3-methoxy-4-hydroxybenzophenone (BRI015) and 1-phenyl-2-butanone [3] produced 2-benzyl-1-(3-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI034) in an 80% yield (Scheme 2.26). The signals in the ^{1}H NMR spectrum in the ranges δ1.06-1.10 (J=7.5Hz) and δ2.12-2.13 (J=7.5Hz) that represent H-4 protons and the methylene H-3 protons are very similar to ^{1}H NMR spectrum for 2-benzyl-1-(4-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI023). Two singlets appear at δ3.75 and δ3.76, which represent the benzyl CH_2 group and can be used to estimate the Z:E isomeric ratio as displayed in Table 2.3. Methoxy group and hydroxy group signals appear as singlets at δ3.84 and δ5.78 respectively. The partially shielded H-3'', H-5'' protons are found as a doublet between δ6.8-6.82 (J=8.04). The remaining aromatic protons are observed as a multiplet in the range δ6.86-7.3.



Scheme 2.27 Mitsunobu method for the synthesis of BRI042

The alkyl side chain addition[136] was achieved using an alternative alkylation based on the Mitsunobu reaction in which the phenol is reacted with triphenylphosphine, 1-(2-hydroxyethyl)-pyrrolidine and diispropyl azodicarboxylate (DIAD)[137]. This reaction produced a yield of 20% product that was still slightly contaminated with triphenylphosphine, which was very difficult to remove. The standard alkylation method for side group addition was then used for this reaction. Using the method from section 2.5.3 the basic side chain[119] was introduced onto 2-benzyl-1-(3-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI034) producing 2-benzyl-1-(3-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI042) in a yield of 44%.

The ^{1}H NMR spectrum is similar to that for 2-benzyl-1-(4-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI040). High resolution mass spectrometry for this compound affords the molecular ion (M^++1) 442.2506 ($C_{30}H_{36}NO_2$) in 100% abundance, calculated for 441.2668 and therefore confirms the synthesis of the desired product.

2.6.1.2.3. Synthesis of 2-benzyl-1-(2-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI041)

4-Hydroxy-2'-methoxy-benzophenone (BRI019) and 1-phenyl-2-butanone [3] were reacted under reductive coupling conditions to produce 2-benzyl-1-(2-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI033)(18%). Basic side chain addition[119] was performed on 2-benzyl-1-(2-methoxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI033) to produce 2-benzyl-1-(2-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI041) using the method described in section 2.5.3. The ^{1}H NMR spectrum once again is very similar to those described above for basic side chain alkylation to methoxy-substituted triarylbutenes. The synthesis of the desired product, 2-benzyl-1-(2-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI041), was confirmed by high resolution mass spectrometry, which afforded the molecular ion (M^++1) 442.0740 ($C_{30}H_{36}NO_2$) in 100% abundance, calculated for 441.2668.

Table 2.2 and Table 2.3 display infrared data for the phenolic analogues and the products of basic side chain addition to these phenolic compounds. The tables allow comparison between the isomeric ratios of the different analogues. On observation, the isomeric ratios

suggest that all of the reactions favour the synthesis of the Z-isomer but also suggest that the simplest of the coupling reactions (BRI030), involving a mono-substituted benzophenone, favours the synthesis of the Z-isomer more than the other couplings displayed in Table 2.2. However, these differences between the analogues tend to balance out after the addition of the basic side chain. In one case (BRI042) the isomeric ratio increases to favour the Z-isomer to a greater extent after basic side chain addition. The infrared data helps to identify the formation of C=C double bonds which are produced by the McMurry coupling reaction (see Table 2.2). Table 2.3 shows the absence of hydroxy signals in the infrared spectra, which confirms the addition of a basic side chain to each of the phenolic compounds.

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})	Z/E isomeric ratio
BRI030	**60**	**1608 (C=C)**	**3360 (OH)**	**5:1**
BRI023	**88**	**1606 (C=C)**	**3402 (OH)**	**1:1**
BRI034	**80**	**1607 (C=C)**	**3346 (OH)**	**3:1**
BRI033	**18**	**1604 (C=C)**	**3490 (OH)**	**3:1**

Table 2.2: Yield and infrared data for compounds BRI030, BRI023, BRI034 and BRI033

Compound	Yield %	IRυ_{max} (film cm^{-1})	Z/E isomeric ratio
BRI039	**22**	**1606 (C=C)**	**2.2:1**
BRI040	**36**	**1606 (C=C)**	**1:1**
BRI042	**42**	**1604 (C=C)**	**4:1**
BRI041	**44**	**1604 (C=C)**	**2:1**

Table 2.3: Yield and infrared data for compounds BRI039, BRI040, BRI042 and BRI041 (basic side chain addition products)

2.6.2. Synthesis of pivaloyl-substituted triarylbutene analogues

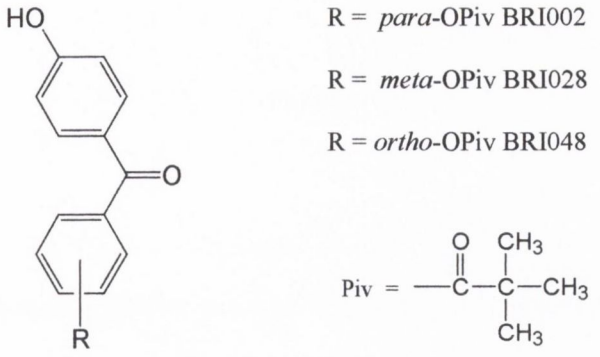
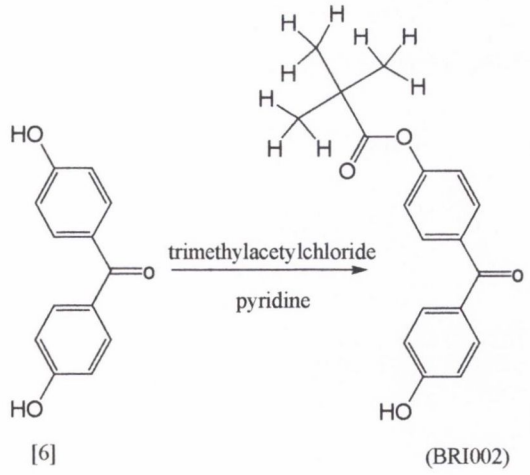


Figure 2.5 Synthesis of *ortho*, *meta* and *para* pivaloyloxy-4'-hydroxybenzophenone

In order to synthesise the pivaloyl-substituted triarylbutenes the initial synthesis of the pivaloyl-substituted benzophenones (Figure 2.5) was necessary. These benzophenones were synthesised using various different Friedel-Crafts, pivaloylation and depivaloylation reactions.

2.6.2.1. Synthesis of *ortho*, *meta* and *para* pivaloyloxy-4'-hydroxybenzophenone

2.6.2.1.1. Pivaloylation of 4,4'-dihydroxybenzophenone [1] (synthesis of 4-hydroxy-4'-pivaloyloxybenzophenone) (BRI002)[119, 139]

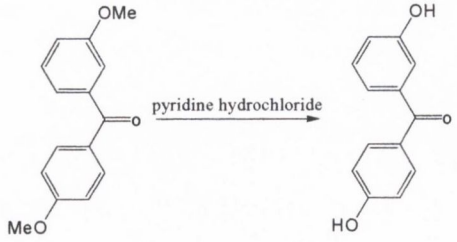


Scheme 2.28 4-Hydroxy-4'-pivaloyloxybenzophenone (BRI002) from 4,4'-dihydroxybenzophenone [1]

4-Hydroxy-4'-pivaloyloxybenzophenone (BRI002)[140] was synthesised (Scheme 2.28) by the mono-pivaloylation of 4,4'-dihydroxybenzophenone [1]. The reaction was carried out many times varying the reaction conditions in order to achieve optimum yield. 4,4'-Dihydroxybenzophenone [1], trimethylacetyl chloride and pyridine[139] were stirred at 50°C for two hours. The progress of the reaction was observed by thin layer chromatography (TLC). The mono-pivaloyloxy and di-pivaloyloxybenzophenones products were separated by flash chromatography. This reaction was successful but produced a very low yield (30%). To increase the yield, the reaction was then attempted with aluminium chloride ($AlCl_3$) and potassium hydroxide using DMSO as the solvent and adding trimethylacetyl chloride to the solution until a colour change occurred. The reaction was left stirring for 3 hours but no product was obtained. Finally the solvent was changed to acetone in the hope that it would make the purification of any product easier. 4,4'-dihydroxybenzophenone [1], potassium hydroxide, and trimethylacetyl chloride in acetone were reacted under two different conditions; a) one reaction was refluxed and the other b) was stirred under gentle heat 30°C. After three hours the reaction was stopped, extracted and purified as before producing yellow crystals in the yields; 27.3% for a) and 40% for b).

The infrared data displayed in Table 2.4 confirms the presence of the pivaloyl group by the carbonyl signal at υ1735cm^{-1}. In the ^{1}H NMR spectrum the pivaloyl group protons are represented by a singlet, which appears at δ1.37 and integrates for nine protons. The proton of the hydroxy group appears at δ6.60 as a singlet. The absence of a second hydroxy signal also confirms its replacement or removal. A series of doublets that represented the aromatic protons are shifted further upfield than in the ^{1}H NMR spectrum of 4,4'-dihydroxybenzophenone [1] confirming a change in the substituent present on one of the rings.

2.6.2.1.2. Synthesis of 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028)[119, 139]

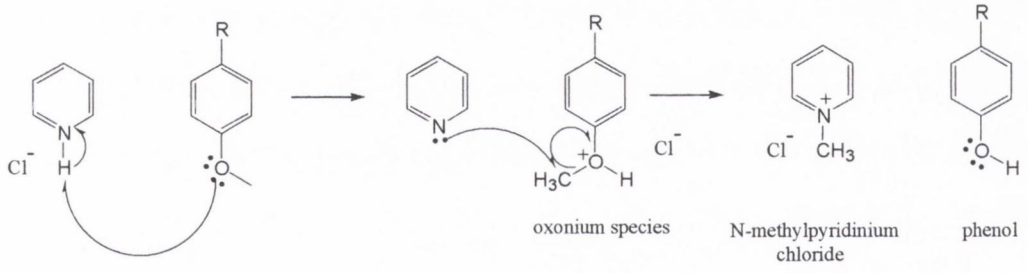


(BRI006) (BRI016)

Scheme 2.29 3,4'-Dihydroxybenzophenone (BRI016) from 3,4'-Dimethoxybenzophenone (BRI006)

The initial synthesis of 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028) was attempted in one step by inducing the complete deprotection 3,4'-dimethoxybenzophenone (BRI006) by refluxing with pyridine and then attempting pivaloylation by adding Trimethylacetyl chloride under the assumption that the pyridine would act as a base for the protection reaction. This reaction was unsuccessful producing a very impure mixture of dihydroxy and dimethoxy benzophenones. The synthesis of 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028) was performed in two steps from the dimethoxybenzophenone. 3,4'-Dimethoxybenzophenone (BRI006)[129] was first melted with an excess of pyridine hydrochloride for 30 minutes (Scheme 2.29) to induce complete demethylation. The mechanism for the deprotection is shown in Scheme 2.30. Nucleophilic attack by a lone pair of electrons of the ether oxygen on the pyridinium ion results in the generation of pyridine and the oxonium species. The product of this reaction, 3,4'-dihydroxybenzophenone (BRI016)[119], was confirmed by the presence of a characteristic OH signal at $\upsilon 3391 cm^{-1}$ in the IR spectrum.

The absence of the methoxy signals in the ^1H NMR spectrum confirms the complete deprotection of 3,4'-dimethoxybenzophenone (BRI006). Also the aromatic signals present in the range $\delta 7.30$-9.00 are further downfield than the protected compound due to deshielding effect by the hydroxy groups.

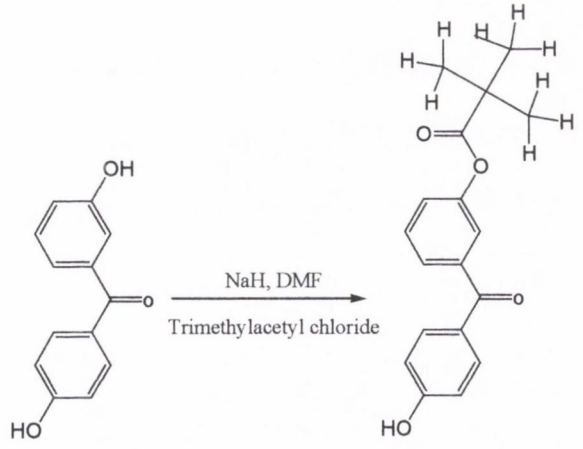


Scheme 2.30 Deprotection mechanism for methyl ethers

Only after minimum purification, this product was selectively protected with trimethylacetyl chloride to produce 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028). 3,4'-Dihydroxybenzophenone (BRI016) dissolved in DMF was reacted with sodium hydride and trimethylacetyl chloride. The base was introduced to promote the formation of an anion from the hydroxy group so substitution with the pivaloylgroup (Scheme 2.31)

could occur. A 50% yield of 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028) was recovered.

The infrared data displayed in Table 2.4 clearly confirms the presence of the pivaloyl group by the carbonyl signal at υ1757cm^{-1}. The ^{1}H NMR spectrum displays a large singlet at δ1.39 that integrates for nine protons and therefore represents the pivaloyl group protons. The ^{1}H NMR spectrum of 4-hydroxy-2'-methoxybenzophenone (BRI019) displays its *para*-positioned hydroxy signal at δ5.58 whereas the ^{1}H NMR spectrum for 2-hydroxy-4'-methoxybenzophenone (BRI018), although very similar, displays the *ortho*-positioned hydroxy group signal at δ11.94 probably due to the deshielding effect of the carbonyl group. A signal at δ5.31 therefore confirms by comparison the presence of the hydroxy group in the *para*-position. A COSY spectrum was produced to confirm the *para*-position of the hydroxy substitutent. The COSY spectrum shows that the distinct aromatic doublet signals δ6.88-6.90 and δ7.77-7.80 of the *para*-substituted ring remain unaffected by the pivaloyloxy group whereas the remaining signals at δ7.28-7.59 that represent the *meta*-substituted ring are affected by the pivaloyloxy group confirming the pivaloylation of the *meta*-hydroxy group and the protection of 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028).



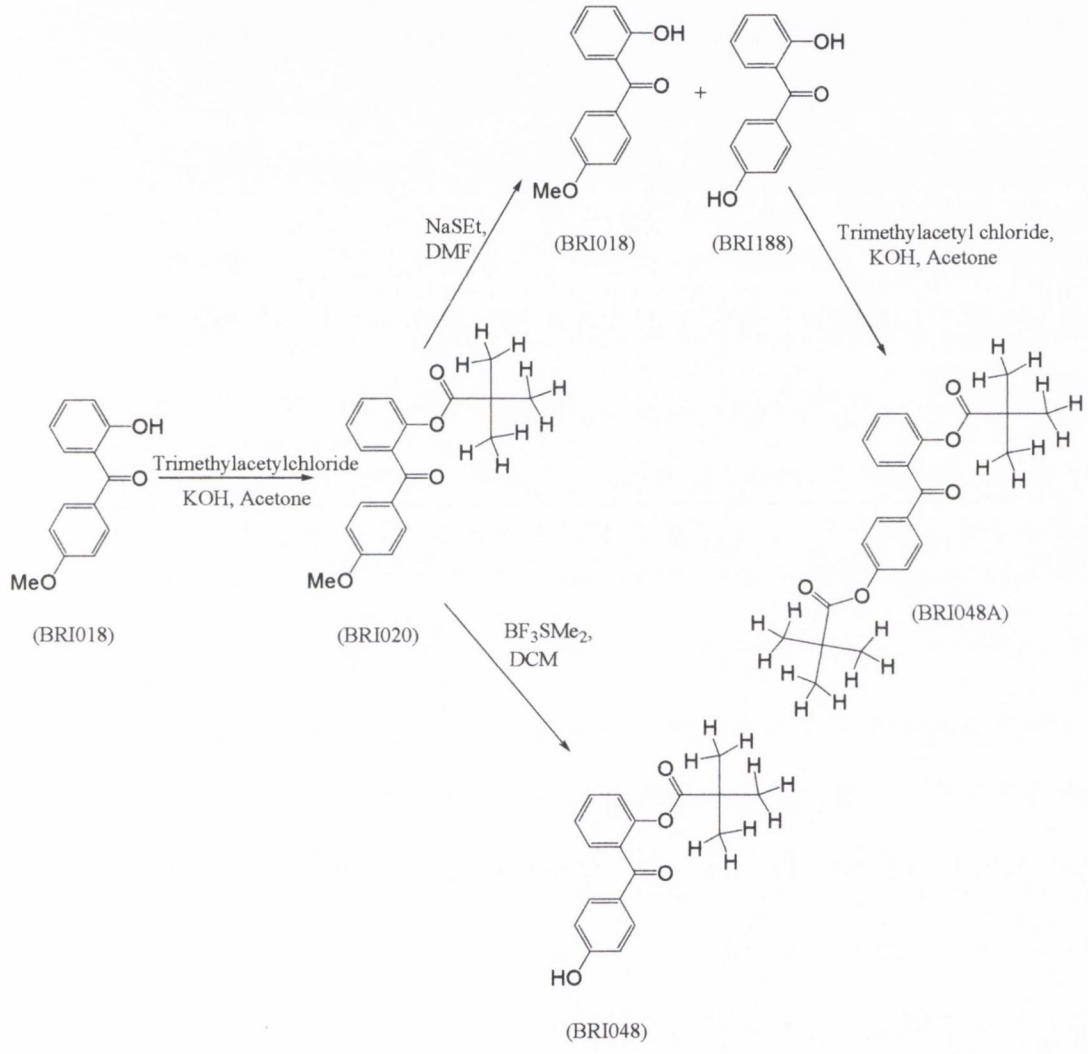
(BRI016) (BRI028)

Scheme 2.31 4-Hydroxy-3'-pivaloyloxybenzophenone (BRI028) from 3',4-hydroxybenzophenone (BRI016)

An explanation for the favoured protection of the *meta*-hydroxy group over the more

available *para*-hydroxy substitutent may lie in the electronegativity values of the hydroxy groups due to their distance from the carbonyl group. The closer the hydroxy group is to the carbonyl group the greater the deshielding effect is that it experiences. This deshielding effect would leave the hydroxy group unstable and more susceptable to substitution or protection then the *para*-hydroxy group.

2.6.2.1.3. Synthesis of 4-hydroxy-2'-pivaloyloxybenzophenone (BRI048)[123, 124, 125]



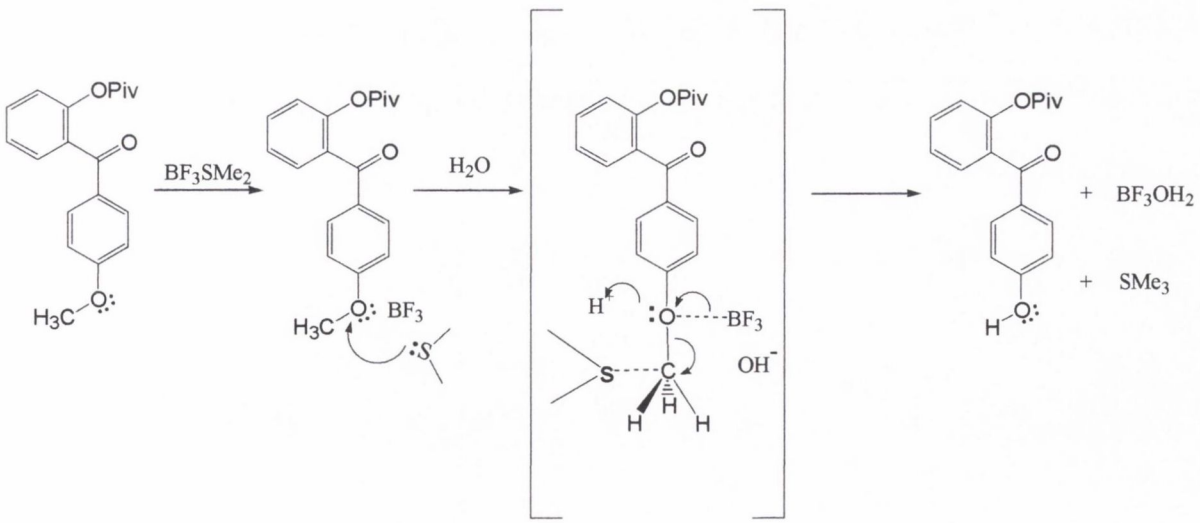
Scheme 2.32 4-Hydroxy-2'-pivaloyloxybenzophenone (BRI048) from 2',4-dihydroxybenzophenone (BRI018)

2-Hydroxy-4'-methoxybenzophenone (BRI018) was produced as a product (50%) of the deprotection of 2,4'-dimethoxybenzophenone (BRI008) in section 2.6.1.1.3 performed

using sodium ethanethiolate. In this reaction the deprotection was confirmed by the presence of a characteristic OH signal at $\upsilon 3448 cm^{-1}$ in the IR spectrum.

Pivaloylation[139] of 2-hydroxy-4'-methoxybenzophenone (BRI018) was performed in order to synthesize 2-pivaloyloxy-4'-methoxybenzophenone (BRI020) (see Scheme 2.32). 2-Hydroxy-4'-methoxybenzophenone (BRI018) was reacted with an excess of potassium hydroxide (KOH) in acetone for one hour. Then trimethylacetyl chloride was added to the mixture and left stirring overnight. This reaction was unsuccessful as only starting material was recovered. The reaction was attempted again but this time the KOH and 2-hydroxy-4'-methoxybenzophenone (BRI018) were left stirring for three hours in acetone (20ml). Then trimethylacetyl chloride was added and the reaction was again left stirring overnight. A yield of yellow crystals (40%) was obtained.

The absence of the hydroxy signal at $\upsilon 3402 cm^{-1}$ in the infrared spectrum plus the presence of a carbonyl signal $\upsilon 1748 cm^{-1}$ suggests the pivaloylation occurred. A large singlet integrating for nine protons in the ^1H NMR spectrum at $\delta 1.07$ represents the pivaloyl group and a singlet at $\delta 3.84$ represents the three protons of the methoxy group. This combined with the absence of the hydroxy signal in the ^1H NMR spectrum and the presence of a carbonyl signal at 192.70 in the ^{13}C NMR spectrum confirmed the pivaloylation of 2-hydroxy-4'-methoxybenzophenone (BRI018).



Transition state

Scheme 2.33 Deprotection mechanism using boron trifluoride-dimethyl sulphide

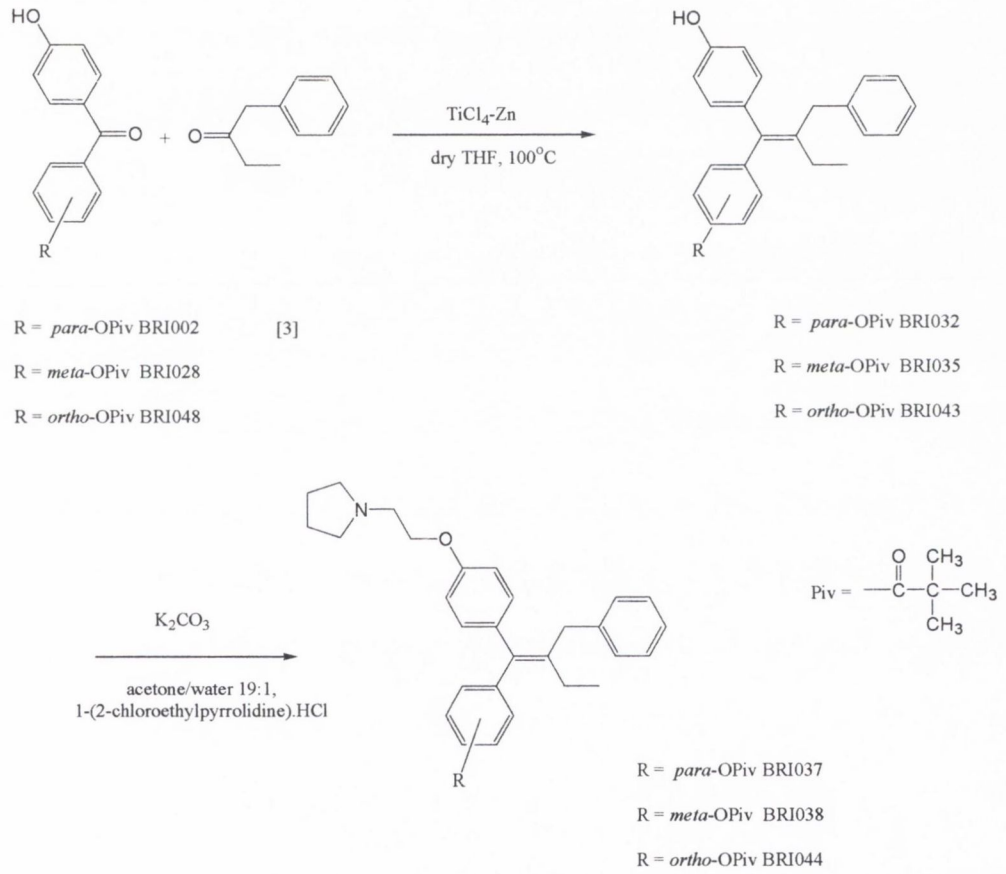
This product was demethylated in order to obtain 4-hydroxy-2'-pivaloyloxybenzophenone (BRI048) (see Scheme 2.33). The deprotection of the methoxy group was performed numerous times on 2-pivaloyloxy-4'-methoxybenzophenonone (BRI020) by varying time, temperature and quantity of sodium ethanethiolate but the pivaloyl group was removed first in each case and then the methoxy. This produced a mixture of the 2-hydroxy-4'-methoxybenzophenonone (BRI018) and the 2,4'-dihydroxybenzophenone (BRI188) (see Scheme 2.32). The next attempt involved selectively pivaloylating the 2,4'-dihydroxybenzophenone (BRI188) by the method described for 2-pivaloyloxy-4'-methoxybenzophenone (BRI020) above. This produced 2,2'-dipivaloyloxybenzophenone (as seen in Scheme 2.32) which was not the desired product, so a deprotection was performed on this in an attempt to remove the *para*-pivaloyl group as its position was known to be more susceptible to deprotection from the initial deprotection of 2-pivaloyloxy-4'-methoxybenzophenonone (BRI020). The deprotection was performed using boron trifluoride-dimethyl sulphide (BF_3SMe_2)[137] and stirring all in dichloromethane (DCM) for two hours. This reagent is a hard Lewis acid/soft nucleophile combination. The reaction performed is an S_n2 reaction and takes place via a transition state. BF_3SMe_2 forms hydrates in the presence of small amounts of water, as seen in scheme 2.32, therefore the reaction was performed under a nitrogen atmosphere and the reagent was deactivated with water when the reaction was complete. Only the desired product, 4-hydroxy-2'-pivaloyloxybenzophenone (BRI048), was produced in this case (see Scheme 2.32) (65%).

The demethylation was confirmed by the presence of a characteristic OH absorption at $\upsilon 2973cm^{-1}$ in the IR spectrum as shown in Table 2.4. The presence of the carbonyl signal of the pivaloyl group at $\upsilon 1751cm^{-1}$in the infrared spectrum combined with the ^1H NMR spectrum, still present, pivaloyl singlet at $\delta 1.37$ integrating for the nine protons of the confirmed the presence of the pivaloyl group in the compound. The demethylation was verified by the absence of the methoxy signal in the ^1H NMR spectrum and also by the absence of the methoxy group characteristic signal in the ^{13}C NMR spectrum. The presence of the pivaloyl carbon signals and carbonyl signal at 26.56ppm, 38.70ppm and 194.73ppm also confirm the presence of the pivaloyl group.

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI002	**40**	**1735 (C=O)**	**1605 (C=C)**	**3458 (OH)**
BRI028	**50**	**1757 (C=O)**	**1601 (C=C)**	**3325 (OH)**
BRI048	**65**	**1751 (C=O)**	**1601 (C=C)**	**2973 (OH)**

Table 2.4: Yield and infrared data for compounds BRI1002, BRI028 and BRI048

2.6.2.2. McMurry coupling of pivaloyl substituted benzophenones to 1-phenyl-2-butanone [3]



Scheme 2.34 Synthesis of pivaloyl-substituted triarylbutenes

The required series of pivaloyl-substituted triarylbutenes[141] were prepared as outlined in Scheme 2.34. Positive identification of the products was obtained from spectroscopic data.

2.6.2.2.1. Synthesis of 2-benzyl-1-(4-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI037)

To synthesise 2-benzyl-1-(4-pivaloyloxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI032), 4-hydroxy-4'-pivaloyloxybenzophenone (BRI002) (Scheme 2.34) was coupled with 1-phenyl-2-butanone [3] under McMurry conditions. 2-Benzyl-1-(4-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI037) was obtained by the side group alkylation[119] of 2-benzyl-1-(4-pivaloyloxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI032) using the method described in section 2.5.3 (% yield and Z:E isomeric ratio, see Table 2.6).

2.6.2.2.2. Synthesis of 2-benzyl-1-(3-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI038).

In a similar fashion 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028) and 1-phenyl-2-butanone [3] were coupled to give 2-benzyl-1-(3-pivaloyloxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI035). The basic side chain alkylation[119] was performed as for the method in section 2.5.3 to 2-benzyl-1-(3-pivaloyloxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI035) to produce 2-benzyl-1-(3-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI038) (% yield and Z:E isomeric ratio, see Table 2.6).

2.6.2.2.3. Synthesis of 2-benzyl-1-(2-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI044)

Synthesis of 2-benzyl-1-(2-pivaloyloxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI043) was achieved by coupling 4-hydroxy-2'-pivaloyloxybenzophenone (BRI048) and 1-phenyl-2-butanone [3] under reductive conditions and was obtained as E/Z mixture (% yield and Z:E isomeric ratio are displayed in Table 2.6).

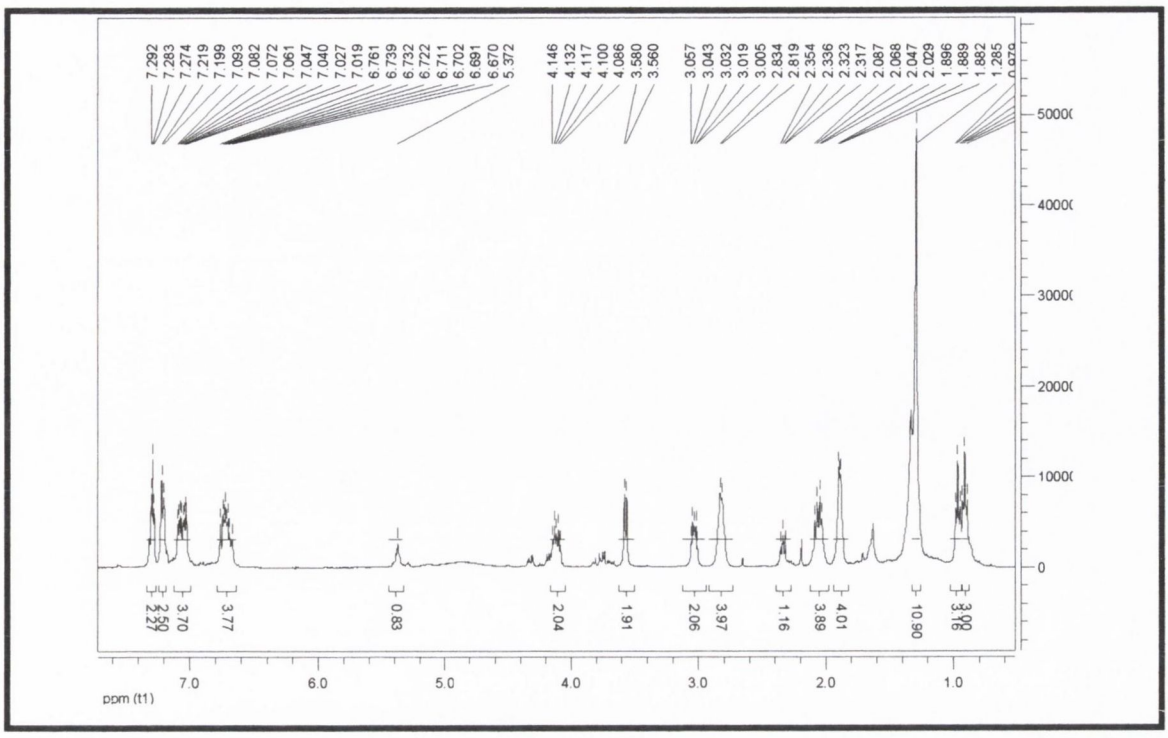


Figure 2.6 ^{1}H NMR spectrum for compound BRI037

In the ^{1}H NMR spectrum (as displayed in Figure 2.6) almost all of the signals are assigned by comparison with the ^{1}H NMR spectra of the related methoxy-substituted compounds. The signals in the ranges δ0.99 (J=7.5Hz) and δ2.02-2.08 (J=7.5Hz) represent the H-4 protons and the methylene H-3 protons. The main feature is the presence of a large singlet that appears at δ1.35 and integrates for nine protons and is assigned to the pivaloyl group. The partially shielded H-3'', H-5'' protons are found as a doublet between δ6.67-6.76 (J=8.04Hz), the remaining aromatic protons are observed as a multiplet in the range δ7.01-7.29.

The ^{13}C NMR spectrum shows the presence of two signals that represent the pivaloyl ester protecting group carbons, which appear at 26.65ppm, 43.08ppm and a signal at 175.00ppm that confirms the presence of its carbonyl group. The aromatic carbon signals appear in the region 114.31ppm to 130.60ppm. The ^{14}C NMR spectrum shows two inverted carbons at 26.70ppm and 22.48ppm, which verifies the existence of two CH_2 groups in the overall structure. High resolution mass spectrometry for this compound affords the molecular ion (M^{+}+1) 415.2438 ($C_{28}H_{30}O_3$) in 100% abundance, calculated for 414.2195.

Basic side chain alkylation[119] was performed on 2-benzyl-1-(2-pivaloyloxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI043) to produce 2-benzyl-1-(2-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI044) (Scheme 2.34) (% yield and Z:E isomeric ratio, see Table 2.6).

The ^{1}H NMR spectrum shows signals at δ0.95-0.98 (J=7.5Hz) and δ2.03-2.08 (J=7.5Hz), which represent H-4 protons and the methylene H-3 protons as a triplet and a quartet respectively. A large singlet appears at δ1.28-1.36 representing nine protons of the pivaloyl-protecting group. Two singlets appear at δ3.56 and δ3.58 representing the benzyl CH_2 group and also allowing the observance of the Z:E isomeric ratio. Signals representing the basic side chain pyrrolidine protons appear at δ1.82 (H-1'''', H-4'''') and δ2.65-2.66 (H-2'''', H-3'''') each integrating for four hydrogens. At δ2.89-2.94 and at δ4.08-4.14 signals integrating for two hydrogens each represent the protons H-7 and H-6 of the alkylated side group. They are more downfield than the pyrrolidine protons of the basic side chain due to the deshielding effects they experience from the oxygen group residing nearby in the structure. The aromatic protons are observed as a multiplet in the range δ6.83-7.32.

In the ^{13}C NMR spectrum it can be seen that some new signals are present in addition to those in the ^{13}C NMR spectrum for 2-benzyl-1-(2-pivaloyloxyphenyl)-1-(4-hydroxyphenyl)-but-1-ene (BRI043). These signals represent the alkylated side chain. Signals at 23.03ppm and 54.17ppm represent the carbons of the pyrrolidine ring of the basic side chain. One methylene signal is shifted dramatically downfield compared to the other. This is due to its position beside the nitrogen group of the basic side chain, which causes it to experience a deshielding effect. The methylene CH_2 groups of the basic side chain also experience different deshielding effects due to their positions in the compound: C-6 appears at 30.39ppm and C-7 appears at 66.41ppm. The aromatic carbons are generally found in the region 113.49ppm to 130.00ppm. High resolution mass spectrometry for this compound affords the molecular ion $M^{+}+1$ 512.3165 ($C_{34}H_{42}NO_3$) in 100% abundance, calculated for 511.3086.

Tables 2.5 and 2.6 present data for the products.

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})	Z/E isomeric ratio
BRI032	**49**	**1610 (C=C)**	**3356 (OH)**	**2:1**
BRI035	**90**	**1605 (C=C)**	**3363 (OH)**	**2:1**
BRI043	**85**	**1607 (C=C)**	**3357 (OH)**	**2:1**

Table 2.5: Yield and infrared data for compounds BRI032, BRI035 and BRI043

Compound	Yield %	IRυ_{max} (film cm^{-1})	Z/E isomeric ratio
BRI037	**40**	**1605 (C=C)**	**2:1**
BRI038	**87**	**1605 (C=C)**	**2:1**
BRI044	**19**	**1607 (C=C)**	**1.4:1**

Table 2.6: Yield and infrared data for compounds BRI037, BRI038 and BRI044

2.6.3. Hydroxy-substituted triarylbutenes *via* demethylation or depivaloylation[123, 139]

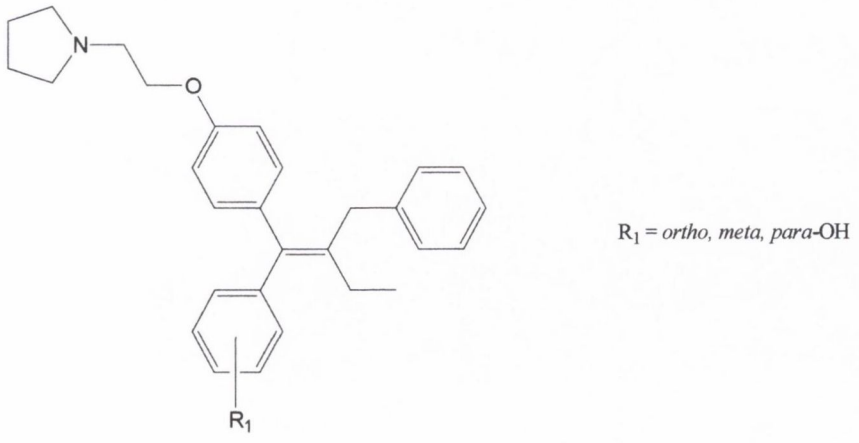
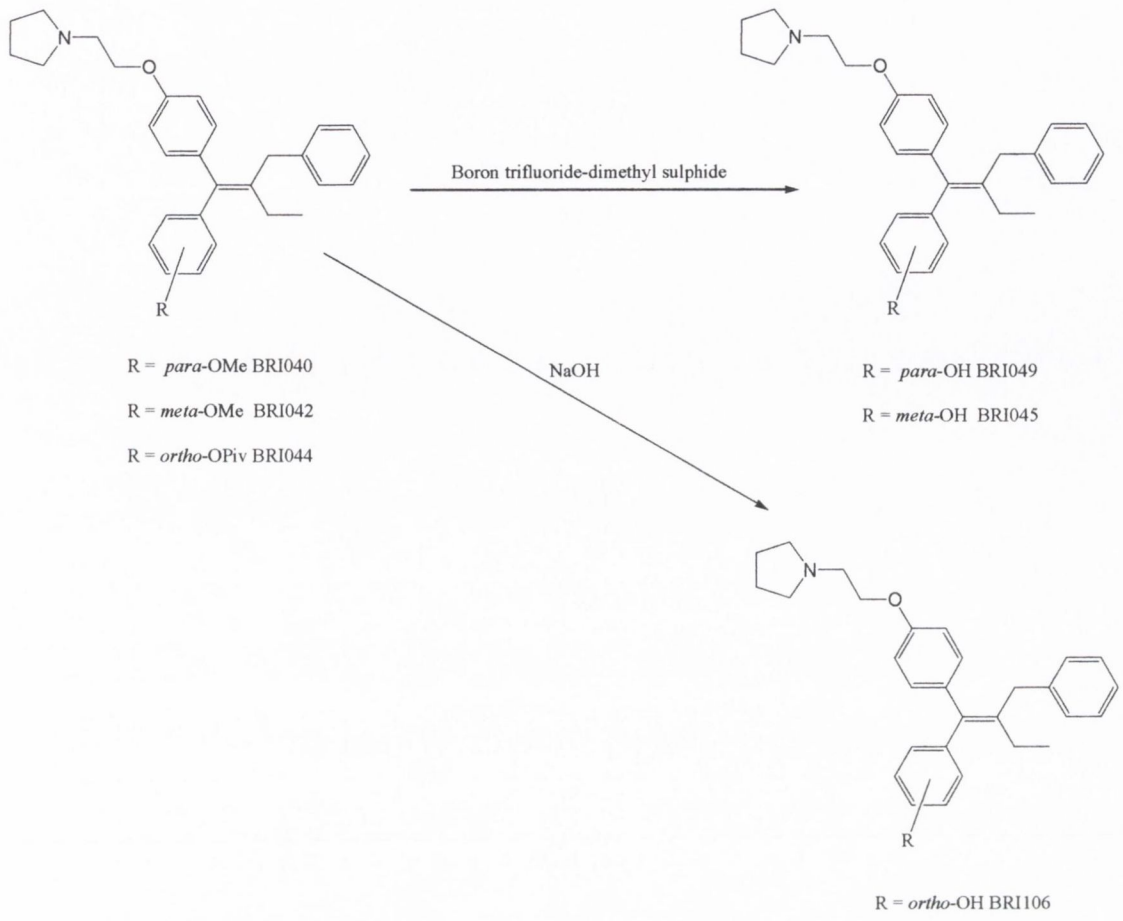


Figure 2.7 Hydroxy-substituted triarylbutenes



Scheme 2.35 Synthesis of hydroxy-substituted triarylbutenes

To complete the synthetic sequence, the preparation of a series of hydroxy-substituted triarylbutenes (Figure 2.7) is now described. Positive identification of all compounds produced was obtained from spectroscopic data.

2.6.3.1. Synthesis of 2-benzyl-1-(3-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI045) *via* demethylation

Boron trifluoride-dimethyl sulphide was used to cleave the methoxy group from 2-benzyl-1-(3-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI042) to yield the product 2-benzyl-1-(3-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene as a yellow oil (BRI045)(Scheme 2.35) (30%). The presence of a broad OH band in the infrared spectrum at $\upsilon 3400 cm^{-1}$ confirmed the demethylation had been successful.

2.6.3.2. Synthesis of 2-benzyl-1-(4-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI049) *via* demethylation

Once again boron trifluoride-dimethyl sulphide was used to demethylate 2-benzyl-1-(4-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI040) to yield the product 2-benzyl-1-(4-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene as a yellow oil (BRI049) (Scheme 2.35) (10%). The infrared spectrum contained a broad OH band at $\upsilon 3300 cm^{-1}$, which represented the deprotected hydroxy group.

In the ^{1}H NMR spectrum of BRI049, the H-4 methyl protons were observed as a triplet at δ0.94 (J=7.28Hz), while H-3 methylene protons appeared as a quartet at δ2.05 (J=7.78Hz). The H-5 benzylic protons were observed as a singlet at δ3.55. A singlet signal at δ1.87 represented the H-2'''', H-3''''protons of the pyrrolidinyl side chain while another singlet at δ2.80 represented the H-1'''', H-4'''' protons of the basic side chain. The protons of the NCH_2 and OCH_2 groups appear as multiplet signals at δ3.0 and δ4.09. The aromatic signals are in the range δ6.67-7.31.

The ^{13}C NMR spectrum of BRI049 presents the carbons C-4, C-3 and C-5 as signals at 12.80ppm, 24.30ppm and 36.82ppm. The pyrrolidinyl side chain carbons (C-2'''', C-3''''), (C-1'''', C-4''''), C-5 and C-4 are found as signals at 22.80ppm, 53.90ppm, 54.39ppm, 65.22ppm. The aromatic carbons are characteristically found in the region 113.46ppm to 156.33ppm. High-resolution mass spectrometry correctly identifies the molecular ion ($M^{+}+1$) of this compound ($C_{29}H_{34}NO_2$), calculated as 427.5779, as 428.2563.

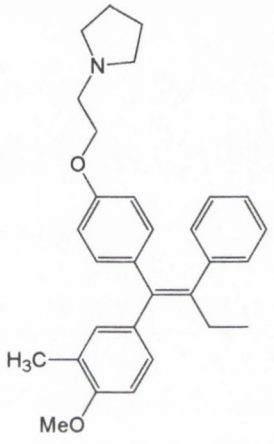
2.6.3.3. Synthesis of 2-benzyl-1-(2-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI106) *via* depivaloylation

The hydrolysis of the pivaloyloxy group ester of 2-benzyl-1-(2-pivaloyloxyphenyl)-1-[4-pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI044) was achieved by stirring with sodium hydroxide in an ethanol:water mixture. 2-Benzyl-1-(4-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene was recovered as a yellow oil (BRI106) (45%). The infrared spectrum confirmed the demethylation by the presence of a hydroxy signal at $\upsilon 3300 cm^{-1}$. The relevant yield and IR data for this series of products are given in Table 2.7 seen below. Again, predominant formation of the Z isomer is observed in each case

Compound	Yield %	IRυ_{max} (film cm^{-1})	Z/E isomeric ratio
BRI045	**30**	**1596 (C=C)**	**6.16:1**
BRI049	**10**	**1604 (C=C)**	**5.71:1**
BRI106	**45**	**1606 (C=C)**	**3:1**

Table 2.7: Yield and infrared data for compounds BRI045, BRI049 and BRI106

2.6.4. Hydroxy, methoxy, methyl-substituted triarylbutenes

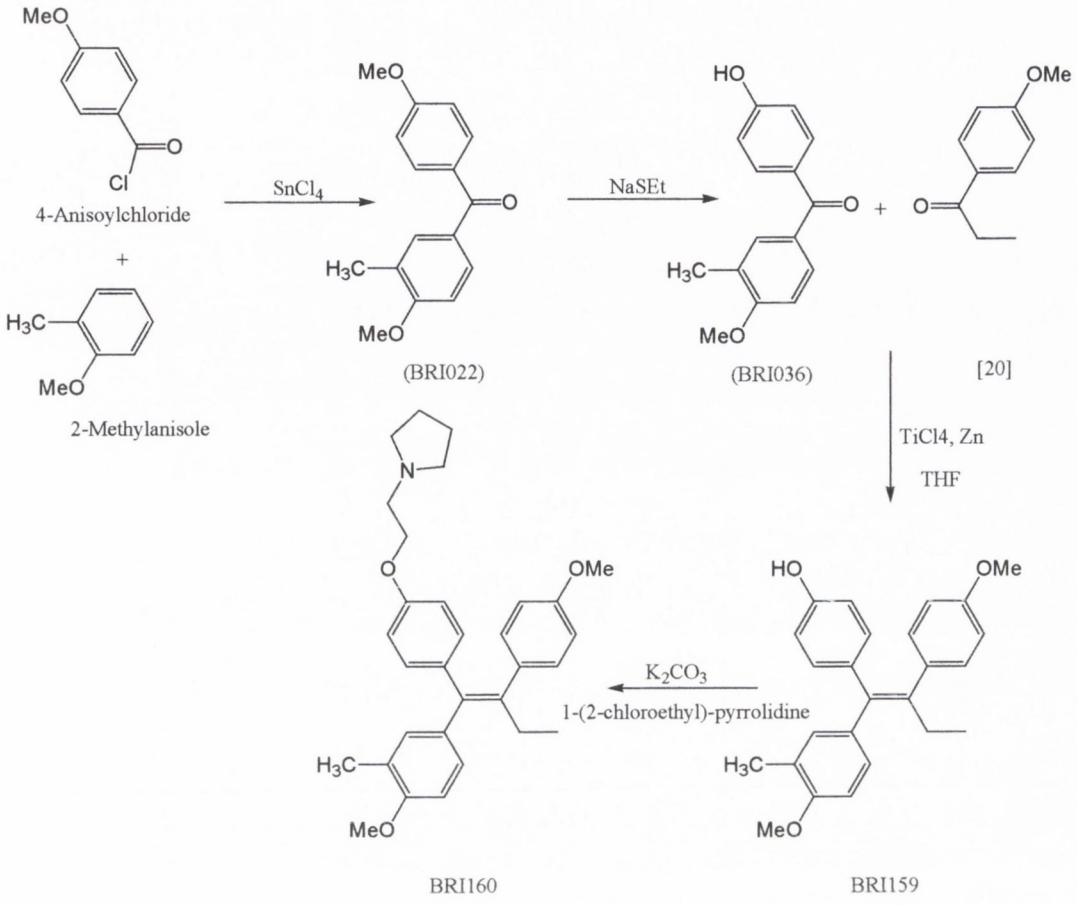


(BRI160)

Figure 2.8 Hydroxy, methoxy, methyl-substituted triarylbutenes

In order to design a product which would retard metabolism or elimination of the tamoxifen type compound, the preparation of a product which would contain the required oxygenated function at C-4 of the Ring B (Figure 2.8) together with a methyl function at C-3 was investigated, BRI160, see Scheme 2.36.

2.6.4.1. Synthesis of 4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenol (BRI159)



Scheme 2.36 Synthesis of 1-(2-{4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI160)

2.6.4.1.1. Synthesis of 4,4'-dimethoxy-3-methylbenzophenone (BRI022)

The synthesis of 4,4'-dimethoxy-3-methylbenzophenone (BRI022)[128] was achieved following the method described for 2,4'-dimethoxybenzophenone (BRI008) in section 2.6.1.1.3, but this time 2-methylanisole , tin (IV) chloride and *para*-anisoyl chloride were dissolved in tetrachloroethane. The product crystallised as clear crystals in a 66% yield in a 2:1 Z:E isomeric ratio. The infrared confirmed the presence of a carbonyl group by a signal at υ1649cm^{-1}. In the ^{1}H NMR spectrum confirmed the presence of a methyl group by a signal at δ2.28 and two methoxy groups at δ3.90 and δ3.93.

2.6.4.1.2. Synthesis of 3-methyl-4-hydroxy-4'-methoxybenzophenone (BRI036)

The demethylation process described in section 2.6.1.1.2 was applied here to 4,4'-dimethoxy-3-methylbenzophenone (BRI022) to produce 4'-hydroxy-4-methoxy-3-methylbenzophenone (BRI036) as colourless crystals (55%). The infrared confirmed the presence of a hydroxy group due to the demethylation by a signal at $\upsilon 3448 cm^{-1}$.

2.6.4.1.3. Synthesis of 4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenol (BRI159)

Following the general method described in section 2.5.3 the McMurry coupling was performed on 4'-hydroxy-4-methoxy-3-methylbenzophenone (BRI036) and 4-methoxypropiophenone [20] to yield 4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxy-phenyl)-but-1-enyl]-phenol (BRI159) as a light yellow oil (60%). The coupling was confirmed by infrared with the presence of a signal at $\upsilon 1607 cm^{-1}$ representing the carbon-carbon double bond and a signal at $\upsilon 3395 cm^{-1}$ indicating the presence of a hydroxy group in the structure.

In the ^{1}H NMR spectrum the methyl H-4 protons are observed as a triplet at $\delta 0.92$ (J=7.5Hz) and the methylene H-3 protons occur as a multiplet at $\delta 2.44$. The methyl substitutent appears as a singlet at $\delta 2.25$. The methoxy groups appear as singlets at $\delta 3.72$ and $\delta 3.78$. The aromatics protons occur in the region $\delta 6.42$ to $\delta 7.15$.

In the ^{13}C NMR spectrum of BRI159 the methyl C-4 is characteristically observed upfield at 13.30ppm. The methylene C-3 appears at 28.55ppm while the methyl substitutent is observed at 15.89ppm. Two methoxy signals appear at 54.64ppm and 54.68ppm. C-2''' is observed at 108.90ppm due to shielding. C-3'', C-5'' and C-3', C-5' also appear upfield to the other aromatic carbons at 112.77ppm and 114.02ppm due to the shielding. The remaining aromatic signals are found between 127.27ppm and 157.13ppm.

2.6.4.2. Synthesis of 1-(2-{4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI160)

Basic side chain alkylation[119] was performed using the general method as in section 2.5.3

on 4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenol (BRI159) to produce the required 1-(2-{4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI160) as a light brown oil in a yield 73%.

In the ^{1}H NMR spectrum, the new pyrrolidine signals that confirm the addition of the basic side chain are identified by a signal at δ1.85 which represents H-2'''' and H-3''''. The signal δ2.65 represents H-1'''', H-4'''' of the basic side chain. Both the NH_2 and OCH_2 protons of the side group appear as multiplets at δ2.95 and δ4.02. The aromatic protons are represented between δ6.56 and 7.17.

The ^{13}C NMR spectrum signals are very similar to those in the ^{13}C NMR spectrum 4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenol (BRI159). The pyrrolidine side chain carbons. C-2'''' and C-3'''' appear at 22.93ppm while the OCH_2 of the alkylated side group appears 54.54ppm. C-1'''', C-4'''' are found at 54.63ppm and the remaining side NCH_2 carbon is observed at 66.55ppm. The aromatic carbon signals appear between 109.82ppm and 157.17ppm.

2.7. Analogue series 2 – bromo-substituted triphenylbutenes

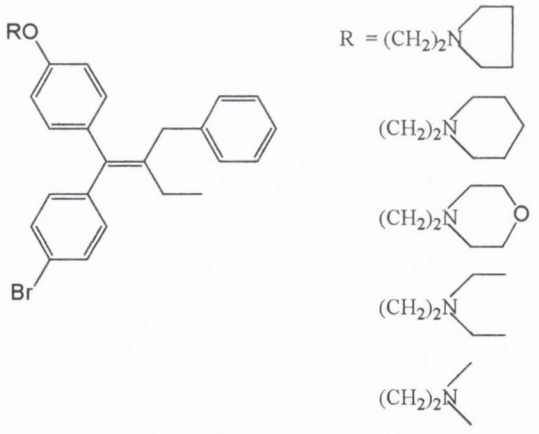


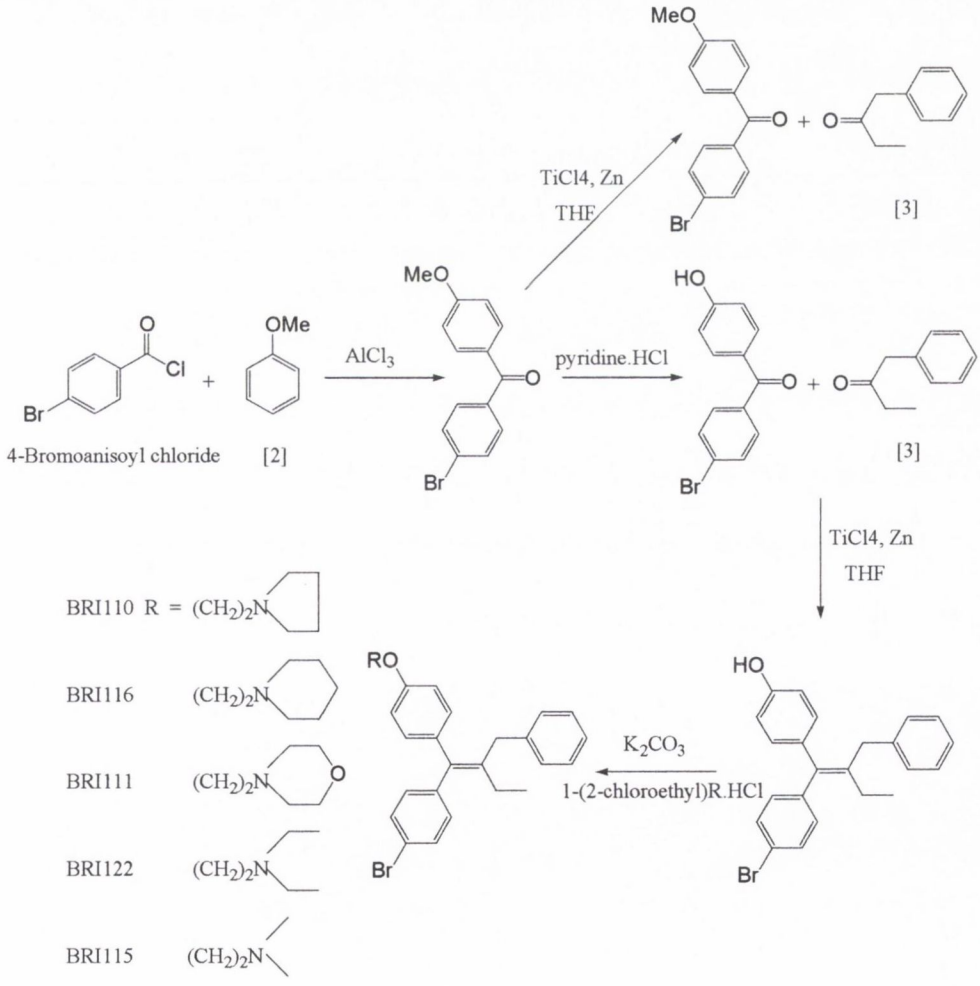
Figure 2.9 Bromo-substituted triphenylbutenes

The objective here was to prepare a bromine-containing molecular scaffold, as seen in figure 2.8 above, onto which long-chain alkyl groups could eventually be introduced. To achieve this bromine-containing hydroxy-substituted benzophenone was synthesised and then coupled to 1-phenyl-2-butanone [3]. The various different side chains displayed in figure 2.8 were introduced to the bromine-containing scaffold at the hydroxy group in the

hope that these variations may lead to beneficial variations in the binding mode of the compounds.

The McMurry coupling of 4-bromoacetophenone compounds was examined by Daik et Feast[142] where 4-bromoacetophenone was coupled to itself in order to synthesise 2,3-bis(4-bromophenyl)-2-butene. The self-coupling of 4-bromobenzophenone was also investigated and resulted in the production of a 1:1 Z:E isomeric mixture. The success of this reaction lead to the conclusion that bromo-substitutents would not interfere with the coupling of carbonyl compounds and so the coupling of bromo-substituted benzophenones with ketones was attempted in order to synthesise the analogues shown in Figure 2.10.

2.7.1. McMurry coupling of bromo-substituted benzophenones and 1-phenyl-2-butanone [3]



Scheme 2.37 Synthesis of bromo-substituted analogues

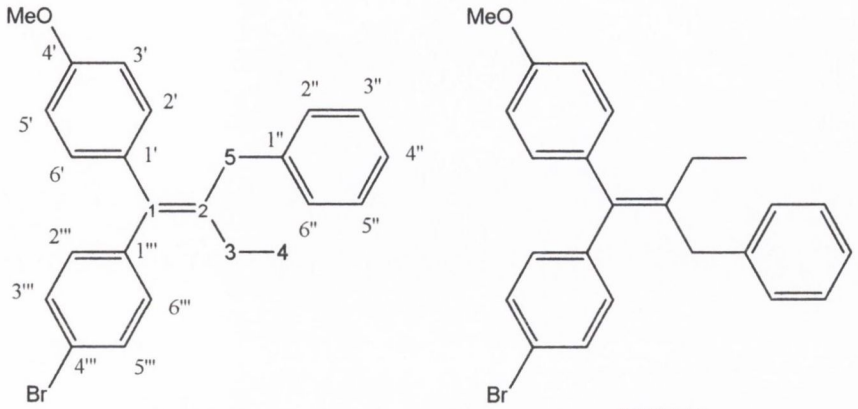
2.7.1.1. Synthesis of 4-bromo-4'-methoxybenzophenone (BRI102)

To extend the structural diversity of the products to be studied for antiproliferative activity, 4-bromo-4'-methoxybenzophenone (BRI102)[143, 144] was prepared for this reaction *via* Friedel-Crafts acylation of anisole [2] with 4-bromoanisoyl chloride to yield 4-bromo-4'methoxybenzophenone, using aluminium trichloride as the Lewis acid (Scheme 2.35). The acylation[145] is accomplished by an electrophilic substitution of the acylium ion generated by the reaction between the acyl halide and aluminium chloride. The methoxy group of anisole is highly electron donating[145] and therefore promotes the acylation reaction from which a high yield of product was obtained as clear crystals (90.3%). The infrared spectrum verified the synthesis of 4-bromo-4'-methoxybenzophenone (BRI102) by a carbonyl signal at υ1639cm^{-1}. The ^{1}H NMR spectrum also verified the success of the reaction by the presence of a methoxy group signal at δ3.04 and aromatic proton signals between δ6.96 and δ7.8.

2.7.1.2. Synthesis of 4-bromo-4'-hydroxybenzophenone (BRI103)[146, 147]

4-Bromo-4'-methoxybenzophenone (BRI102) was demethylated with pyridine hydrochloride (Scheme 2.37) to afford the required product as colourless crystals (30%). The demethylation of 4-bromo-4'-methoxybenzophenone (BRI102) was then attempted with sodium ethanethiolate[124, 125]. The desired product (BRI103) was recovered as colourless crystals (55%). An absence of a methoxy signal in the ^{1}H NMR spectrum confirmed the demethylation of 4-bromo-4'-hydroxybenzophenone (BRI103).

2.7.1.3. Synthesis of 1-(2-4-{-[1-(4-bromo-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-methoxy (BRI112)



Z-BRI112 *E*-BRI112

Figure 2.10 NMR assignment for bromo-substituted analogues

The McMurry coupling was performed on 4-bromo-4'-methoxybenzophenone (BRI102) and 1-phenyl-2-butanone [3] (Scheme 2.37) (Figure 2.9) to produce the required product 1-(2-4-{-[1-(4-bromo-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-methoxy (BRI112) a colourless oil (100%) and therefore ensure the coupling reaction conditions are suitable for bromo-substituted compounds. The infrared spectrum contained a signal at υ1605cm^{-1}, which represented the carbon-carbon double bond.

The ^{1}H NMR spectrum of BRI112 contained the usual triplet and quartet signals at δ1.0 (J=6.82Hz) and δ 2.06 (J=7.5Hz) that represented the H-4 and H-3 protons. A singlet signal at δ3.59 represents the benzyl CH_2 group while the following singlet δ3.81 represents the methoxy group. The aromatics signals appear between δ6.86 and δ7.5.

In the ^{13}C NMR spectrum the methyl C-4 signal is characteristically observed upfield at 12.92ppm while the corresponding methylene C-3 is found at 24.37ppm. The benzyl CH_2 appears at 37.02ppm. The methoxy group signal is observed at 54.76ppm. C-3''', C-5''' aromatic carbons are the most shielded of the aromatic carbons and are found at 113.23ppm. The remaining aromatic carbons are found between 119.86ppm and 157.9ppm.

2.7.1.4. Synthesis of 1-(2-benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104)[148]

The McMurry coupling of 4-bromo-4'-hydroxybenzophenone (BRI103) and 1-phenyl-2-butanone [3] (Figure 2.9) using the method described in section 2.5.3 produced 1-(2-benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104), as a brown oil (100%). The coupling was confirmed by the presence of a carbon-carbon double bond signal at ν1594cm^{-1} in the IR spectrum.

In the ^{1}H NMR spectrum the methyl H-4 protons are observed as a triplet at δ0.98 (J=7.52Hz), while the corresponding methylene H-3 protons are observed as a quartet at δ2.03 (J=7.52Hz). The benzyl CH_2 group exists as singlets at δ3.60 and δ3.56, the signal integration of which represents a 2:1 ratio of Z:E isomers. H-3', H-5', being the most shielded aromatic protons, are represented by signals between 6.77-6.79.

The ^{13}C NMR spectrum methyl group, C-4, is observed upfield at 13.70ppm while the corresponding methylene C-3 is found at 24.28ppm. The benzyl CH_2 appears at 36.70ppm. Due to the partial shielding the aromatic carbons C-3' and C-5' are more upfield, at 114.70ppm, than the remaining aromatic carbons, which appear in the range 125.47-154.05ppm.

2.7.2. Basic side group addition[119]

Basic side group addition was completed as outlined in Scheme 2.37. The ^{1}H NMR and ^{13}C NMR spectra of analogue series 2 compounds are all very similar. I have previously discussed the compounds baring the pyrrolidinyl side chain so for comparison purposes I will discuss it in reference to this series of compounds (1-(3-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenyl}-propyl)-pyrrolidine (BRI110)). I will also discuss the spectra of (2-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenoxy}-ethyl)-diethyl-amine (BRI115), which contains the diethylamine side chain.

2.7.2.1. Synthesis of 1-(3-{4-[2-benzyl-1-(4-bromophenyl)-but-1-enyl]-phenyl}-propyl)-pyrrolidine (BRI110)

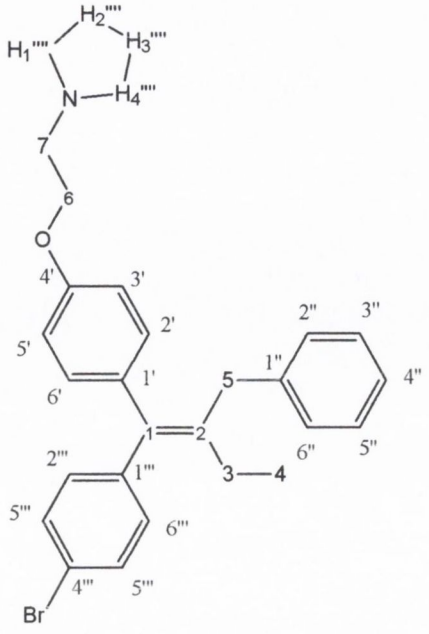


Figure 2.11 NMR assignment for bromo-substituted analogues

1-(2-Benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104) was reacted with 1-(2-chloroethyl)pyrrolidine.HCl and K_2CO_3 (Scheme 2.37) to produce 1-(3-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenyl}-propyl)-pyrrolidine (BRI110) as a light brown oil in a yield 100%.

In the ^{1}H NMR spectrum of BRI110 the pyrrolidinyl side chain protons (Figure 2.11) H-2'''', H-3'''' and H-1'''', H-4'''' are observed at δ1.84 and δ2.66. H-7 and H-6 protons of the basic side chain appear as signals at δ2.90 and δ4.08. The benzyl CH_2 group is found as a singlet signal at δ3.58. The most shielded of the aromatic protons H-3', H-5' appear as a doublet at δ6.81 while the remaining aromatic protons are found between δ7.08 and δ7.44.

In the ^{13}C NMR spectrum of BRI110 the carbons C-4, C-3 and C-5 appear characteristically at 12.79ppm, 24.24ppm and 36.70ppm. The pyrrolidinyl side chain C-2'''', C-3'''' and C-1'''', C-4'''' are observed at 22.98ppm and 54.17ppm. The remaining side group carbons C-6 and C-7 are observed more downfield at 54.52ppm and 66.27ppm. The partially shielded C-3', C-5' carbons appear at 113.79ppm. All the remaining aromatic carbons are found between 119.72ppm and 141.87ppm.

2.7.2.2. Synthesis of (2-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenoxy}-ethyl)-diethyl-amine (BRI115)

1-(2-Benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104) was reacted with 2-diethylaminoethylchloride.HCl to produce (2-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenoxy}-ethyl)-diethyl-amine (BRI115) (Scheme 2.37) as a light brown oil (80%).

Once again the ^{1}H NMR spectrum of BRI115 shows characteristic signals that represent the protons H-4 and H-3. The diethylamino side chain protons H-2'''', H-3'''' appear as a multiplet at δ1.07. The remaining side chain protons H-1'''', H-4'''', H-7 and H-6 are found at δ2.64, δ2.88 and δ4.03. The aromatic protons H-3', H-5' appear as signals at δ6.80 while the remaining aromatic protons are all found between δ7.08 and δ7.44.

In the ^{13}C NMR spectrum of BRI115 the basic side chain carbons C-2'''', C-3'''' and C-1'''', 4'''' are observed at 12.82ppm and 47.24ppm, while the remaining side chain carbons $N\underline{C}H_2$ and $O\underline{C}H_2$ appear at 51.15ppm and 65.67ppm respectively. The aromatic carbons C-3', C-5' are partially shielded and therefore appear upfield from the other aromatic protons at 113.70ppm.

2.8. Analogue series 3 - fluoro substituted compounds

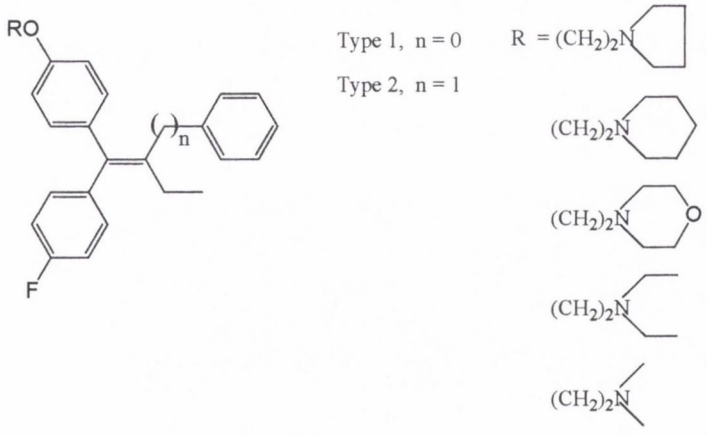
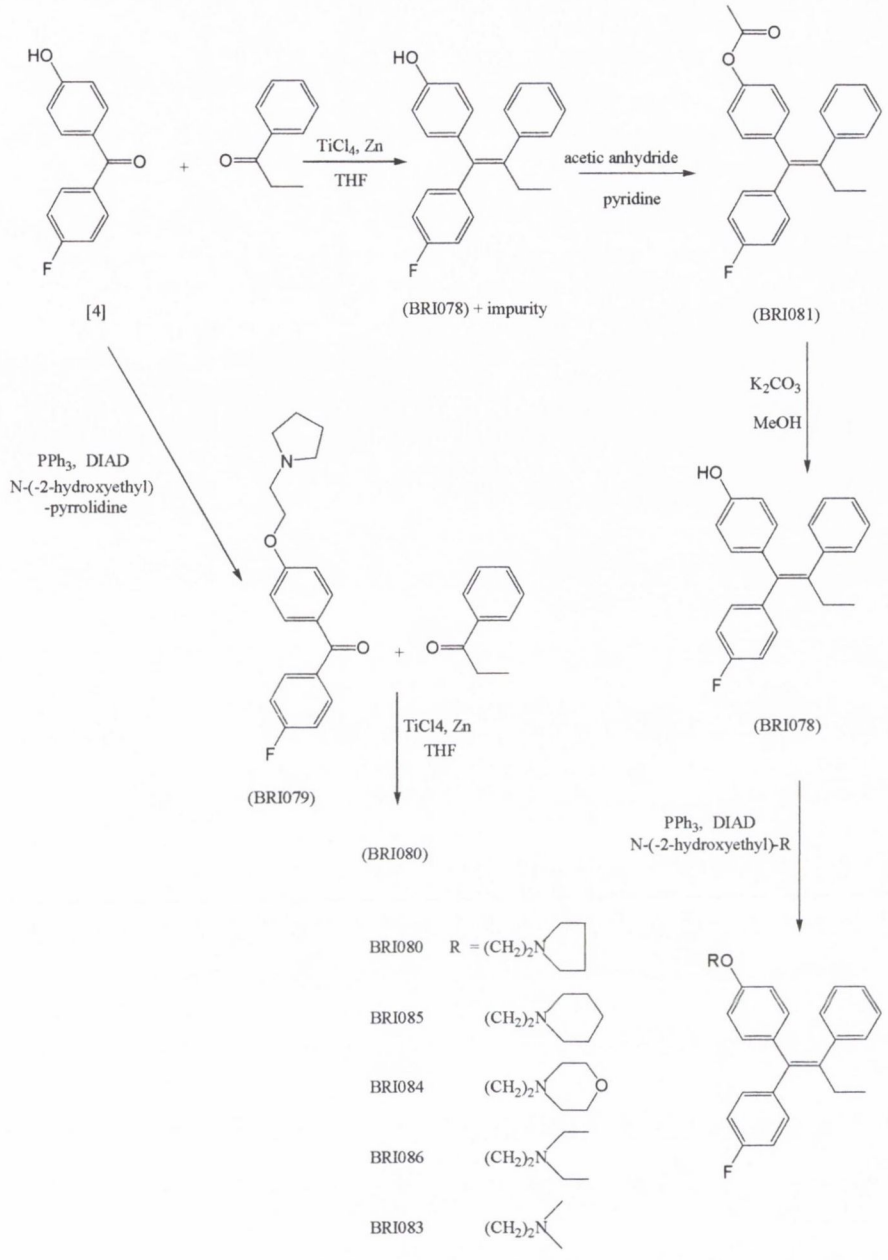


Figure 2.12 Fluoro-substituted analogues

2.8.1. Analogue series 3-Type 1

The synthesis of fluoro-substituted compounds was suggested in light of the fact that the bromo-substituted analogues were synthesised with little difficulty and presented some reasonable results when tested on breast cancer cells (see chapter 4). The fluoro-substituted compounds, due to similar size but the different electronegativity values of the halogens, may just offer a more efficient binding to the estrogen receptor and could therefore be more effective anti-estrogens. Another influence that encouraged the examination of fluoro-analogues as anti-estrogens was a study performed on fluorotamoxifen[149] that suggested its potential use as an anti-estrogen. Fluorotamoxifen was compared with tamoxifen in terms of its uterotrophic activity and receptor binding capacity and although it proved to be highly competitive to estradiol, its abilities were not greater than the renowned tamoxifen. However a major advantage of using fluorotamoxifen is its metabolic stability, which occurs due to the fact that the fluorine atom was inserted in the same position of the tamoxifen molecule where one of the hydroxylative metabolism occurs, thus preventing the tamoxifen metabolism through one specific route. Despite not being as effective as tamoxifen, fluorotamoxifen has good estrogen receptor binding ability, which could be useful in diagnostics where ^{18}F-labelled compounds are considered near-physiological markers for positron-emission tomography in estrogen receptor studies for medicine.

Considering these studies on fluorotamoxifen allowed the conclusion that this area of research has great potential and further development of fluoro-substituted analogues was necessary. So both tamoxifen-like and flexible scaffolds were investigated in this section, as seen in Scheme 2.38.



Scheme 2.38 Synthesis of fluoro-substituted analogues

The McMurry coupling of 4-benzyloxybenzophenone and propiophenone [18][150] was examined as a more direct means of synthesising tamoxifen. Despite the bulky nature of the substituted benzophenone the coupling was a success. Also in another study the McMurry coupling of carbonyl containing amides to ketones[151] was assessed and was successful even though it was assumed that the alleged inertness of the amide would prevent the reaction from taking place. The results of these studies lead to the suggestion that, due to the difficulty of separation of the fluoro-substituted coupled products, that the basic side chain addition could be performed on the 4-hydroxy-4'-fluorobenzophenone and

the subsequent coupling of this compound to the ketone could then be attempted. If successful, due to the immediate difference in polarity between the expected product and self-coupled propiophenone, separation by column chromatography should be sufficient to purify the product.

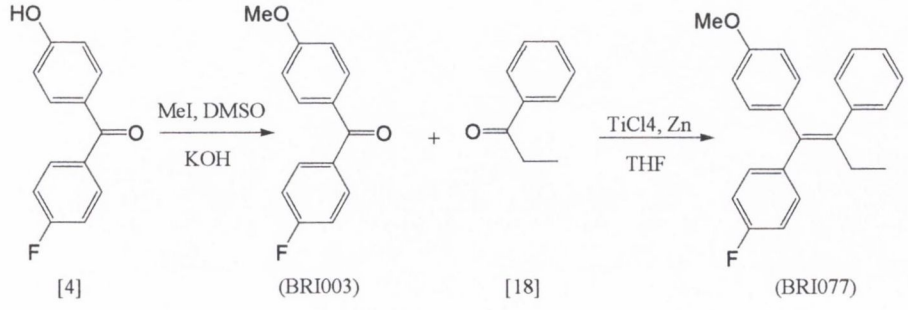
2.8.1.1. Synthesis of 4-methoxy-4'-fluorobenzophenone (BRI003)[152, 143, 149]

4-Methoxy-4'-fluorobenzophenone (BRI003)[153] was obtained in a 50% yield by stirring 4-hydroxy-4'-fluorobenzophenone [4] with potassium hydroxide and methyl iodide in DMSO[122] as shown in Scheme 2.38. The infrared spectrum carbonyl signal at υ1641cm^{-1} and the ^{1}H NMR spectrum methoxy signal at δ3.90 confirmed the synthesis of the required product.

2.8.1.2. Synthesis of (4-fluoro-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone (BRI079) using basic side chain addition[136] methods

As in the general method 2.6.1.1.2, 4-hydroxy-4'-fluorobenzophenone [4] was reacted in base to afford (4-fluoro-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone (BRI079) (36%) (see Scheme 2.38). Synthesis was also achieved using the Mitsunobu procedure side chain addition method where 4-hydroxy-4'-fluorobenzophenone [4] was reacted with triphenylphosphine, 1-(2-hydroxyethyl)pyrrolidine and diispropyl azodicarboxylate. The product was recovered as yellow oil (70%).

2.8.1.3. Synthesis of 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-methoxy (BRI077)[149, 154]



Scheme 2.39 Synthesis of 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-methoxy (BRI077)

As a trial coupling to ensure the reaction conditions are suitable for fluoro-substituted compounds, 4-fluoro-4'-methoxybenzophenone[152] and propiophenone [18] were coupled under McMurry coupling reaction conditions described in section 2.5.3, to give 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-methoxy (BRI077) as a colourless powder (80%) in a 2:1 Z:E isomeric ratio.

General assignment of the Z isomer in the ^{1}H NMR spectrum is based on the more downfield shift of the benzyl CH_2 group signal, which correlated with the downfield shift of all other protons related to that isomer. Therefore the E-isomer was assigned to the more upfield benzyl signal and, by the exclusion of Z-isomer signals, all remaining upfield signals. This rule of assignment was applied to analogues series 3- Type 1 and Type 2.

In the ^{1}H NMR spectrum the methyl H-4 protons are observed at δ0.91 (J=7.76Hz) as a triplet while the methylene H-3 protons appear at δ2.48 (J=7.52Hz) as a quartet. The methoxy group is found as a singlet at δ3.72. H-3', H-5' are more upfield at δ6.60-6.62 than the other aromatic protons due to the partial shielding effect of the neighbouring methoxy group. The remaining aromatic protons appear between δ6.80 and δ7.23.

In the ^{13}C NMR spectrum, the methyl C-4 signal is characteristically observed upfield at 13.16ppm while the methylene C-3 is found at 28.62ppm. The methoxy protons are observed at 54.56ppm. C-3''', C-5''' and C-3', C-5' signals are the most shielded of the aromatic protons and appear more upfield at 112.38ppm and 113.12ppm than the remaining protons that found between 114.70ppm and 157.13ppm. The ^{19}F NMR spectrum confirms the presence of a fluoro-substitutent by a signal at -116.47ppm also indicating that the Z-isomer is the more dominant isomer with a minor signal at 117.21ppm representing the E-isomer.

2.8.1.4. Synthesis of 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078)[155]

A McMurry coupling reaction, as seen in Scheme 2.39 was performed between 4-hydroxy-4'-fluorobenzophenone [4] and propiophenone [18] to produce 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078), as an oil (55%).

2.8.1.4.1. Acetylation of BRI078 to produce BRI081 {4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol}-acetic acid [156, 157]

In an attempt to purify the product from the reaction above, 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol was reacted with acetic anhydride and pyridine and afforded the acylated product in a 64% yield (see Scheme 2.39).

The ^{1}H NMR spectrum showed the presence of an acetyl group as a singlet signal at δ2.23. Also the ^{13}C NMR spectrum verified the presence of an acetyl group as a singlet at 20.70ppm.

2.8.1.4.2. Deacetylation of BRI081 to produce 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078)

{4-[1-(4-Fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol}-acetic acid (BRI081), was then deacetylated by refluxing at 80°C for 24 hours in methanol with water and potassium carbonate. A yellow oil (70%) was recovered and identified as 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078).

The ^{1}H NMR spectrum showed the characteristic signals for protons H-4 and H-3 at δ1.00 and δ2.52. H-3', H-5' aromatic signals are partially shielded and appear at δ6.55.
The ^{13}C NMR spectrum also has the carbon signals for C-4 and C-3 at 13.21ppm and 28.64ppm. C-3', C-5' and C-3'', C-5'' carbons are found at 114.54ppm and 114.79ppm due to partial shielding as seen in the ^{1}H NMR spectrum. The remaining carbon signals appear between 127.58ppm and 162.49ppm. The ^{19}F NMR spectrum showed the ratio of isomers Z:E to be 1:1 with the fluoro-substituents represented by signals at -116.99ppm and –116.99ppm.

2.8.1.5. Introduction of basic side chains[136]

2.8.1.5.1. Synthesis of 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-pyrrolidine (BRI080)

As in the basic side chain addition method 2.6.1.1.2, 2 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078) was reacted with triphenylphosphine, 1-(2-hydroxyethyl)-pyrrolidine and diispropyl azodicarboxylate (see Scheme 2.39) (Figure 2.12). 1-(2-4-{-[1-(4-Fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-pyrrolidine (BRI080) was obtained as a yellow oil (78%) from which triphenylphosphineoxide was very difficult to remove.

A McMurry coupling[149] was then carried out on 4-fluoro-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone (BRI079) and propiophenone [18] in an attempt to produce 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-pyrrolidine (BRI080) in a more efficient way without the difficulty of the removal of triphenylphosphineoxide. The product was obtained in a 78% yield as a yellow oil. No difference was observed between the yields of the two reaction sequences in the synthesis of 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-pyrrolidine (BRI080) so the previous method of synthesis was opted for in relation to the synthesis of all compounds to follow (Scheme 2.39).

In the ^{1}H NMR spectrum of BRI080, the protons H-4 and H-3 are observed as a triplet and a quartet at δ0.93 (J=7.28Hz) and δ2.43 (J=7.26Hz). The pyrrolidinyl side chain protons H-2'''', H-3'''' and 1'''', 4''''are found as signals at δ1.81 and δ2.62. The remaining side chain protons H-7 and H-6 are more deshielded due to the neighbouring oxygen and therefore appear as triplets downfield at δ2.84 (J=6.02Hz) and δ3.99 (J=5.76Hz). In comparison to the other aromatic protons H-3', H-5' are shielded and appear upfield as a doublet at δ6.57. All remaining protons are observed between δ6.76 and δ7.22.

In the ^{13}C NMR spectrum C-4 and C-3 are found as signals at 13.14ppm and 28.56ppm. The side chain carbons are observed in positions similar to those in the ^{13}C NMR spectra of other compounds that contain a pyrrolidinyl side chain. Signals at 23.00ppm, 54.25ppm, 54.60ppm and 66.20ppm are in order of representation for the basic side chain carbons the (C-2'''', C-3''''), (C-1''''and C-4''''), C-7 and C-6. Finally the partially shielded aromatic carbons C-3', C-5' and C-3''', C-5''' are found at 112.99ppm and 114.6ppm while all

remaining aromatic carbons are observed between 125.70ppm and 157.00ppm. The ^{19}F NMR spectrum shows the Z-isomer to be the major isomer with a signal at -116.53ppm in a 4:1 ratio with the E-isomer which is observed at –117.27ppm. High Resolution Mass Spectrometry calculated the molecular ion (M^++1) for $C_{28}H_{31}FNO$ as 415.5423 and correctly observed it as 416.2390.

2.8.1.5.2. Synthesis of 4-(2-{4-[1-4-fluoro-phenyl]-2-phenyl-but-1-enyl}-[phenoxy]-ethyl)-morpholine (BRI084)

Using the basic side chain addition method 2.6.1.1.2, 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078) was reacted with triphenylphosphine, N-(-2-hydroxyethyl)-morpholine and diispropyl azodicarboxylate (see Scheme 2.39) (Figure 2.12). The required product was recovered as a yellow oil in 86% yield. The infrared spectrum confirmed the addition of alkyl side group by the presence of a signal at υ1497cm^{-1}which represented the N-CH_2 bond.

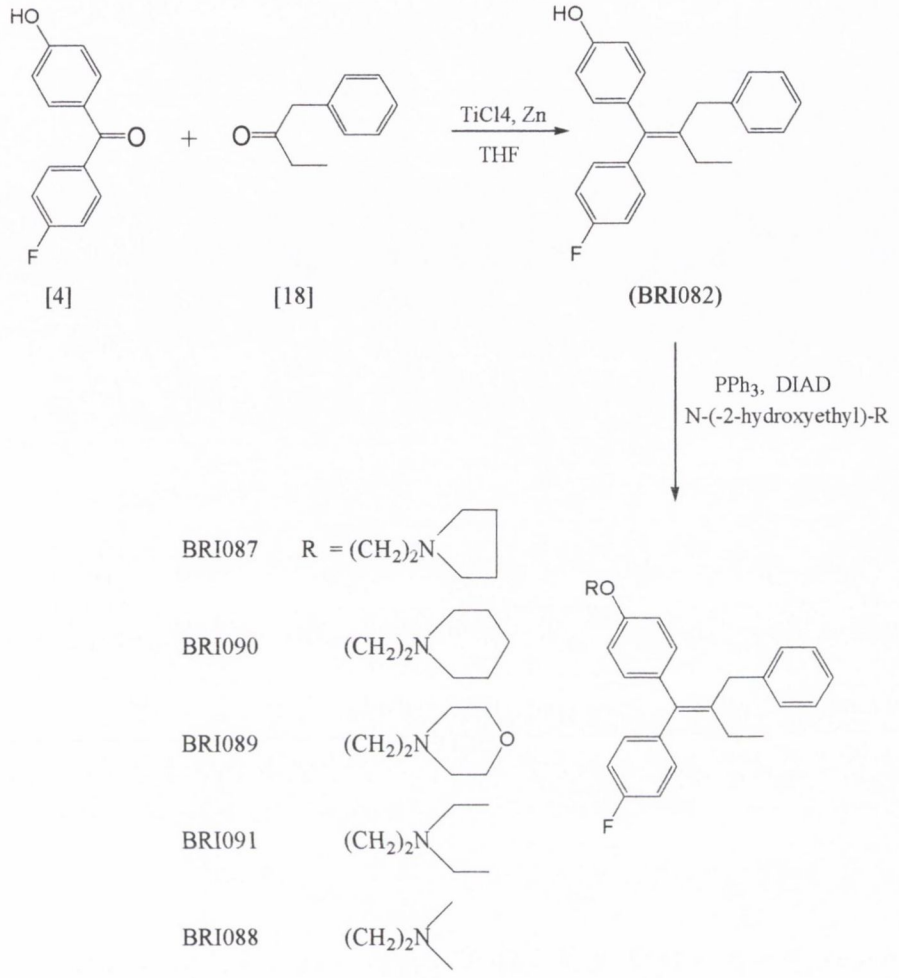
The ^1H NMR spectrum displays the required signals representing the basic side chain protons 2'''', 3'''' and 1'''', 4'''', H-6 and H-7 at δ2.57, δ3.72, δ2.74 and δ3.99.

The ^{13}C NMR spectrum also confirms the presence of the basic side chain carbons. Signals at 53.57ppm and 66.35ppm represent the carbons C-2'''', C-3'''' and C-1'''', C-4'''' while C-7 and C-6 carbons are represented by signals at 64.90ppm and 57.17ppm. The ^{19}F NMR spectrum shows once again the Z-isomer to be the major isomer with a signal at -116.52ppm but this time in a 5:1 ratio with the E-isomer appearing as a signal at -117.27. High-resolution mass spectrometry correctly observed the molecular ion of this compound as 432.2339, while it calculated as $C_{28}H_{31}FNO$ 431.5417 (M^++1).

2.8.2. Analogue series 3-Type 2

Following the study of the fluoro-substituted analogues in section 2.81, this section will study the effect on the anti-estrogen activity of the fluoro-substituted analogues with introduction of flexiblity. The means by which this was achieved was by replacing

propiophenone [18] with 1-phenyl-2-butanone [3] and coupling, as in Scheme 2.40, to 4-hydroxy-4'-bromobenzophenone.



Scheme 2.40 Synthesis of flexible fluoro-substituted analogues

2.8.2.1. Synthesis of 4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082)

The synthetic sequence required a McMurry coupling (Scheme 2.40) to be performed between 4-hydroxy-4'-fluorobenzophenone [4] and propiophenone [18] to produce 4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082) as a brown oil (100%). The coupling reaction was confirmed in the infrared spectrum of the product by the presence of a carbon-carbon double bond signal at $\upsilon 1601 cm^{-1}$.

In the ^{1}H NMR spectrum of BRI082 a triplet appears at δ1.0 (J=6.78Hz), which corresponds to the H-4 of the major E-isomer. Similarly a quartet appears at δ2.43 (J=7.52Hz) that represents the H-3 protons. The benzylic protons appear as two singlets at δ3.65 and δ3.60 and are integrated to give an E/Z isomer ratio of 4.75:1. In the ^{19}F NMR spectrum, due to the higher priority of fluorine than oxygen, the E-isomer is the major isomer in this case. The assigned fluorine signal of the major E-isomer appears at -117.00ppm while the minor Z-isomer resonates at 116.94ppm. H-3' and H-5' protons were partially shielded and therefore appeared upfield at δ6.85-6.87 to the other aromatic protons. All of the remaining aromatic protons appear in the range δ7.00-7.34.

In the ^{13}C NMR spectrum the C-4 methyl signal is observed upfield at 12.85ppm while the adjoining C-3 methylene signal is found at 24.30ppm. The benzylic C-5 CH_2 group appears at 36.78ppm. C-3''', C-5''' and C-3', C-5' are partially shielded and therefore are found most upfield of the aromatic carbons at 114.36ppm and 114.80ppm. The remaining aromatic carbons appear in the range 125.47-162.17ppm. High-resolution mass spectrometry determined the molecular weight of the product, as a molecular ion, as $(C_{23}H_{22}FO)\,M^{+}$+1 333.1576.

2.8.2.2. Basic side group addition[119]

2.8.2.2.1. Synthesis of 1-(2-{4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI087)

Following the basic side chain addition method 2.6.1.1.2, 4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082), was treated with triphenylphosphine, 1-(2-hydroxyethyl)-pyrrolidine and diispropyl azodicarboxylate (Figure 2.12) (Scheme 2.40). The required product was recovered as a yellow oil (80%). The infrared spectrum signal at υ1505cm^{-1} represents the N-CH_2 bond verifying the success of the side group addition reaction.

In the ^{1}H NMR spectrum of BRI087 H-4, H-3 and H-5 are observed as a triplet at δ0.97 (J=7.56Hz), a quartet at δ2.06 (J=7.3Hz) and a singlet at δ3.58 respectively. The pyrrolidinyl protons H-2'''', H-3'''' and 1'''', 4''''appear as singlet signals at δ1.85 and δ2.65 while the H-7 and H-6 protons of the basic side chain appear as triplets at δ2.92

(J=6.0Hz) and δ4.1 (J=5.98Hz). The aromatic carbons are represented by signals in the range δ6.83-7.31.

In the ^{13}C NMR spectrum the methyl group, C-4, is observed as a signal at 12.80ppm. The signal at 24.23ppm represents the C-3 methylene CH_2 group, while the benzylic CH_2 group is represented by a signal at δ36.7. The pyrrolidinyl side chain carbons C-2'''', C-3'''' and C-1'''', C-4'''' appear as signals at 23.00ppm and 54.18ppm. The C-5 and C-4 carbons of the side group are identified as signals at 54.60ppm and 66.20ppm. The aromatic signals are characteristically found between 113.75ppm and 159.70ppm. The ^{19}F NMR spectrum shows the E-isomer to be the predominant isomer in this case with a signal appearing at -117.12ppm. The molecular ion of this compound was calculated as ($C_{29}H_{34}FNO$) (M^++1) 429.5503 and correctly observed by high-resolution mass spectrometry as 430.2546.

2.8.2.2.2. Synthesis of 1-(2-{4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenoxy}-ethyl)-piperidine (BRI090)

This reaction was carried out according to the method 2.6.1.1.2. 4-[2-Benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082) was treated with triphenylphosphine, N-(-2-hydroxyethyl)piperidine and diispropyl azodicarboxylate (Figure 2.12) (Scheme 2.40). The product was recovered as a yellow oil (73%)

In this case the piperdinyl side chain protons H-3'''', (H-2'''', H-4'''') and (H-1'''', H-5'''') are represented in the ^1H NMR spectrum by a singlet at δ1.28, a multiplet at δ1.62 and a singlet at δ2.55 respectively. The H-7 and H-6 protons of the basic side chain appear as triplets at δ2.78 (J=6.02Hz) and δ4.09 (J=6.02Hz).

In the ^{13}C NMR spectrum the basic side chain carbons C-3'''', (C-2'''', C-4''''), (C-1'''', C-5''''), C-7 and C-6 are observed as signals at 24.23ppm, 25.29ppm, 54.33ppm 57.40ppm and 65.16ppm respectively. The ^{19}F NMR spectrum shows the E-isomer to be the major isomer with a signal at -117.04ppm. High-resolution mass spectrometry confirmed the molecular ion (M^++1) of the compound ($C_{30}H_{35}FNO$) to be 444.2703, calculated for 443.5955.

2.9. Summary of analogue series 1,2,3 and 4

In conclusion, a series of novel antiestrogens, as shown in Figure 2.13, of various stereochemical aspects, structurally related to tamoxifen were prepared via the McMurry coupling reaction. The modifications that were applied to the tamoxifen structure, which allowed the design of these new analogues, involved the variation of the basic side chain on the phenolic ring, the introduction of flexibility by means of an ethylene group and the type and position of substitutents placed around Ring B of the structure while Ring C of the structure remained unsubstituted.

The biochemical evaluation and estrogen receptor binding results for these compounds can be found in chapter 4.

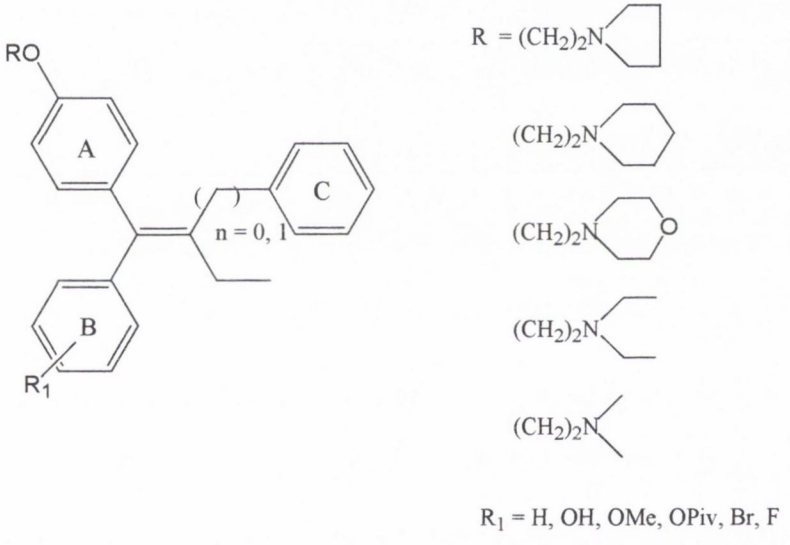


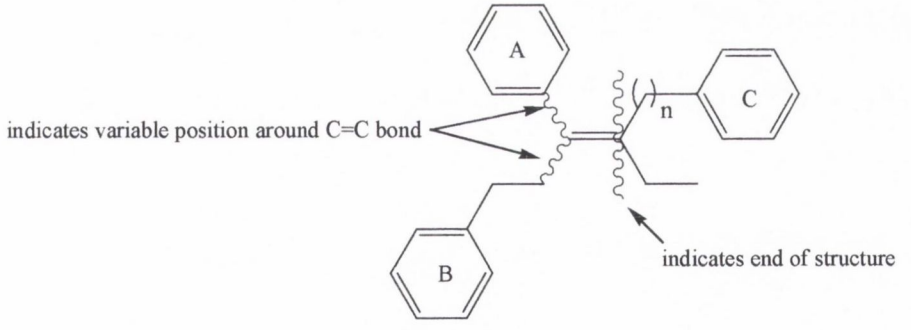
Figure 2.13 Analogues discussed in chapter 2

3. Synthesis of 1,3-Diphenylpropanones and Flexible Tamoxifen Analogues

3.1. Introduction

In this chapter a further series of compounds were synthesised by introducing more flexibility into the triphenylethylene structure than seen in the previous chapter, with the addition of additional ethylene spacing groups. This investigation is pursued under the theory that flexibility could increase the binding affinity of compounds to the estrogen receptor.

The McMurry coupling (low valent titanium)[158] is a very versatile reaction and can be applied to the coupling of many different types of carbonyl compounds. In one case the couplings of acetophenones[159] were discussed, using similar reaction conditions to the couplings of benzophenones, the reactions were successful. More challenging couplings were investigated by Furstner and Bogdanovic[158] where functional groups, which were considered previously to be hardly reactive or even inert for example amides, were found to undergo intramolecular cross-coupling with ketones quite smoothly. The target products required the initial synthesis of chalcones (α,β-unsaturated ketones) after which couplings of these with selected ketones would then be attempted. It was suggested that the double bond of the chalcone[142] structure may interfere with the coupling reaction so in that case the chalcones would be hydrogenated to produce a new series of 1,3-diphenylpropan-1-ones and the coupling of these compounds to selected ketones would then be performed to afford the target product illustrated in Figure 3.1 and Figure 3.2.



Ring A and B = chalcones, dihydrochalcones

Ring A, B and C = triarylpentenyls, triarylbutenyls (n = 1, 0)

Ring C = 1-phenyl-2-butanones, propiophenones (n = 1, 0)

Figure 3.1 General ring assignment for analogues Type 1-10

3.1.1. Lead structures of analogues Types 1-10

The various target products Types 1 – 10 are illustrated in Figure 3.1.

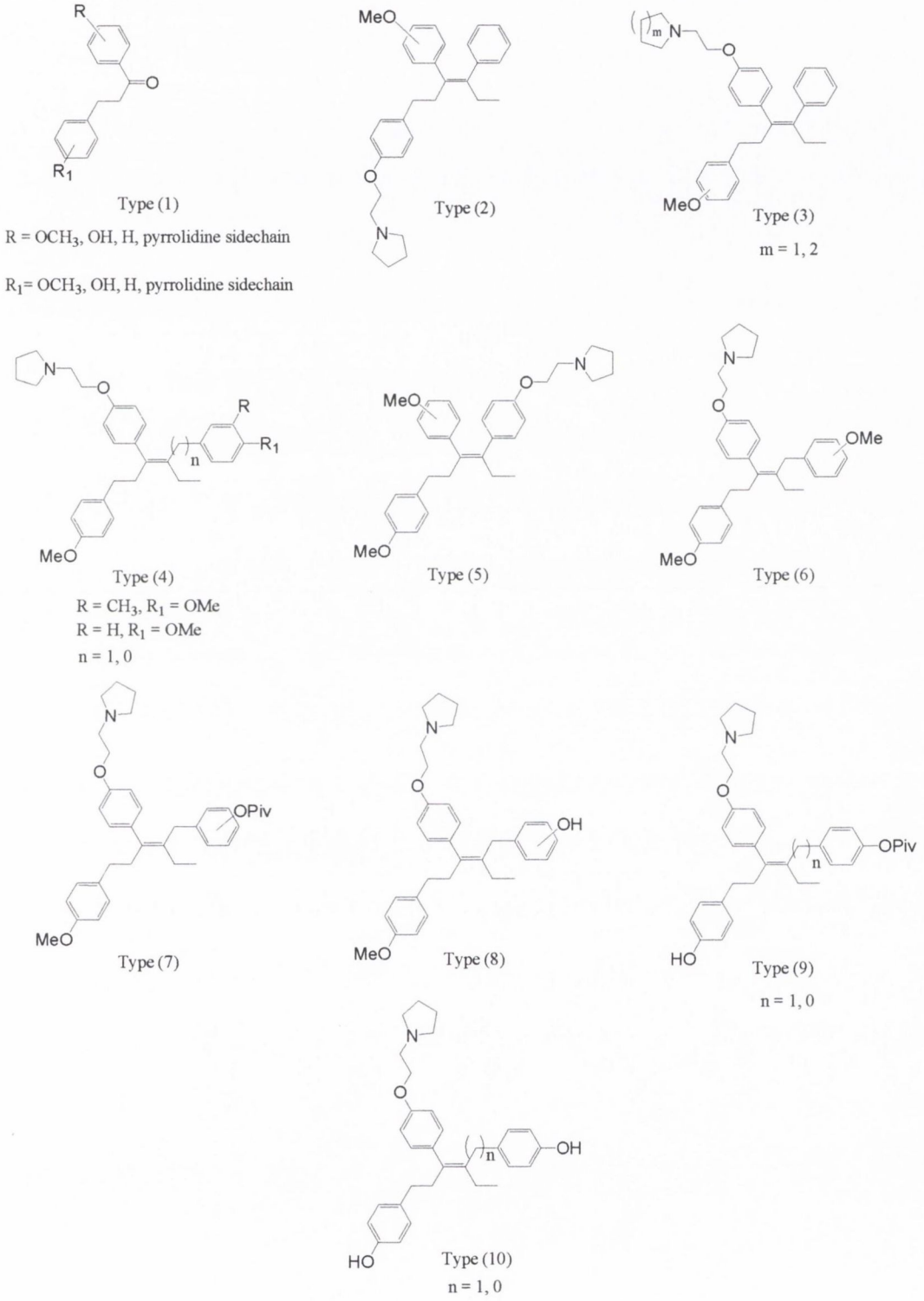
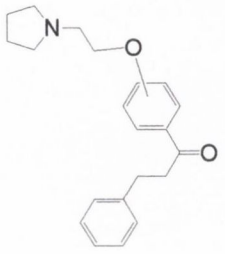


Figure 3.2 Lead structures of analogues Types 1-10

3.2. Analogue series Type 1

The analogue series Type 1 ketones are dihydrochalcones containing hydroxy and methoxy substitutents arranged at *ortho*, *meta* and *para* positions on Rings A and B as shown in figure 3.2. A basic pyrrolidine side chain is being used throughout this series of analogues.



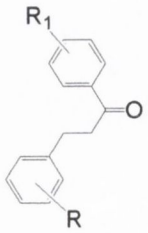
Type 1.1

BRI165 *ortho*-side chain

BRI173 *meta*-side chain

BRI172 *para*-side chain

para-side chain = OCH_2CH_2N



Type 1.2

BRI068 R = *ortho*-OMe, R_1 = side chain

BRI166 R = *meta*-OMe, R_1 = side chain

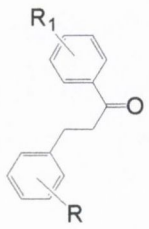
BRI145 R = *para*-OMe, R_1 = side chain

BRI167 R_1 = *ortho*-OMe, R = side chain

BRI136 R_1 = *meta*-OMe, R = side chain

BRI168 R_1 = *para*-OMe, R = side chain

para-side chain = OCH_2CH_2N



Type 1.3

BRI183 R_1= *ortho*-OH, R = side chain

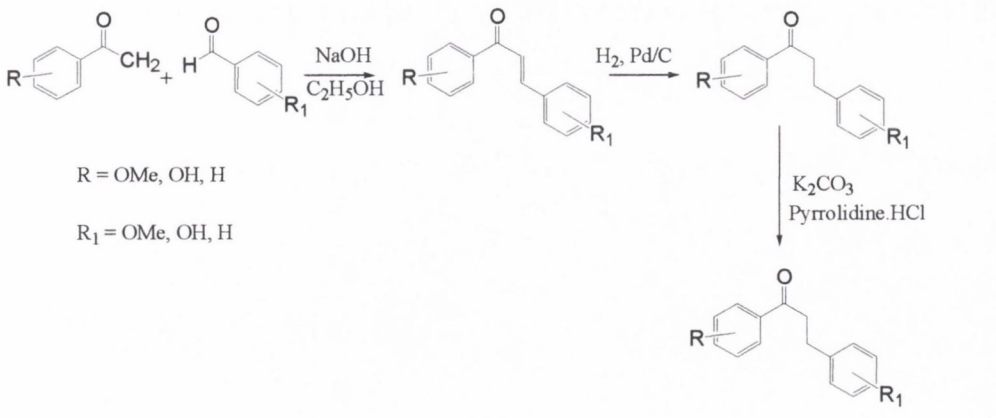
BRI177 R = *para*-OH, R_1= side chain

BRI178 R = *meta*-OH, R_1= side chain

para-side chain = OCH_2CH_2N

Figure 3.3 Analogue series Type 1

3.2.1. Synthesis of analogue series 1



R = OMe, OH, H

R_1 = OMe, OH, H

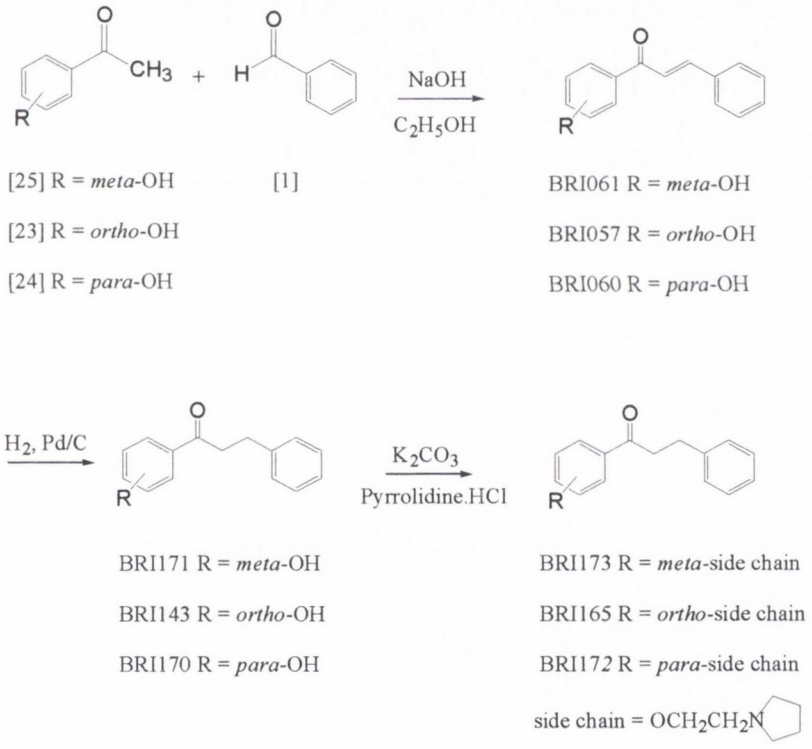
R = OMe, pyrrolidine side chain, H

R_1 = OMe, pyrrolidine side chain, H

Scheme 3.1 Pathway for synthesis of analogue series 1

In order to synthesise the ketones displayed in Figure 3.3 above, a series of related chalcones[160] were first synthesised. The general method for the synthesis of these chalcones was a base catalysed aldol condensation (Scheme 3.1) reaction of aryl aldehydes and ketones. Following the isolation of these chalcones they were then subjected to a hydrogenation[161] in preparation for their subsequent coupling, via carbonyl groups, with selected ketones. But in this section, to complete the study of these compounds as potential anti-estrogens, it was necessary to add a basic side chain to the hydrogenated phenolic chalcones before biochemical evaluation could be performed. A deprotection of the hydroxy groups was performed on selected compounds in an attempt to optimise their binding ability to the estrogen receptor.

3.2.2. Analogue series Type 1.1 – synthesis of chalcones via the Aldol condensation reaction[160]



Scheme 3.2 Pathway for synthesis of analogues Type 1.1

These products contain pyrrolidine basic side chains on the aromatic rings. Products BRI061, BRI057 and BRI060 were obtained by aldol condensation of the acetophenones [23], [6] and [8] with benzaldehyde [5]. Normally the product precipitated out of solution and the crystalline product was shown to be of high purity by melting point. Positive identification was obtained from spectroscopic data (shown in Table 3.1).

3.2.3. Synthesis of 1,3-diphenylpropenones

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI061[162]	**45**	**1654 (C=O)**	**1597 (C=C)**	**3433 (OH)**
BRI057[163]	**34**	**1638 (C=O)**	**1573 (C=C)**	**3310 (OH)**
BRI060[164]	**60**	**1654 (C=O)**	**1597 (C=C)**	**3124 (OH)**

Table 3.1: Yield and infrared data for compounds BRI061, BRI057 and BRI060

3.2.4. Synthesis of 1,3-diphenylpropan-1-ones

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI171[165]	**72**	**1713 (C=O)**	**3072 (OH)**
BRI143[166]	**79**	**1683 (C=O)**	**3028 (OH)**
BRI170[164]	**57**	**1607 (C=O)**	**3369 (OH)**

Table 3.2: Yield and infrared data for compounds BRI171, BRI143 and BRI170

BRI171, BRI143 and BRI170 were obtained by hydrogenation of the corresponding chalcones in ethanol with 10% palladium on charcoal. The reaction was observed by TLC for product development. The reaction was stopped when a third spot started to appear on TLC as this indicated completion of the hydrogenation[161] of the chalcone (Scheme 3.2) and a slight over hydrogenation to the reduced carbonyl product[167]. The products were then identified spectroscopically. On comparison of the infrared spectra of the starting material 1-(4-hydroxyphenyl)-3-phenylpropenone (BRI060)[164] and the product 1-(4-hydroxyphenyl)-3-phenyl-propan-1-one (BRI170)[164] a C=C double bond signal is present in the starting material spectrum but absent in the product spectra therefore confirming the success of the hydrogenation reaction which can be seen by comparing tables 3.2 and 3.1.

3.2.5. Addition of basic side chains to 1,3-diphenylpropan-1-ones[119]

Compound	Yield %	IRυ_{max} (film cm^{-1})
BRI173	**73**	**1611 (C=O)**
BRI165	**40**	**1612(C=O)**
BRI172[168]	**65**	**1612(C=O)**

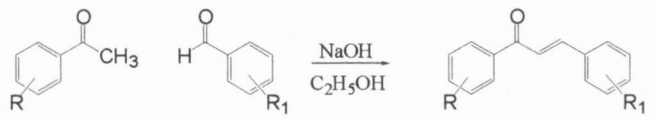
Table 3.3: Yield and infrared data for compounds BRI173, BRI165 and BRI172

Alkylation of the phenolic functional group on compounds BRI171, BRI170 and BRI143 with a basic side chain affords the products BRI173, BRI165 and BRI172. The addition of the basic side chain[119] to produce these compounds was confirmed using infrared, ^{1}H NMR and ^{13}C NMR spectroscopy. Details of the carbonyl signals in the infrared spectra 3-phenyl-1-[2-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI165) of the compounds are listed in Table 3.3 above. The ^{1}H NMR spectrum of BRI165 confirms basic side chain addition also by the presence of signals at δ1.89, and δ2.95 which represent the protons H-3'''', H-5'''' and H-2'''', H-6''''of the pyrrolidine ring of the basic side chain as they each integrate for four. The chain of the basic side group contains protons H-4 and H-3 that is represented by signals at δ2.61 and δ2.73 each integrating for two protons. In the ^{13}C NMR spectrum the pyrrolidine side chain is also confirmed by the presence of signals at 32.70ppm and 54.68ppm that represent C-1'''', C-4'''' and C-2'''', C-3'''' of the basic side chain. The remaining carbons of the basic side chain C-3 and C-4 are verified by the presence of signals at 54.25ppm and 66.54ppm.

3.2.6. Analogue series Type 1.2

Type 1.2 products will contain methoxy substitutents on one aromatic ring and another substitutent on the other aromatic ring. In the case of hydroxy substituted analogues a side chain is added at the hydroxy position.

3.2.6.1. Aldol condensation to afford chalcones Type 1.2.1



[21] R = H [1] R_1 = H BRI050 R = H, R_1 = H

[20] R = *meta*-OMe [1] R_1 = H BRI046 R = *meta*-OMe, R_1 = H

[22] R = *para*-OMe [1] R_1 = H BRI055 R = *para*-OMe, R_1 = H

[20] R = *meta*-OMe [3] R_1 = *para*-OMe BRI047 R = *meta*-OMe, R_1 = *para*-OMe

[21] R = *ortho*-OMe [1] R_1 = *para*-OMe BRI056 R = *ortho*-OMe, R_1 = *para*-OMe

[22] R = *para*-OMe [3] R_1 = *para*-OMe BRI054 R = *para*-OMe, R_1 = *para*-OMe

Scheme 3.3 Aldol condensations to afford chalcones Type 1.2.1

The synthesis of these chalcones (BRI050, BRI046, BRI055, BRI047, BRI056 and BRI054)[160] was achieved using the method described in section 3.2.2. The aldol condensation is performed between the appropriate benzaldehydes and the ketones (Scheme 3.3) in order to obtain the desired chalcones.

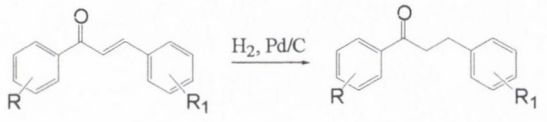
Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI046[169]	**75**	**1681 (C=O)**	**1596 (C=C)**
BRI047[170]	**71**	**1682 (C=O)**	**1597 (C=C)**
BRI050[171]	**73**	**1664 (C=O)**	**1608 (C=C)**
BRI054[172]	**40**	**1647 (C=O)**	**1599 (C=C)**
BRI055[173]	**98**	**1656 (C=O)**	**1605 (C=C)**
BRI056[174]	**60**	**1629 (C=O)**	**1601 (C=C)**

Table 3.4: Yield and infrared data for compounds BRI046, BRI047, BRI050, BRI054, BRI055 and BRI056

On examination of the infrared spectrum of 3-(methoxyphenyl)-1'-phenylpropenone (BRI055)[173], the presence of a carbonyl group signal and a C=C double bond signal are

acknowledged. These signals are characteristic of chalcones and are listed in Table 3.4 above for the identification of the range of chalcones synthesised. The infrared spectrum of 3-(4-methoxyphenyl)-1'-(2-methoxyphenyl)-propenone (BRI056)[174] also has the same characteristic carbonyl and double bond signals as seen in Table 3.4 above. The ^{1}H NMR spectrum differs from that of BRI055 with two singlets at δ3.78 and δ3.82 that integrate for three protons each therefore representing the methoxy groups. 3-(Methoxyphenyl)-1'-phenylpropenone (BRI055) is mono-substituted and so its ^{1}H NMR spectrum is only shows one methoxy signal, a singlet, integrating for three protons and signals in the range δ7.44-8.06 which represent the C=C bond protons and all aromatic protons.

3.2.6.2. Hydrogenation of Type 1.2.1 chalcones



BRI050 R = H, R_1 = H

BRI046 R = *meta*-OMe, R_1 = H

BRI055 R = *para*-OMe, R_1 = H

BRI047 R = *meta*-OMe, R_1 = *para*-OMe

BRI056 R = *ortho*-OMe, R_1 = *para*-OMe

BRI054 R = *para*-OMe, R_1 = *para*-OMe

BRI051 R = H, R_1 = H

BRI052 R = *meta*-OMe, R_1 = H

BRI059 R = *para*-OMe, R_1 = H

BRI053 R = *meta*-OMe, R_1 = *para*-OMe

BRI137 R = *ortho*-OMe, R_1 = *para*-OMe

BRI058 R = *para*-OMe, R_1 = *para*-OMe

Scheme 3.4 Hydrogenation of Type 1.2.1 chalcones to corresponding 1,3-diphenylpropan-1-ones

The hydrogenation[161] of chalcones BRI051, BRI052, BRI059, BRI053, BRI137 and BRI058 were achieved using the method described in section 3.2.4. Scheme 3.4 shows the hydrogenation of each of the chalcones to their subsequent diphenylpropan-1-ones[175] (Table 3.5).

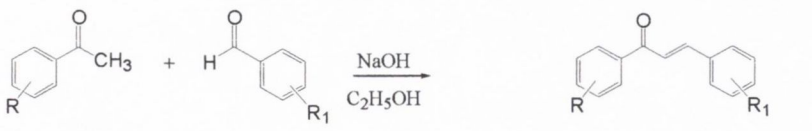
Compound	Yield %	IRυ_{max} (film cm^{-1})
BRI052[162]	**38**	**1689 (C=O)**
BRI053	**68**	**1686 (C=O)**
BRI051[176]	**68**	**1664 (C=O)**
BRI058[177]	**40**	**1670 (C=O)**
BRI137	**88**	**1656 (C=O)**
BRI059[178]	**50**	**1670 (C=O)**

Table 3.5: Yield and infrared data for compounds BRI052, BRI053, BRI051, BRI058, BRI137 and BRI059

The infrared spectrum of 3-(methoxyphenyl)-1'-phenylpropan-1-one (BRI059)[178] contains the characteristic carbonyl signal as seen in Table 3.5 above. When compared with the infrared spectrum of 3-(methoxyphenyl)-1'-phenylpropan-1-one (BRI055), the absence of the C=C double bond signal is observed. This clearly shows that the hydrogenation reaction was successful. The success of the reaction is also confirmed by the upfield shift of the four protons of the CH_2-CH_2 protons to δ3.07 (J=7.76Hz) and δ3.25 (J=7.78Hz) which now appear as triplets.

The infrared spectrum of 3-(4-methoxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI137) also contains the characteristic carbonyl signal as listed in Table 3.5 above. The C=C bond signal is also absent in this spectrum confirming the success of the hydrogenation reaction. The ^{1}H NMR spectrum of BRI137 also shows the upfield shift of two triplets to δ2.97 (J=7.78Hz) and δ3.28 (J=7.78Hz) which each integrate for two protons. This ^{1}H NMR spectrum also contained two singlets at δ3.79 and δ3.88 each integrating for three that represent two methoxy groups.

3.2.6.3. Aldol condensation to afford chalcones Type 1.2.2



[24] R = *para*-OH [3] R_1= *para*-OMe

[24] R = *para*-OH [3] R_1= *meta*-OMe

[24] R = *para*-OH [3] R_1= *ortho*-OMe

[20] R = *meta*-OMe [15] R_1= *para*-OH

[23] R = *ortho*-OMe [15] R_1= *para*-OH

[22] R = *para*-OMe [15] R_1 = *para*-OH

BRI062 R = *para*-OH, R_1 = *para*-OMe

BRI066 R = *para*-OH, R_1 = *meta*-OMe

BRI070 R = *para*-OH, R_1 = *ortho*-OMe

BRI074 R = *meta*-OMe, R_1 = *para*-OH

BRI075 R = *ortho*-OMe, R_1 = *para*-OH

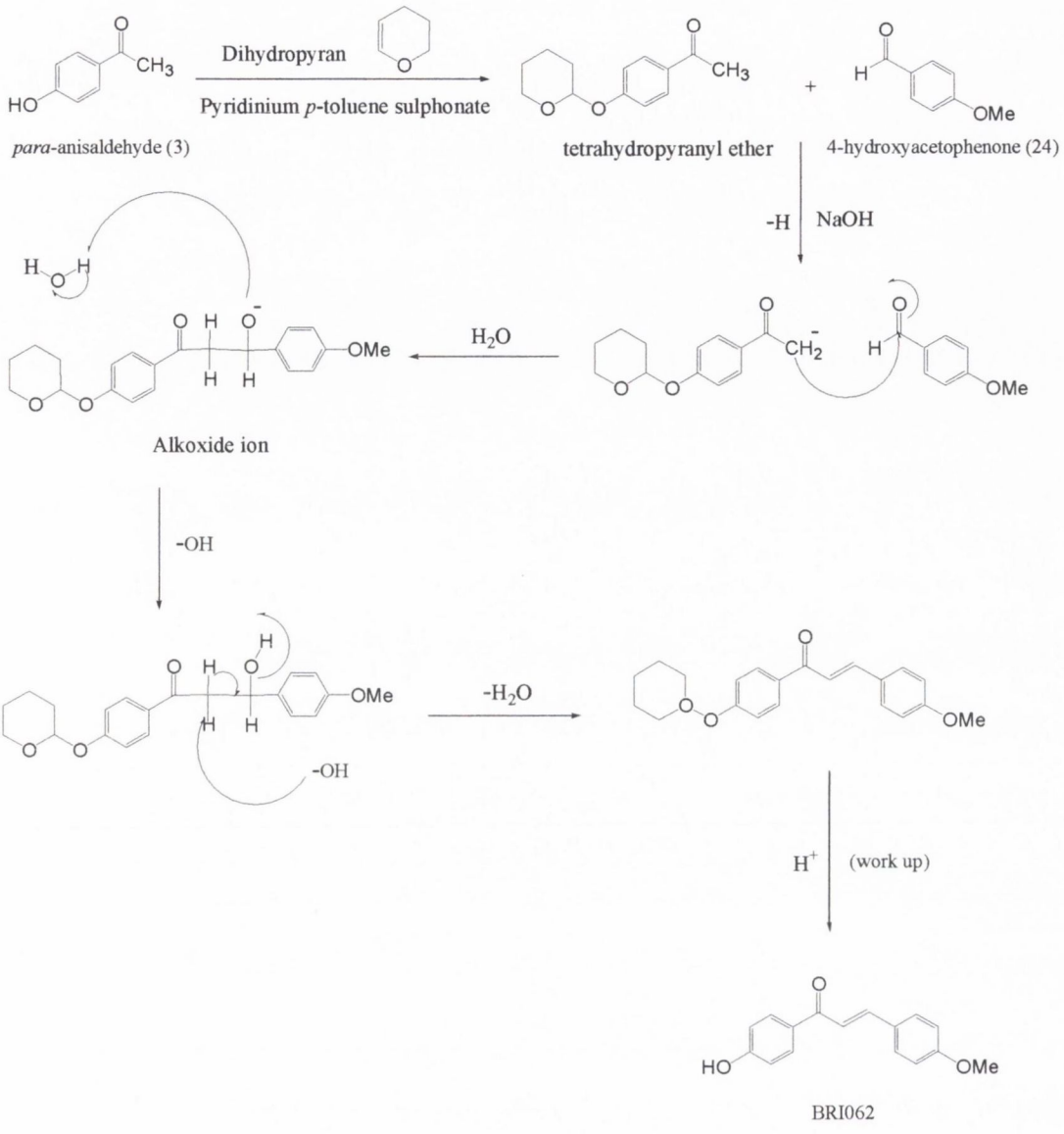
BRI073 R = *para*-OMe, R_1 = *para*-OH

Scheme 3.5 Aldol condensations to afford chalcones Type 1.2.2

The synthesis of these chalcones[160] (BRI062, BRI066, BRI070, BRI074, BRI075 and BRI073) was achieved using the general method (Scheme 3.5) and produced a yield of 23.7%. A new method was attempted in order to increase the yield of these hydroxylated chalcones. To improve the yield an alternative procedure involving the protection of the phenolic group as the dihydropyran derivative was explored.

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI062[179]	**46**	**1654 (C=O)**	**1610 (C=C)**	**3322 (OH)**
BRI066[180]	**53**	**1654 (C=O)**	**1577 (C=C)**	**3301 (OH)**
BRI070[181]	**70**	**1647 (C=O)**	**1578 (C=C)**	**3187 (OH)**
BRI074[182]	**55**	**1648 (C=O)**	**1568 (C=C)**	**3294 (OH)**
BRI075[183]	**63**	**1643 (C=O)**	**1598 (C=C)**	**3317 (OH)**
BRI073[184]	**72**	**1643 (C=O)**	**1598 (C=C)**	**3269 (OH)**

Table 3.6: Yield and infrared data for compounds BRI062, BRI066, BRI070, BRI074, BRI075 and BRI073



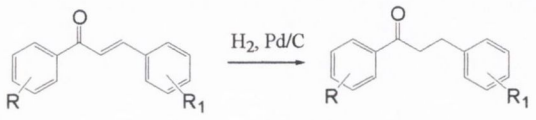
Scheme 3.6 Chalcones Type 1.2.2 formation via tetrahydropyranyl (THP) ether

The alternative reaction sequence involved the treatment of 4-hydroxyacetophenone (6), pyridinium *para*-toluene sulphonate and dihydropyran in dichloromethane for 4 hours at room temperature (Scheme 3.6). The newly formed THP protected acetophenone was reacted with *para*-anisaldehyde [12] in sodium hydroxide solution. Column chromatography afforded the product as yellow crystals (46%). This new method was then applied to 1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)-propenone (BRI066)[180] and resulted in a 53% yield. The reaction was tedious and with the necessary purification was quite time

consuming so despite the increased yields via this reaction the general method was returned to for chalcone synthesis as it was much less time consuming.

The synthesis of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062)[179] as confirmed on observation of the infrared spectrum carbonyl and C=C double bond signals as seen in Table 3.6 above. The presence of a broad hydroxy band at 3301cm^{-1} also identified an OH group on the chalcone structure. In the ^{1}H NMR spectrum a methoxy group appears as a singlet at δ3.88 integrating for three protons.

3.2.6.4. Catalytic hydrogenation of Chalcones



BRI062 R = *para*-OH, R_1 = *para*-OMe — BRI072 R = *para*-OH, R_1 = *para*-OMe

BRI066 R = *para*-OH, R_1 = *meta*-OMe — BRI071 R = *para*-OH, R_1 = *meta*-OMe

BRI070 R = *para*-OH, R_1 = *ortho*-OMe — BRI142 R = *para*-OH, R_1 = *ortho*-OMe

BRI074 R = *meta*-OMe, R_1 = *para*-OH — BRI067 R = *meta*-OMe, R_1 = *para*-OH

BRI075 R = *ortho*-OMe, R_1 = *para*-OH — BRI017 R = *ortho*-OMe, R_1 = *para*-OH

BRI073 R = *para*-OMe, R_1 = *para*-OH — BRI097 R = *para*-OMe, R_1 = *para*-OH

Scheme 3.7 Hydrogenation of Type 1.2.2 chalcones to corresponding 1,3-diphenylpropan-1-ones

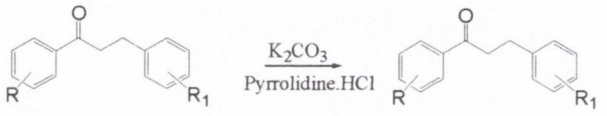
The hydrogenation[161] of these chalcones (Scheme 3.7) was achieved using hydrogen and a palladium catalyst as described in the method in section 3.2.4.

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI072[164]	**80**	**1648 (C=O)**	**3179 (OH)**
BRI071[180]	**73**	**1651 (C=O)**	**3362 (OH)**
BRI142	**86**	**1684 (C=O)**	**2939 (OH)**
BRI067[187]	**80**	**1677 (C=O)**	**3433 (OH)**
BRI017	**67**	**1668 (C=O)**	**3381 (OH)**
BRI097[164]	**65**	**1613 (C=O)**	**3394 (OH)**

Table 3.7: Yield and infrared data for compounds BRI072, BRI071, BRI142, BRI067, BRI017 and BRI097

The infrared spectrum for 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072)[164] was compared to that of the starting material, 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062), and the absence of a C=C double bond signal was noted therefore confirming the hydrogenation of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062). Table 3.7 lists the carbonyl signals for each of the compounds. The ^{1}H NMR spectrum presented an upfield shift of two triplets to δ2.99 (J=7.5Hz) and δ3.22 (J=7.5Hz) that were originally two singlets in the δ7.00-8.00 range and integrated for one proton each but now integrate for two each. This confirms the hydrogenation reaction success.

3.2.6.5. Basic side chain addition[119] to 1,3-diphenylpropan-1-ones



BRI072 R = *para*-OH, R_1 = *para*-OMe

BRI071 R = *para*-OH, R_1 = *meta*-OMe

BRI142 R = *para*-OH, R_1 = *ortho*-OMe

BRI067 R = *meta*-OMe, R_1 = *para*-OH

BRI017 R = *ortho*-OMe, R_1 = *para*-OH

BRI097 R = *para*-OMe, R_1 = *para*-OH

BRI145 R = *para*-side chain, R_1 = *para*-OMe

BRI166 R = *para*-side chain, R_1 = *meta*-OMe

BRI068 R = *para*-side chain, R_1 = *ortho*-OMe

BRI136 R = *meta*-OMe, R_1 = *para*-side chain

BRI167 R = *ortho*-OMe, R_1 = *para*-side chain

BRI168 R = *para*-OMe, R_1 = *para*-side chain

side chain = OCH_2CH_2N 

Scheme 3.8 Basic side chain additions to Type 1.2.2 1,3-diphenylpropan-1-ones

Scheme 3.8 demonstrates the pyrrolidine side chain addition to each of the diphenylpropanones synthesised in section 3.2.6.4. The addition of basic side chains was performed in order to produce compounds more suitable for estrogen receptor antagonism. The method involved the reaction of the hydroxy substituted 1,3-diphenylpropan-1-ones with 1-(2-chloroethyl)pyrrolidine.HCl in acetone:water (19:1) with potassium carbonate.

The absence of a broad hydroxy band combined with the presence of an N-H signal in the infrared spectrum as displayed in Table 3.8 for each of the compounds BRI145, BRI166,

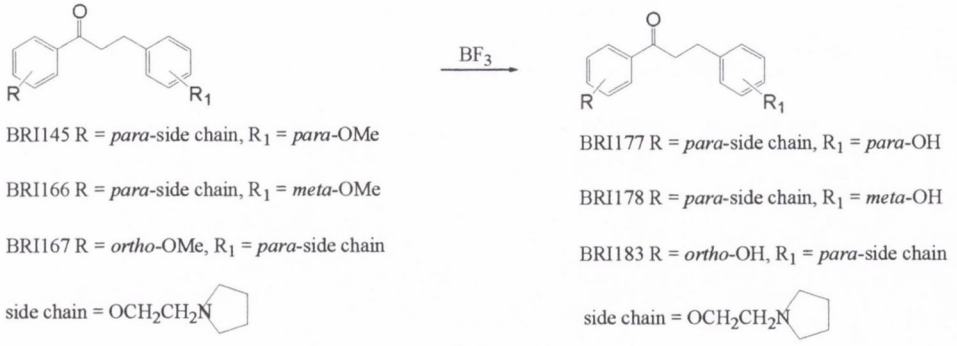
BRI068, BRI137, BRI167 and BRI168 indicates that the basic side chain additions at the hydroxy groups had been successful. The ^{1}H NMR spectrum of BRI145 shows many new signals that are not observed in the ^{1}H NMR spectrum of the starting material. A singlet at δ1.68 that integrates for four protons represents H-3'''' and H-5'''' of the basic side chain was observed as was the singlet at δ2.51 represent H-2'''' and H-6'''' of the basic side chain. The remaining protons of the basic side chain are represented by triplets at δ3.07 (J=5.8Hz) and δ4.02 (J=5.8Hz) each integrating for two protons.

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI145	**70**	**1675 (C=O)**	**1513 (N-CH$_2$)**
BRI166	**80**	**1640 (C=O)**	**1513 (N-CH$_2$)**
BRI068	**55**	**1675 (C=O)**	**1494 (N-CH$_2$)**
BRI137	**88**	**1656 (C=O)**	**1464 (N-CH$_2$)**
BRI167	**36**	**1606 (C=O)**	**1509 (N-CH$_2$)**
BRI168	**70**	**1676 (C=O)**	**1510 (N-CH$_2$)**

Table 3.8: Yield and infrared data for compounds BRI145, BRI166, BRI167, BRI068, BRI137 and BRI168

3.2.7. Analogue series Type 1.3

Type 1.3 products will contain a hydroxy substitutent on one aromatic ring and a side chain on the other Ring.



Scheme 3.9 Deprotection[185] of 1,3-diphenylpropan-1-ones containing basic side chains

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})
BRI177	**76**	**1673 (C=O)**	**3400 (OH)**
BRI178	**55**	**1674 (C=O)**	**3436 (OH)**
BRI183	**71**	**1674 (C=O)**	**3300 (OH)**

Table 3.9: Yield and infrared data for compounds BRI177, BRI178 and BRI183

It is well known that ER binding ability[186] increases with the introduction of a hydroxy group into a structure. Therefore deprotections were performed (Scheme 3.9) on some of the compounds BRI145, BRI166 and BRI167 synthesised in section 3.2.6.5 in order to increase the binding ability of these compounds to the estrogen receptor and hopefully create analogues of higher affinity for the ER protein.

Boron trifluoride-dimethyl sulphide was used to remove the methoxy protecting groups from BRI145, BRI166 and BRI167 at room temperature, in order to liberate the hydroxy groups, and subsequently synthesise BRI177, BRI178 and BRI183.

Table 3.9 above shows the carbonyl signals that are still present in the infrared spectrum of each of the compounds BRI177, BRI178 and BRI183. The absence of a methoxy signal in the ^{1}H NMR spectrum and the presence of all other expected signals, confirmed the synthesis of compounds BRI177, BRI178 and BRI183.

3.3. Analogue series Type 2

3.3.1. Unsubstituted coupled compounds - McMurry coupling of 1,3-diphenylpropan-1-ones and propiophenone[159]

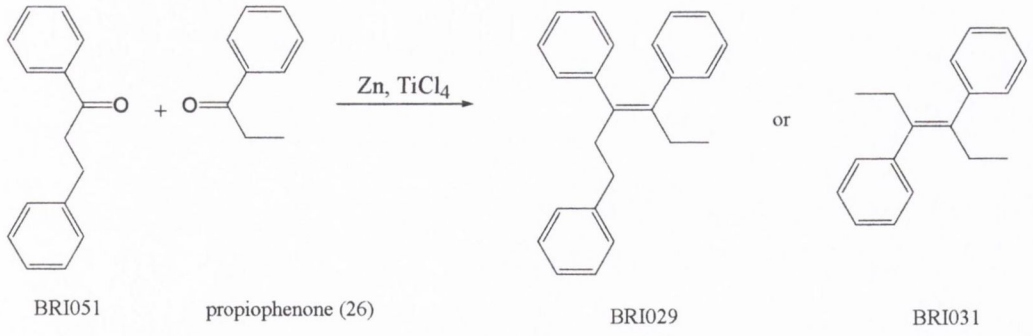
The McMurry coupling of propiophenones with benzophenones[92] has been discussed in Chapter 3 experiments with the coupling of more hindered ketones i.e. dihydrochalcones with propiophenones and phenylbutanones. Initially it was thought that the coupling of chalcones with propiophenones and phenylbutanones could be directly pursued. This assumption was based on the couplings of α,β-unsaturated ketones such as (R)-(4-

methylcyclohexylidene)acetone with acetone[92] documented by McMurry which were successful despite the presence of a C=C double bond in the ketone which is thought to hinder the reaction. McMurry reports the coupling of many different types of ketones that vary in size, functional group and aromatic ring presence and many other categories.

The McMurry reaction conditions[188] applied to reactions discussed in chapter two, which involved the use of a reaction carousel, were initially used for this reaction in an attempt to couple the unsubstituted 1,3-diphenylpropan-1-one (BRI051) to propiophenone [18] (Scheme 3.10). The self-coupled ketones are common by-products of these types of coupling reactions and are easily identified by NMR.

Only one product was isolated from this reaction and this was identified by ^{1}H NMR as (*E*)-3,4-diphenyl-3-hexene (BRI031) the spectrum of which contained a characteristic triplet integrating for six protons at δ0.99 and a characteristic quartet at δ2.57 that integrated for four protons both of which represent the protons of the ethyl groups. The aromatic signals appeared in the region δ6.97 to δ7.09 and integrated for 20 protons. The ^{13}C NMR spectrum confirms the presence of the ethyl groups by signals at 12.87ppm and 26.86ppm. All the remaining aromatic signals appear in the region 125ppm to 142.76ppm.

Because this reaction only produced the self-coupled propiophenone (BRI031) another attempt was made but this time performing the reaction in a two-neck round bottom flask in dioxane[189] while maintaining the temperature of the reaction between 0 and 5°C. This was also unsuccessful. A final attempt was made using titanium tetrachloride and zinc in dry THF as was attempted before but the reaction was performed in a two-neck round bottom flask. From this reaction the product 3,4,6-triphenyl-hex-3-ene (BRI029) was recovered as yellow oil (80%). Only one isomer was present for this product and so assignment of E/Z was only possible when a similar product was synthesised containing a mixture of both isomers.



Scheme 3.10 McMurry coupling of 1,3-diphenylpropan-1-one (BRI051) and propiophenone [18]

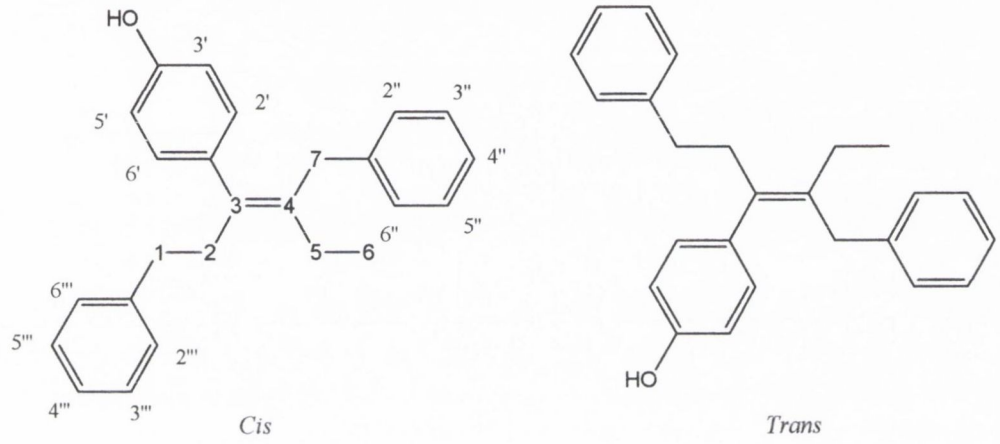
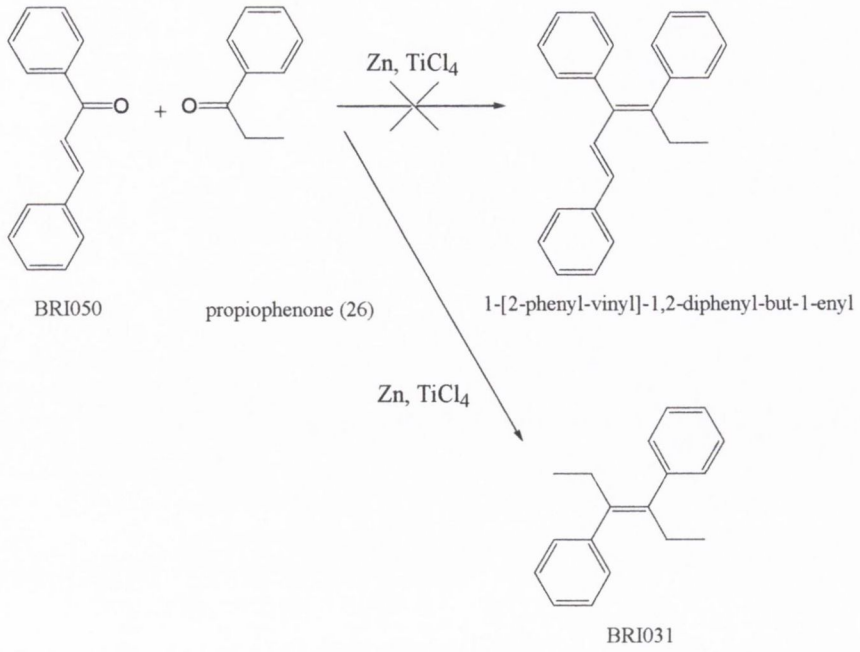


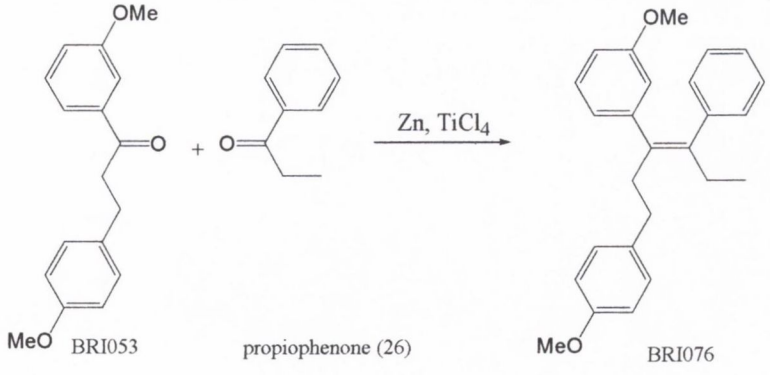
Figure 3.4 NMR assignment of *Cis* and *Trans* lead structures

In the ^{1}H NMR spectrum of BRI029, triplet and quartet signals are observed at δ0.92 (J=7.5Hz) and δ2.44 (J=7.5Hz) the first integrating for three protons and the latter integrating for three protons. These signals represent an ethyl group in the structure of 3,4,6-triphenyl-hex-3-ene (BRI029) (Figure 3.4). Triplet signals at δ2.66 (J=8.16) and δ2.87 (J=8.2) represent H-1 and H-2 represent the two CH_2 groups that connect the two aromatic rings of the left hand side of the structure. The aromatic protons appear in the region δ6.94 to δ7.34. The ^{13}C NMR spectrum supports the presence of an ethyl group by the signals 12.64ppm (CH_3) and 26.94ppm (CH_2). The C-1 and C-2 carbons appear as signals at 34.28ppm and 35.86ppm. Two signals at 135.99ppm and 140.20ppm represent the carbon to C=C double bond. The remaining aromatic carbons appear in the region 125.11ppm to 142.56ppm.



Scheme 3.11 McMurry coupling of 1,3-diphenylpropenone (BRI050) and propiophenone [18]

Following the success of this coupling, attempts were then made to couple 1,3-diphenylpropenone (BRI050) to propiophenone [18] (Scheme 3.11) to produce 1-[2-phenyl-vinyl]-1,2-diphenyl-but-1-enyl. The first reaction was attempted using a reaction carousel. Only self-coupled propiophenone (BRI031) was recovered from this reaction. The reaction was attempted again but this time it was carried out in a two-neck round bottom flask using additional THF solvent. Once again the self-coupled propiophenone (BRI031)[190] was the only product recovered.

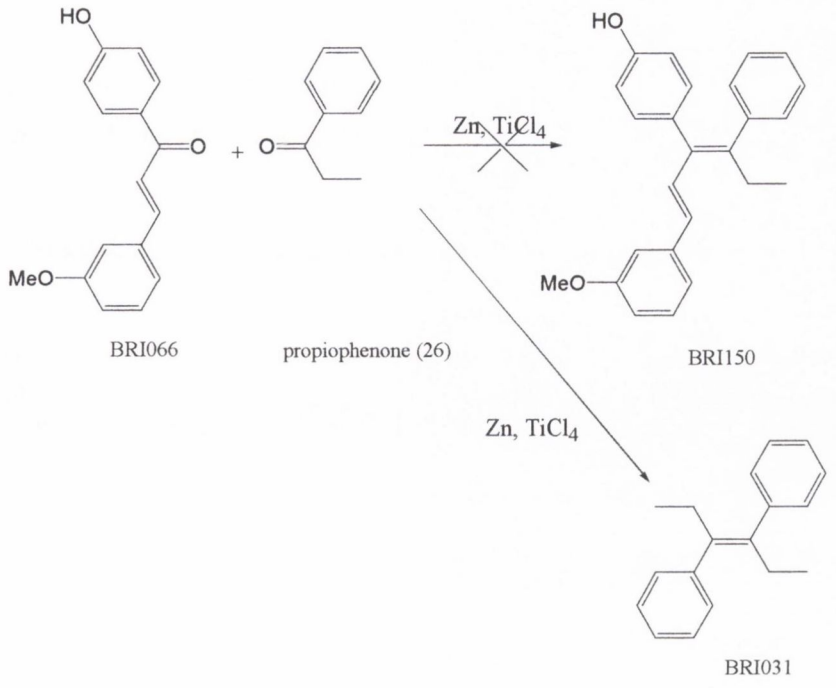


Scheme 3.12 McMurry coupling of 3-(4-methoxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI053) and propiophenone [18]

On discovery of this result, the coupling of 1,3-diphenylpropan-1-ones with propiophenones was returned to, using the general method for the coupling of 3-(4-methoxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI053) and propiophenone [18] (Scheme 3.12) in order to synthesise the methoxy substituted analogue 3-phenyl-4-(4-methoxyphenyl)-6-(3-methoxyphenyl)-hex-3-ene (BRI076). The product was obtained as light yellow oil.

The ^{1}H NMR spectrum for 3-phenyl-4-(4-methoxyphenyl)-6-(3-methoxyphenyl)-hex-3-ene (BRI076) contains the same characteristic signals of coupled 1,3-diphenylpropan-1-ones as does BRI029. The only difference between the two spectra is the presence of methoxy group signals in the spectra of BRI076. These appear as singlets at δ3.62 and δ3.81 each integrating for three protons indicating that there are two methoxy groups present in the structure. The aromatic protons are found in the usual aromatic area of the spectra in the region δ6.52 to δ7.14. The ^{13}C NMR spectra are also very similar to the spectra of BRI029 except for the methoxy signals, which occur at 54.56ppm and 54.83ppm.

General assignment of the Z isomer, in the ^{1}H NMR spectrum in chapter 2, was based on the more downfield shift of the benzyl CH_2 group signal which correlated with the downfield shift of all other protons related to that isomer. As for the E-isomer, assigned to more upfield benzyl signal, by means of exclusion of Z-isomer signals all remaining upfield signals, present to the same extent, were assigned to the E-isomer. A similar rule of assignment was applied to this chapter where the more downfield signals were assigned to the Z-isomer and the upfield signals were assigned to the E-isomer. For the product BRI076 a two isomers were present in the ^{1}H NMR spectrum in a 30:1 ratio. The Z isomer was assigned to the more downfield signals and the E isomer was assigned to the upfield remaining signals presenting the isomeric ratio of the mixture as Z:E 30:1. Regarding compound BRI029 this rule allowed the identification of the isomer as the Z-isomer.



Scheme 3.13 McMurry coupling of 1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)-propenone (BRI066) and propiophenone [18]

Despite the failure of the previous coupling attempts for α,β-unsaturated ketones the coupling of 1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)-propenone (BRI066) and propiophenone [18] (Scheme 3.13) was attempted in the hope of producing a hydroxy and methoxy substituted product 4-{1-[2-(3-methoxyphenyl)-vinyl]-2-phenyl-but-1-enyl}-phenol (BRI150). A mixture of two inseparable products of the same polarity was recovered including the self-coupled propiophenone (BRI031).

3.3.2. Analogue series Type 2- methoxy substituted compounds

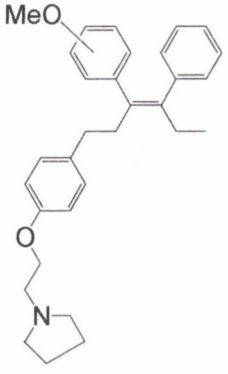
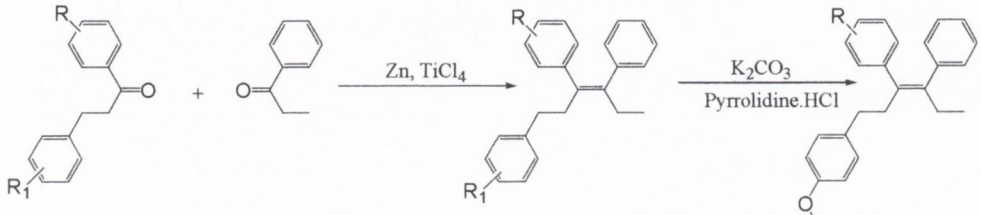


Figure 3.5 Methoxy substituted compounds

Type 2 analogues were synthesised to produce compounds with methoxy substitutents on Ring A and a pyrrolidine side chain on Ring B leaving Ring C unsubstituted.

3.3.2.1. Synthesis of analogues Type 2



BRI051 R = H, R_1 = H [26]

BRI053 R = *para*-OMe, R_1 = *meta*-OMe

BRI067 R = *meta*-OMe, R_1 = OH

BRI017 R = *ortho*-OMe, R_1 = OH

BRI097 R = *para*-OMe, R_1 = OH

BRI029 R = H, R_1 = H

BRI076 R = *para*-OMe, R_1 = *meta*-OMe

BRI094 R = *meta*-OMe, R_1 = OH

BRI095 R = *ortho*-OMe, R_1 = OH

BRI108 R = *para*-OMe, R_1 = OH

BRI179 R = *meta*-OMe

BRI182 R = *ortho*-OMe

BRI109 R = *para*-OMe

Scheme 3.14 Synthesis of analogues Type 2

Following the previous synthesis of methoxy substituted analogues in section 3.3.1 the synthesis of a hydroxy substituted analogue (Scheme 3.13) was then pursued so the required basic side chain introduction could be attempted. 3-(4-Hydroxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI017) (0.0017M) and propiophenone [18] (Scheme 3.14) were coupled using the general method for McMurry coupling described in section 3.3.1 to produce 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI095). A mixture of product and self-coupled propiophenone (BRI031) was produced as oil. Separation of this product from the self-coupled propiophenone (BRI031) was difficult. The presence of a signal at 1610 cm^{-1} in the infrared confirmed the formation of a C=C bond. The ^{1}H NMR and the ^{13}C NMR spectra were difficult to identify due to the presence of the self-coupled propiophenone (BRI031) (appeared to be a 1:1 mixture of BRI095:BRI031) so the product mixture was carried on to the next step, which was basic side group addition.

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratio Z:E
BRI029	**80**	**1600 (C=C)**	**5:1**
BRI076	**50**	**1609 (C=C)**	**20:1**
BRI095	**43**	**1610 (C=C)**	**3:1**
BRI094	**49**	**1604 (C=C)**	**6:1**
BRI108	**43**	**1601 (C=C)**	**20:1**

Table 3.10: Yield and infrared data for compounds BRI029, BRI076, BRI095, BRI094 and BRI108

The synthesis of 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI182) was accomplished according to section 3.2.6.5 where 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI095) was refluxed for 5 hours in acetone:water 19:1 with potassium carbonate and 1-(2-chloroethyl)pyrrolidine.HCl . The product was much more polar than the concurring product, self-coupled propiophenone (BRI031), and was therefore easily separated from it by column chromatography and obtained as a light brown oil in a yield 80%. Isomeric assignment for Z and E isomers as displayed in Table 3.10 above was performed following the assignment rules applied for compounds BRI076 and BRI029 in section 3.3.1.

The infrared spectrum for 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI094) indicates the presence of a hydroxy group by the broad band at 3408cm^{-1}. In the ^{1}H NMR spectrum signals observed at δ0.90 (J=7.52Hz) and δ2.49 (J=7.28Hz) represent the characteristic ethyl group of this type of structure. The CH_2 group protons of the butylene appear at δ2.63 (J=7.76Hz) and δ2.88 (J=7.78Hz) as triplets, while the methoxy group appears as a singlet at δ3.64. The aromatic protons are observed in the region δ6.84 to δ7.02. The ^{13}C NMR spectrum contains signals, which are characteristic of carbons in these type structures and appear at 12.69ppm (CH_3), 30.06ppm (CH_2), 33.49ppm (CH_2) and 36.06ppm (CH_2). A methoxy group is found at 54.63ppm. Finally the aromatic carbons appear in the range 111.15-158.2ppm.

The infrared, ^{1}H NMR and ^{13}C NMR spectra for 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI095) and 4-[3-(4-methoxyphenyl)-4-phenyl-hex-3-enyl]-phenol (BRI108) are very similar to the spectra of 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI094) and are not discussed further

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI179	**70**	**1606 (C=C)**	**20:1**
BRI182	**80**	**1597 (C=C)**	**3:1**
BRI109	**75**	**1607 (C=C)**	**Z only**

Table 3.11: Yield and infrared data for compounds BRI179, BRI182 and BRI109

The infrared spectra for the compounds BRI179, BRI182 and BRI109 shows a C=C alkene stretch in the region 1597-1607cm^{-1} as seen in Table 3.11 above. The ^{1}H NMR and ^{13}C NMR spectra of 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI182) will be discussed in detail as representative of this series of products.

In the ^{1}H NMR spectrum of BRI182 triplet and quartet signals at δ0.93 (J=7.54Hz) and δ2.39 (J=7.4Hz) represent the characteristic ethyl group of this type of structure. The pyrrolidine ring protons H-2'''', H-6'''' and H-3'''', H-5'''' appear as singlets at δ2.80 and δ1.90 integrating for four protons each. The remaining basic side chain protons appear as triplets integrating for two hydrogen's at δ3.02 (J=5.5Hz) and δ4.17 (J=5.78Hz). The CH_2 group protons of the butylene appear at δ2.55 and δ2.59 as multiplets. The methoxy group appears as a singlet at δ3.74. The aromatic protons are found in the region δ6.70 to δ7.13. In the ^{13}C NMR spectrum characteristic signals for carbons of compound BRI182 appear at 12.78ppm (CH_3), 26.31ppm (CH_2), 33.32ppm (CH_2) and 34.98ppm (CH_2). Pyrrolidine side chain carbons C-2'''', C-3'''' and C-1'''', C-4'''' also appear characteristically at 22.96ppm and 54.44ppm. The remaining carbons of the basic side chain are observed at 54.96ppm and 65.71ppm. A methoxy group is found at 54.15ppm. Finally the aromatic carbons appear downfield in the region 110.64-156.5ppm.

3.4. Analogue series Type 3

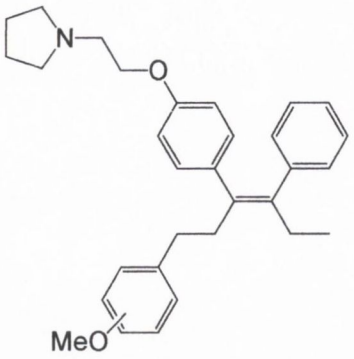
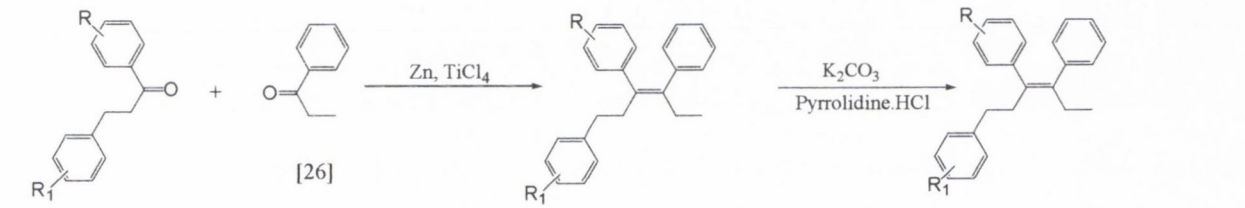


Figure 3.6 Analogue series Type 3

Type 3 analogues were synthesised to produce compounds alternative to Type 2 analogues with the methoxy substitutents on Ring B and a pyrrolidine side chain on Ring A leaving Ring C unsubstituted.

3.4.1. Synthesis of analogues Type 3



BRI072 R = *para*-OH, R_1 = *para*-OMe

BRI071 R = *para*-OH, R_1 = *meta*-OMe

BRI099 R = *para*-OH, R_1 = *para*-OMe

BRI092 R = *para*-OH, R_1 = *meta*-OMe

BRI101 R = *para*-pyrrolidine side chain, R_1 = *para*-OMe

BRI096 R = *para*-piperidine side chain, R_1 = *meta*-OMe

BRI093 R = *para*-pyrrolidine side chain, R_1 = *meta*-OMe

Scheme 3.15 Synthesis of analogues Type 3

The synthesis of inverted methoxy substituted compounds (Figure 3.6), similar to those in section 3.3, was pursued in this section. Scheme 3.15 shows the pathway for the synthesis of these structures.

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI099	**80**	**1610 (C=C)**	**3391 (OH)**	**3:1**
BRI092	**43**	**1602 (C=C)**	**3372 (OH)**	**3:1**

Table 3.12: Yield and infrared data for compounds BRI099 and BRI092

The synthesis of 4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI099) and 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI092) were performed using the same McMurry coupling reaction as that employed in section 3.3.1. Once again the spectra for both are very similar so only one will be discussed in detail. Isomeric assignment for Z and E isomers was performed following the assignment rules applied for compounds BRI076 and BRI029 in section 3.3.1.

The infrared spectrum for 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI092), as displayed in Table 3.12, contains a hydroxy signal at 3372cm^{-1}, which is necessary for basic side group addition in the next step of the reaction. Although these structures are inverted in comparison to the compounds in section 3.2 they still have very similar spectra. So the ^{1}H NMR spectrum signals are observed that are characteristic of these type of structures such as δ0.83 (CH_3), δ2.45 (CH_2), δ2.62 (CH_2), δ2.82 (CH_2), δ3.73 (OCH_3). The ^{13}C NMR spectrum of 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI092) has characteristic signals at 12.65ppm (CH_3), 28.13ppm (CH_2), 34.52ppm (CH_2), 37.14ppm (CH_2).

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI101	**70**	**1606 (C=C)**	**3:1**
BRI096	**34**	**1597 (C=C)**	**3:1**
BRI093	**38**	**1607 (C=C)**	**3:1**

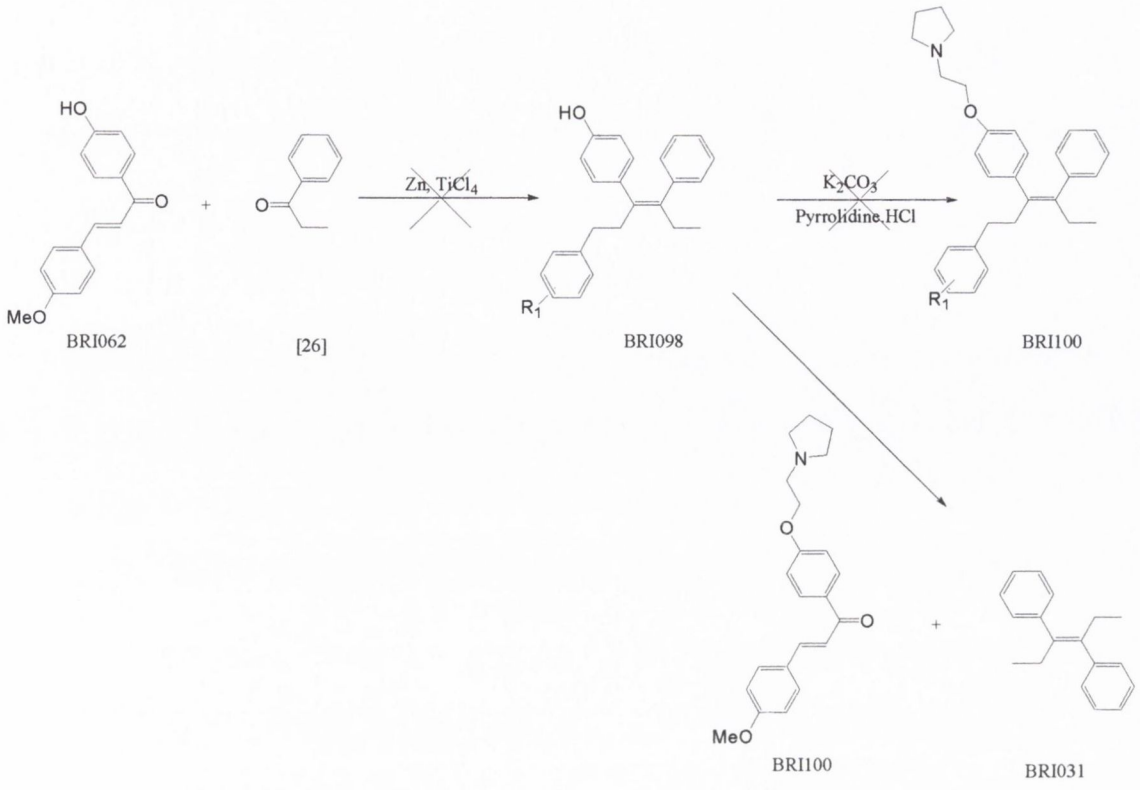
Table 3.13: Yield and infrared data for compounds BRI101, BRI096 and BRI093

The addition of an ethoxy-pyrrolidine and a piperdine basic side chain[119] to 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI092) and 4-{1-[2-(4-

methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI099) was achieved using the basic alkylation method described in section 3.2.6.5 to produce BRI101, BRI096 and BRI093

Compounds 1-[2-(4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-piperidine (BRI096), [2-(4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI093) and 1-[2-(4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI101) have all very similar structures therefore their identification data are also very similar. Therefore only the identification data for 1-[2-(4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI101) will be discussed in detail below.

The C=C alkene stretch of each of the compounds is displayed in Table 3.13 above. In the ^{1}H NMR spectrum of 1-[2-(4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI101) ethyl group protons are appear as signals at δ0.78 and δ2.18. The pyrrolidine ring protons H-2'''', H-6'''' and H-3'''', H-5'''' appear as singlets at δ1.84 and δ2.79 integrating for four protons each. The remaining basic side chain protons appear as triplets integrating for two hydrogen's at δ3.00 and δ4.20. The two CH_2 group protons H-1, H-2 and appear at δ2.38 and δ2.59. The methoxy group appears as a singlet at δ3.76. The aromatic protons are found in the region δ6.72 to δ7.37. It can be seen that all of the signals discussed here are shifted slightly downfield when compared with the spectrum of 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI182). From examination of the Figures 3.5 and 3.6 it could be suggested that is related to the longer aliphatic chain between the phenol ring and the alkene group as this also increases the distance between the C=C group and the pyrrolidine side chain. In the ^{13}C NMR of BRI101 spectrum characteristic signals for carbons of these type structures appear at 13.20ppm (CH_3), 28.54ppm (CH_2), 33.80ppm (CH_2) and 37.80ppm (CH_2). Pyrrolidine side chain carbons C-2'''', C-3'''' and C-1'''', C-4'''' also appear characteristically at 23.41ppm and 54.65ppm. Respectively the remaining carbons of the basic side chain are observed at 55.20ppm and 62.54ppm. A methoxy group is found at 55.15ppm. High resolution mass spectrometry for this compound affords the molecular ion M+1 at 456.2880 ($C_{31}H_{37+1}NO_2$) in 100% abundance (calculated for M+1 = 456.2876).



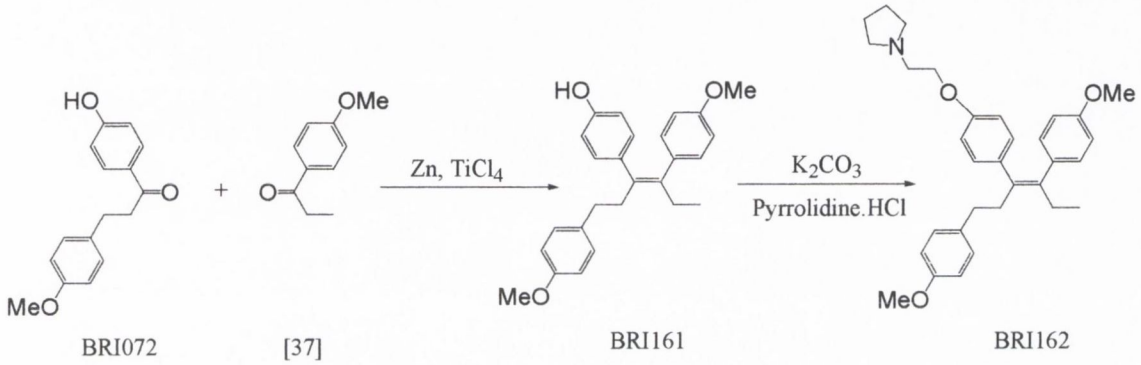
Scheme 3.16 Attempted synthesis of BRI098 and BRI100

The synthesis of 4-{1-[2-(4-methoxyphenyl)-vinyl]-2-phenyl-but-1-enyl}-phenol (BRI098) was attempted by performing the McMurry coupling reaction on 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062) and propiophenone [18] (Scheme 3.16). Instead basic side chain addition was proceeded with on the impure product mixture, because the basic side chain would make the product more polar than the self-coupled propiophenone (BRI031) and therefore they could be easily separated. The synthesis of [2-(4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI100) was attempted by applying the method from section 3.2.6.5 to the mixture. The products were separated but it was discovered that the desired product had not actually being produced. Only starting material 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062) which now had a basic side chain added (BRI062B) and self-coupled propiophenone (BRI031) were recovered from the reaction.

3.5. Analogues series Type 4

Analogues of Type 4 contained methoxy substitutents on aromatic Ring C in order to be able to examine the interaction of these type of subtitutents on the histidine residue (His) of the ER ligand binding domain.

3.5.1. Analogues Type 4.1 – methoxy substituted propiophenone component



BRI072 [37] BRI161 BRI162

Scheme 3.17 Synthesis of analogues Type 4

3.5.1.1. Synthesis of using 4-{2-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI161)

A coupling reaction was performed on 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) and 4-methoxypropiophenone [20] (Scheme 3.17) in order to synthesise 4-{2-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI161). A mixture of the self-coupled propiophenone (BRI031) and the desired product was obtained from the reaction. After much column chromatography a mixture of the two compounds was recovered as yellow oil (36%). Because the self-coupled propiophenone (BRI031) could not be removed, the basic side group addition[119] was proceeded with, as this change in polarities of the desired product would allow easy separation by column chromatography.

3.5.1.2. Synthesis of 1-[2-(4-{2-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI162)

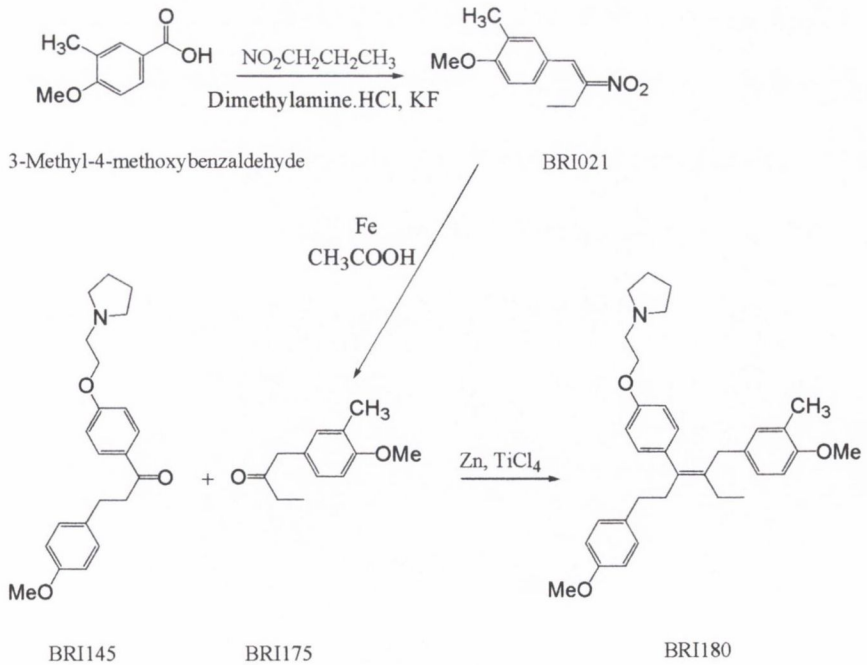
The addition[119] of 1-(2-chloroethyl)pyrrolidine.HCl to 4-{2-(4-methoxyphenyl)-1-[2-(4

-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI161) (Scheme 3.17) was performed using the method described in section 3.2.6.5. This time the product (BRI161) separated easily from the self-coupled propiophenone (BRI031) as light brown oil (70%) and was identified by spectroscopic data.

Infrared data confirmed the presence of a C=C by a signal at 1608cm^{-1}. The ^{1}H NMR spectrum of BRI161 contained the characteristic ethyl group triplet and quartet signals at δ0.78 (J=7.28Hz) and δ2.27 (J=7.02Hz). The presence of a basic side group was verified by signals at δ1.85 and δ2.70 that represented the protons H-3'''', H-5'''', H-2'''' and H-6''''. The remaining pyrrolidine protons were identified by signals at δ2.50 and δ4.15. The CH_2 groups adjacent to the alkene appear at δ2.69 and δ2.95. Two methoxy groups were found at δ3.85. All the aromatic protons were located in the region δ6.63 to δ7.18. The assignment for these protons was supported by the presence of the corresponding carbon signals in the ^{13}C NMR spectrum.

3.5.2. Analogue Type 4.2 – methoxy, methyl substituted 1-phenyl-2-butanone component

This analogue was synthesised in order to investigate the effect of extra substituents on Ring C on the ER binding of the analogue.



Scheme 3.18 Synthesis of analogues Type 4

3.5.2.1. Synthesis of 1-(-2-nitro-but-1-enyl)-2-methoxybenzene (BRI021)[191]

The synthesis of 1-(-2-nitro-but-1-enyl)-2-methoxybenzene (BRI021) was achieved by refluxing 3-methyl-4-methoxybenzaldehyde, nitropropane, potassium fluoride and N,N-dimethyamine.HCl in toluene with a Dean-Stark condenser attached to the apparatus (Scheme 3.18). The condensation of aldehydes with nitropropane is achieved by the Henry reaction[192]. A yellow oil was recovered from the reaction in a 45% yield. The infrared spectrum confirmed the presence of a C=C bond by a signal at 1644cm^{-1}. The ^{1}H NMR spectrum identified an ethyl group by triplet and quartet signals at δ1.29 (J=7.52Hz) and δ2.89 (J=7.52Hz). The methyl group was observed as a singlet at δ2.27 and a methoxy group appeared as a singlet at δ3.90. The ^{13}C NMR spectrum supports the presence of the ethyl group by signals at 11.84ppm and 20.34ppm. The methyl group carbon is observed at 15.81ppm while the methoxy group appears at 55.0ppm.

3.5.2.2. Synthesis of 1-(3-methyl-4-methoxyphenyl)-butan-2-one (BRI175)[193]

The general method for the reduction of the nitrostyrene, 1-(2-nitrobutyl-1-enyl)-3-methyl-4-methoxybenzene (BRI021), to a ketone involved the addition of the nitrostyrene to a suspension of iron powder in glacial acetic acid at 100°C. The reaction was stirred for 2 hours and yielded the product as a colourless oil in a 78% yield.

A carbonyl signal was observed for BRI021 in the infrared spectrum at ν1714cm^{-1} indicating that the reaction was a success. The presence of a singlet at in the ^{1}H NMR spectrum at δ3.60 confirmed the existence of a newly formed CH_2 group due to the reduction. The ^{13}C NMR spectrum supported the presence of this CH_2 group with the signal at 48.50ppm. Finally the carbonyl at 209.16ppm identified the product as the required ketone.

3.5.2.3. Synthesis of 1-[2-(4-{2-(4-methoxy-3-methyl-phenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI180)

The McMurry coupling of 1-(3-methyl-4-methoxyphenyl)-butan-2-one (BRI175) and 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) was

performed using the method described in section 3.19 and brown oil was produced in an 85% yield, Scheme 3.18, in a Z:E 1:1 isomeric ratio.

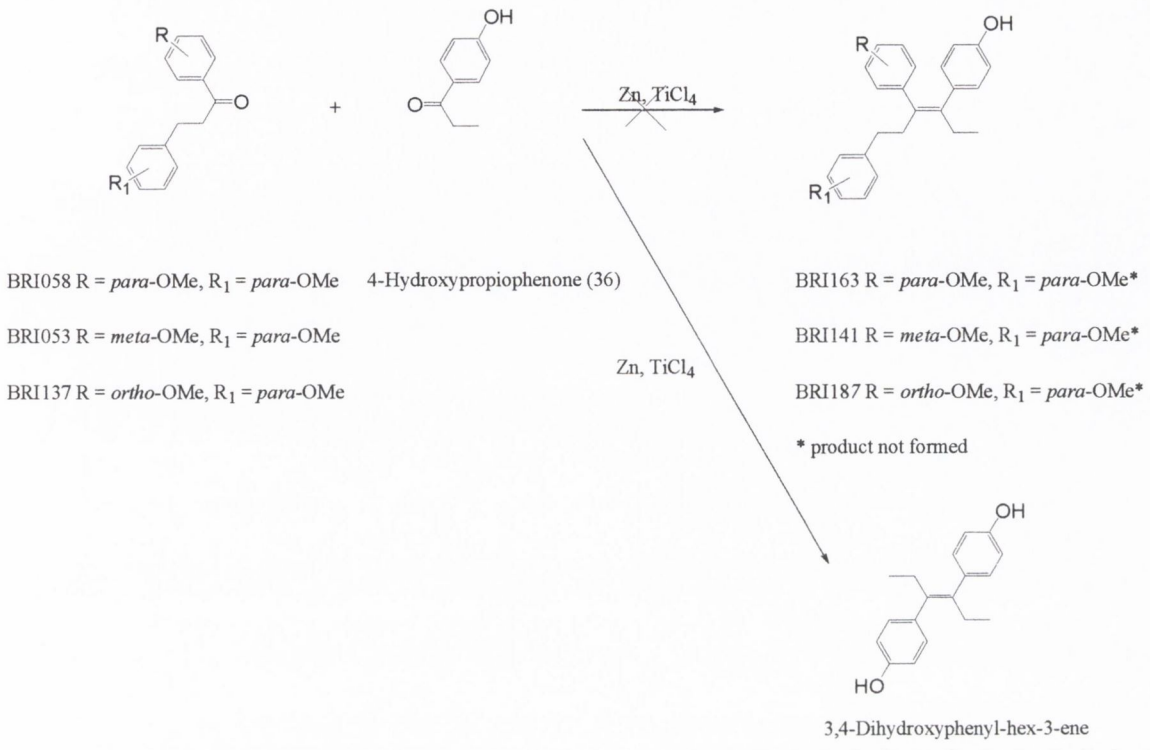
The infrared spectrum of BRI180 exhibited a signal at $\nu 1607 cm^{-1}$ that could be identified as a C=C double bond signal. In the ^{1}H NMR spectrum a triplet at δ0.86 (J=7.54Hz) that integrated for three protons and a quartet signal at δ2.02 (J=7.04Hz) that integrated for two protons were characteristic of these types of structures and verified the presence of an ethyl group. H-2'''', H-6'''' and H-3'''', H-5'''' were observed as singlet signals at δ1.85 and δ2.73. The benzylic CH_2 group was found as a singlet at δ3.21. The methyl substitutent appeared as a singlet at δ2.23 whereas the two methoxy groups appeared as singlets at δ3.80 and δ3.82. H-8 and H-9 protons were observed as multiplets at δ2.49 and δ2.55 while the remaining basic side chain protons appeared as triplet signals at δ2.92 (J=6.04Hz) and δ4.12 (J=6.04Hz). The ^{13}C NMR spectrum supported the presence of the ethyl group with signals such as 12.98ppm and 29.65ppm. The methyl group appeared at 15.90ppm while the methoxy groups were found more downfield at 54.25ppm and 54.28ppm. Side group carbons C-2'''', C-3'''' and C-1'''', C-4'''', C-6 and C-7 were observed as signals at 23.04ppm, 54.69ppm, 54.89ppm and 66.43ppm. The benzylic carbon was observed as a signal at 33.36ppm while the remaining CH_2 groups appeared at 36.63ppm and 45.17ppm. The aromatic carbons were all observed in the region 109.35ppm to 157.23.

3.6. Analogue series 5 – alternative positioned basic side chain analogues

3.6.1. Synthesis of analogue Type 5 - McMurry coupling of pyrrolidine basic side chain substituted propiophenone with 1,3-diphenylpropan-1-ones

Type 5 analogues were synthesised to produce compounds where the pyrrolidine side chain was on Ring C instead of the usual Ring B. It was hoped that the placement of the pyrrolidine side chain on an alternative Ring would increase affinity of the analogues for the ER ligand binding domain.

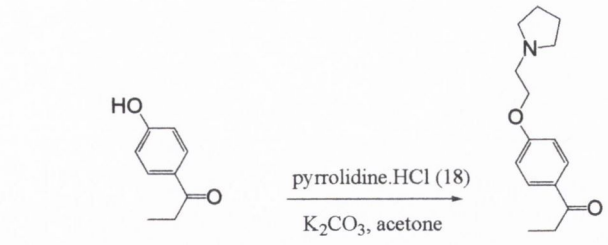
3.6.1.1. Initial attempt of coupling of hydroxy substituted propiophenone with dihydrochalcones



Scheme 3.19 Attempted coupling of 4-hydroxypropiophenone (36) with 1,3-diphenylpropan-1-ones

The synthesis of 4-[2-ethyl-5-(4-methoxyphenyl)-3-(3-methoxyphenyl)-pent-2-enyl]-phenol (BRI187) was attempted by the performing of the McMurry coupling on 3-(4-methoxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI137) (Scheme 3.19) and 4-hydroxypropiophenone (36). Column chromatography was performed many times to try and separate the mixture of products. It was discovered that only self-coupled 4-hydroxypropiophenone (36) was produced by the reaction. A similar result was obtained for the attempted couplings between BRI053 and BRI137 and 4-hydroxypropiophenone. Because of the difficulty in using phenolic ketones it was necessary to protect the hydroxy group in some way before attempting the coupling reaction. Addition of a pyrrolidine side chain was performed on 4-hydroxypropiophenone, as seen in Scheme 3.20 below, and the coupling of this with the various dimethoxy substituted diphenypropan-1-ones was then attempted.

3.6.1.2. Synthesis of 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (BRI174)

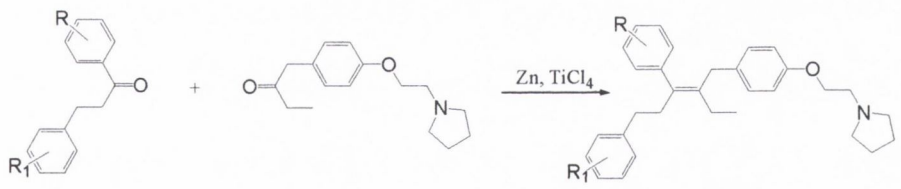


4-Hydroxypropiophenone (36) BRI-174

Scheme 3.20 Basic side chain addition to 4-hydroxypropiophenone

Basic side chain addition[119] of 1-(2-chloroethyl)pyrrolidine.HCl was performed on 4-hydroxypropiophenone (36) using the method described in section 3.2.6.5 (Scheme 3.20). The resulting product was light yellow oil (92%). The infrared spectrum of BRI174 showed the absence of the broad hydroxy band due to the basic side chain addition. The ^{1}H NMR spectrum a triplet signal was observed at δ0.77 (J=7.52Hz) and a quartet observed at δ2.22 (J=7.52Hz). H-2'''', H-6'''' and H-3'''', H-5'''' were represented by signals at δ1.71 and δ2.58. The remaining basic side chain protons appear as triplets at δ2.83 (J=4.5Hz) and δ4.03 (J=4.5Hz). The aromatic protons were observed in the region δ6.77 to δ6.93. In the ^{13}C NMR spectrum a carbonyl signal at 266.13ppm confirms the ketone structure.

3.6.2. McMurry coupling of 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (BRI174) and selected methoxy substituted ketones



BRI058 R = *para*-OMe, R_1 = *para*-OMe BRI174

BRI053 R = *meta*-OMe, R_1 = *para*-OMe

BRI137 R = *ortho*-OMe, R_1 = *para*-OMe

BRI169 R = *para*-OMe, R_1 = *para*-OMe

BRI144 R = *meta*-OMe, R_1 = *para*-OMe

BRI138 R = *ortho*-OMe, R_1 = *para*-OMe

Scheme 3.21 McMurry coupling of 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (BRI174) with 1,3-diphenylpropan-1-ones

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI138	**30**	**1608 (C=C)**	**4:1**
BRI144	**42**	**1607 (C=C)**	**15:1**
BRI169	**57**	**1606 (C=C)**	**10:1**

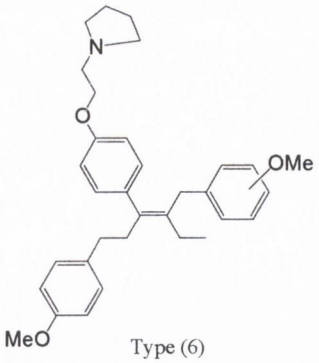
Table 3.14: Yield and infrared data for compounds BRI138, BRI144 and BRI169

The McMurry coupling to produce each of the compounds BRI138, BRI144 and BRI169 listed in Table 3.14 was performed following the method in section 3.3.1. One of the reactions involved the coupling of 3-(4-methoxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI137) and 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (BRI174) (Scheme 3.21) in order to produce 1-(2-{4-[2-ethyl-5-(4-methoxyphenyl)-3-(2-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI138). The product was recovered as yellow oil. Spectroscopic data was used to identify the product as discussed below.

Table 3.14 displays infrared data that verifies the success of the coupling reaction by the presence of C=C signals. When examining the ^{1}H NMR spectrum of 1-(2-{4-[2-ethyl-5-(4-methoxyphenyl)-3-(2-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI138) it can be seen that many signals present are characteristic of these compounds such as the ethyl group signals δ0.91 (J=7.28Hz, CH_3) and δ2.42 (CH_2). There are two methoxy groups and they appear as singlets at δ3.70 and δ3.79 each integrating for three protons. The ^{13}C NMR spectrum confirms the presence of these two methoxy groups by signals at 54.11ppm and 54.16ppm. The existence of these two methoxy groups plus the presence of basic side chain methylenes and a C=C double bond confirms the identity of the product as 1-(2-{4-[2-ethyl-5-(4-methoxyphenyl)-3-(2-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI138).

The synthesis of BRI144 and of BRI169 (Scheme 3.21) were performed in the same manner as for BRI138.

3.7. The McMurry coupling of dihydrochalcones with 1-phenyl-2-butanones

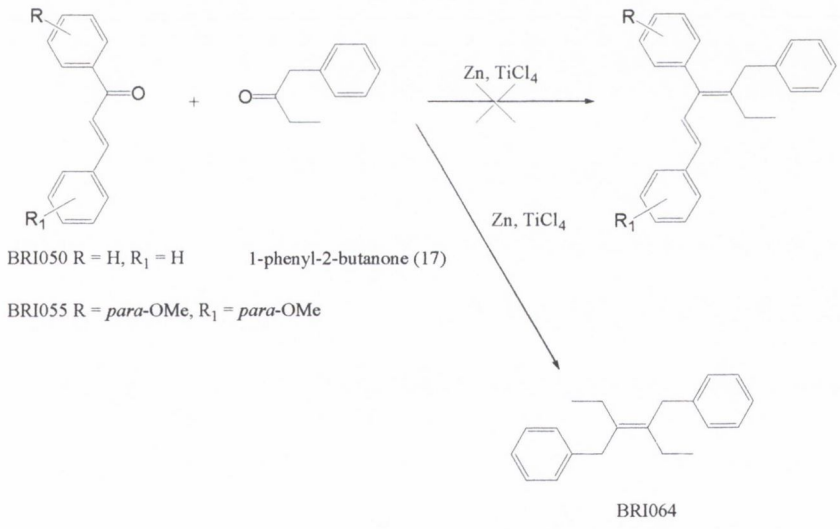


Type (6)

Figure 3.7 Dimethoxy substituted analogues

Type 6 analogues were synthesised to produce compounds with methoxy substitutents on Rings B and C and a pyrrolidine side chain on Ring A.

3.7.1. McMurry coupling reaction



Scheme 3.22 McMurry coupling of substituted 1,3-diphenylpropenones and 1-phenyl-2-butanone [3]

Initial reductive couplings of unsubstituted chalcones and ketones with 1-phenyl-2-butanone [3] were attempted as shown in Scheme 3.22 above in order to confirm that these compounds would couple by the McMurry coupling reaction and to elucidate the correct conditions for the reaction. The coupling reactions of 1,3-diphenylpropenone (BRI050) and

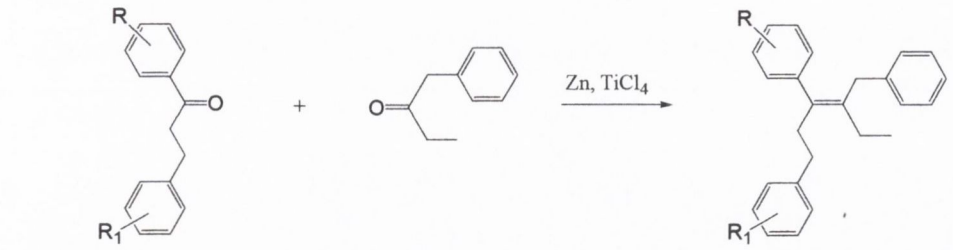
1,3-diphenylpropan-1-one (BRI051) each with 1-phenyl-2-butanone [3] were first performed simultaneously in a reaction carousel. Only self-coupled 1-phenyl-2-butanone (BRI064) was recovered from both reactions. The coupling of the unsubstituted ketone, 1,3-diphenylpropan-1-one (BRI051) with 1-phenyl-2-butanone [3], was repeated in a two-neck round bottom flask with increased equivalents of $TiCl_4$ and Zn. This coupling was successful producing 3,4,6-triphenyl-hex-3-enyl (BRI063) as seen in Scheme 3.23 below.

The ^1H NMR spectrum of 3,4,6-triphenyl-hex-3-enyl (BRI063) contained the signals characteristic of these types of structures; a triplet at δ1.14 (J=7.18Hz) and a quartet at δ2.23 (J=7.8Hz) that represent the ethyl group protons. Two triplets appear at δ2.76 (J=4.12Hz) and δ2.91 (J=4.12Hz) each integrating for two protons therefore representing the two CH_2 groups connecting one of the aromatic ring B to the C=C double bond. A singlet at δ3.72 that represents the CH_2 group that connects the other aromatic ring C to the C=C double bond. The remaining aromatic protons appear in the region δ7.16 to δ7.42 and integrate for fifteen protons. In the ^{13}C NMR spectrum signals are present at 13.10ppm and 24.20ppm that represent the C-6 and C-5 carbons. C-1 and C-2 appear as signals at 34.40ppm and 37.87ppm. The signal at 36.35ppm is identified as the C-7 CH_2 group. The aromatic carbons are represented by signals that occur in the region 125.46ppm to 140.71ppm.

Then a coupling of the unsubstituted chalcone 1,3-diphenylpropenone (BRI050) with 1-phenyl-2-butanone [3] was attempted in the same way but once again the self-coupled 1-phenyl-2-butanone (BRI064), 1,4-diphenyl-2,3-diethyl-but-2-ene (BRI064) was the only product recovered from the reaction. The synthesis 1-(4-methoxyphenyl)-1-(2-phenylvinyl)-2-phenyl-but-1-enyl (BRI065) was then attempted in the reaction carousel by the coupling of 1-(4-methoxyphenyl)-3-phenylpropenone (BRI055)[173] and 1-phenyl-2-butanone [3]. Once again only self-coupled 1-phenyl-2-butanone (BRI064) was produced.

The ^1H NMR spectrum of 1,4-diphenyl-2,3-diethyl-but-2-ene (BRI064) is very similar to that of (*E*)-3,4-diphenyl-3-hexene (BRI031) described in section 3.3.1. The main difference is the presence of an extra singlet at δ3.61 that integrates for four protons therefore representing the two CH_2 groups of the self-coupled 1-phenyl-2-butanone structure. The aromatic protons all appear in the region δ7.20 to δ7.37 and integrate for ten protons. The ^{13}C NMR spectrum is also very similar to that of BRI031 except for the two signals at

36.21ppm and 36.65ppm, which represent the two CH_2 groups of the self-coupled self-coupled 1-phenyl-2-butanone (BRI064).



BRI051 R = H, R_1 = H

BRI059 R = *para*-OMe, R_1 = H

BRI058 R = *para*-OMe, R_1 = *para*-OMe

1-phenyl-2-butanone (17)

BRI063 R = H, R_1 = H

BRI153 R = *para*-OMe, R_1 = H

BRI069 R = *para*-OMe, R_1 = *para*-OMe

Scheme 3.23 McMurry coupling of 1,3-diphenyl-propanones and 1-phenyl-2-butanone [3]

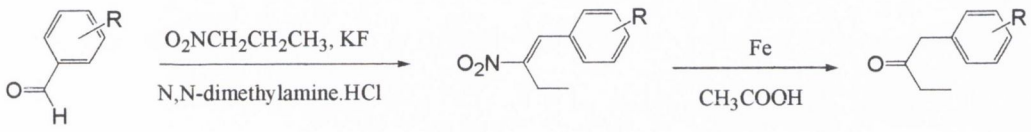
The synthesis of 3-phenyl-4-(4-methoxyphenyl)-6-phenyl-hex-3-enyl (BRI153) was not successful when attempted in the reaction carousel and after some investigation into these reactions it is clear that compounds such as 1-(4-methoxyphenyl)-3-phenyl-propan-1-one (BRI059)[178] and 1-phenyl-2-butanone [3] do not couple in this type of vessel and the self-coupled 1-phenyl-2-butanone (BRI064) is the only product produced. As a similar compound to 1-(4-methoxyphenyl)-3-phenyl-propan-1-one (BRI059), 1,3-bis-(4-methoxyphenyl)-propan-1-one (BRI058)[177] was reacted in a 250ml two-neck round bottom flask with 1-phenyl-2-butanone [3] in order to synthesise 3-phenyl-4,6-(4-methoxyphenyl)-hex-3-enyl (BRI069). The product was obtained as light yellow oil in a 50% yield (isomeric ratio of Z:E 1:1) proving that surface area on the catalyst for reaction of the reagents is a limiting factor for this reaction.

The ^1H NMR spectrum for 3-phenyl-4,6-(4-methoxyphenyl)-hex-3-enyl (BRI069) two methoxy singlets at δ3.86 and δ3.90 are observed each integrating for three protons. An additional singlet at δ3.37 that integrates for two protons represents the CH_2 group of the coupled structure. The ^{13}C NMR spectrum also bore many similarities with the spectrum of BRI076 except for one signal at 33.26ppm that indicated the presence of an extra CH_2 group.

3.8. Analogue series 6 – Methoxy-substituted coupled compounds

In this section analogues of the structure shown in Figure 3.7 derived from methoxy substituted 1-phenyl-2-butanones will be synthesised. This protocol required an initial synthesis of methoxy-substituted ketones and their subsequent coupling to 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) the synthesis of which had been discussed in section 3.2.6.3. The final step in this synthesis is the basic side group addition to the coupled compounds.

3.8.1. Methoxy-substituted ketone synthesis



R = *meta*-OMe
R = *para*-OMe
R = *ortho*-OMe

BRI005 R = *meta*-OMe
BRI001 R = *para*-OMe
BRI007 R = *ortho*-OMe

BRI123 R = *para*-OMe
BRI124 R = *meta*-OMe
BRI117 R = *ortho*-OMe

Scheme 3.24 Methoxy-substituted ketone synthesis

3.8.1.1. Nitrostyrene Synthesis – General method[191, 194]

The appropriate benzaldehyde [5], nitropropane [3], potassium fluoride and N,N-dimethyamine.HCl were all reacted to afford the products BRI125, BRI124 and BRI117. Table 3.15 below presents the relevant yields and signals in the infrared spectra of each of the nitrostyrenes synthesised (Scheme 3.24) that are necessary for confirmation of the synthesis of the compounds.

Compound	Yield %	IRυ_{max} (film cm^{-1})	^{13}C NMR ppm ($CDCl_3$)
BRI001[195]	**84**	**1648 (C=C)**	**151.30 (C-NO_2)**
BRI005[196]	**56**	**1656 (C=C)**	**151.30 (C-NO_2)**
BRI007[197]	**20**	**1652 (C=C)**	**152.30 (C-NO_2)**

Table 3.15: Yield and infrared data for compounds BRI001, BRI005 and BRI007

The infrared spectrum for 1-(-2-nitro-but-1-enyl)-2-methoxybenzene (BRI007)[197], as for each of the other two nitrostyrenes, contains a signal at v1652cm^{-1} the represents the C=C double bond. In the ^{1}H NMR spectrum a triplet and a quartet are present at δ1.27 and δ2.82 the first integrating for three therefore representing the methyl group and the latter integrating for two the CH_2 group protons. The methoxy group is observed as a singlet at δ3.90 that integrates for three protons and the remaining protons, which are aromatic, appear in the region δ6.95 to δ8.20. The ^{13}C NMR spectrum also helps in the identification of each of the nitrostyrenes with the carbon, bearing the nitro group, appearing as a signal around 152.30ppm as seen in Table 3.15 above.

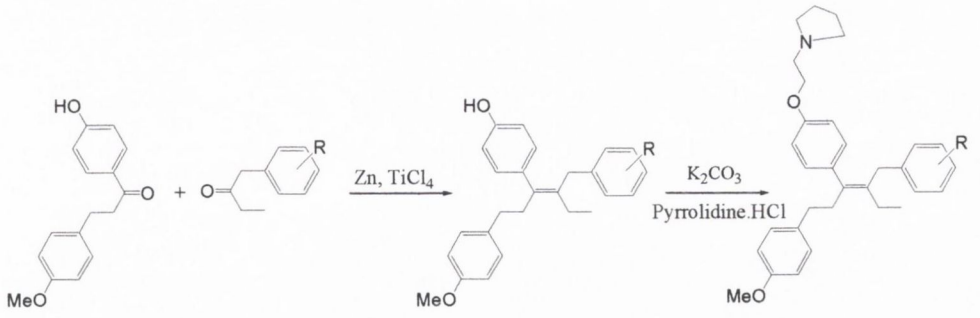
3.8.1.2. Reduction of nitrostyrenes to ketones[193]

Compound	Yield %	IRυ_{max} (film cm^{-1})
BRI123[198]	**88**	**1714 (C=O)**
BRI124[199]	**100**	**1712 (C=O)**
BRI117[200]	**100**	**1713 (C=O)**

Table 3.16: Yield and infrared data for compounds BRI117, BRI123 and BRI124

The reduction of 1-(-2-nitro-but-1-enyl)-2-methoxybenzene (BRI007) to 1-(2-methoxyphenyl)-butan-2-one (BRI117)[200] (Scheme 3.24) was achieved by treatment with iron powder and glacial acetic acid. Table 3.16 above lists the yields for each of the reactions and also the carbonyl signal from the infrared spectra of the ketone products. In the ^{1}H NMR spectra the usual triplet and quartet signals at δ1.04 and δ2.44 are present and represent the ethyl group. A new CH_2 group is present in the structure due to the reduction reaction and appears as a singlet at δ3.68 that integrates for two protons. In the ^{13}C NMR spectrum a new signal is also present that represents the new CH_2 group at δ43.90pm.

3.8.2. Synthesis of analogue Type 6 via coupling of dihydrochalcones with substituted ketones



BRI072

BRI123 R = *para*-OMe
BRI124 R = *meta*-OMe
BRI117 R = *ortho*-OMe

BRI132 R = *para*-OMe
BRI131 R = *meta*-OMe
BRI119 R = *ortho*-OMe

BRI135 R = *para*-OMe
BRI134 R = *meta*-OMe
BRI133 R = *ortho*-OMe

Scheme 3.25 Synthetic route for methoxy substituted analogues

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI119	**49**	**1610 (C=C)**	**15:1**
BRI131	**80**	**1609 (C=C)**	**20:1**
BRI132	**95**	**1610 (C=C)**	**3:1**

Table 3.17: Yield and infrared data for compounds BRI119, BRI131 and BRI132

The synthesis of 4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI119), 4-{2-(3-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI131) and 4-{2-(4-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI132) were performed according to the method in Scheme 3.25 above, the yields of which are found in Table 3.17. The compounds were obtained as oils in a light brown colour. The presence of a C=C bond in the infrared spectra of each of the compounds (Table 3.17) verifies the coupling of the ketones.

The infrared spectrum for 4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI119) contains a hydroxy signal at $\nu 3396 cm^{-1}$, which is necessary for basic side group addition in the next step of the reaction. In the ^1H NMR spectrum signals are observed that are characteristic of each of these types of structures such as $\delta 0.95$ (CH_3),

δ2.0 (CH_2), δ2.55 (CH_2), δ2.70 (CH_2), δ3.24 (benzylic CH_2), δ3.76 (OCH_3) and δ3.82 (OCH_3). The aromatic protons occur in the region δ6.74 to δ7.10. The ^{13}C NMR spectrum of 4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI119) has characteristic signals at 13.05ppm (CH_3), 23.81ppm (CH_2), 31.10ppm (benzylic CH_2), 33.32ppm (CH_2), 35.63ppm (CH_2) 54.70ppm (OCH_3) and 54.80ppm (OCH_3) all of which are discussed in section 3.3.2. The aromatic carbons appear as usual in the region 109.30ppm to 157.25ppm.

3.8.3. Addition of a basic side chain to coupled methoxy substituted ketones

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI133	**34**	**1609 (C=C)**	**1:1**
BRI134	**43**	**1606 (C=C)**	**1:1**
BRI135	**22**	**1608 (C=C)**	**1:1**

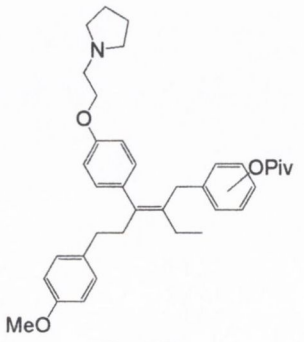
Table 3.18: Yield and infrared data for compounds BRI133, BRI134 and BRI135

The pyrrolidine side group addition[119] (Scheme 3.25) to each of the compounds listed in Table 3.17 was performed using the method described in section 3.2.6.5. The synthesis of the analogues BRI133, BRI134 and BRI135 was successful and the products were obtained as oils light brown in colour the yields of which are displayed in Table 3.18 above. Identification of the compounds was initially performed by infrared spectroscopy where the disappearance of the broad hydroxy band (Table 3.17) present in the starting material indicated the presence of a basic side chains.

Further identification was carried out by examination of the ^1H NMR spectra of the products. The ^1H NMR spectrum of 1-[2-(4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI133) new signals, that were not present in the starting material, were identified as the protons of the pyrroidine side group. H-3'''', H-5'''' and H-2'''', H-6'''' protons appeared as singlets at δ1.87 and δ2.72 each integrating for four protons. Signals at δ3.02 and δ4.20 represent the remaining basic side chain protons. The ^{13}C NMR spectrum also presents some new signals that are representative of the basic side chain. C-2'''', C-3'''' and C-1'''', C-4'''' carbons

appear as signals at 22.98ppm and 53.73ppm. The remaining carbons of the basic side group are found as signals at 54.16ppm and 65.95ppm. High resolution mass spectrometry for this compound affords the molecular ion M+1 500.3165 ($C_{33}H_{41+1}NO_3$) in 100% abundance (calculated for M+1 = 499.3102).

3.9. Analogue series 7 – Pivaloyl-substituted coupled compounds



Type (7)

Figure 3.8 Pivaloyl-substituted analogues

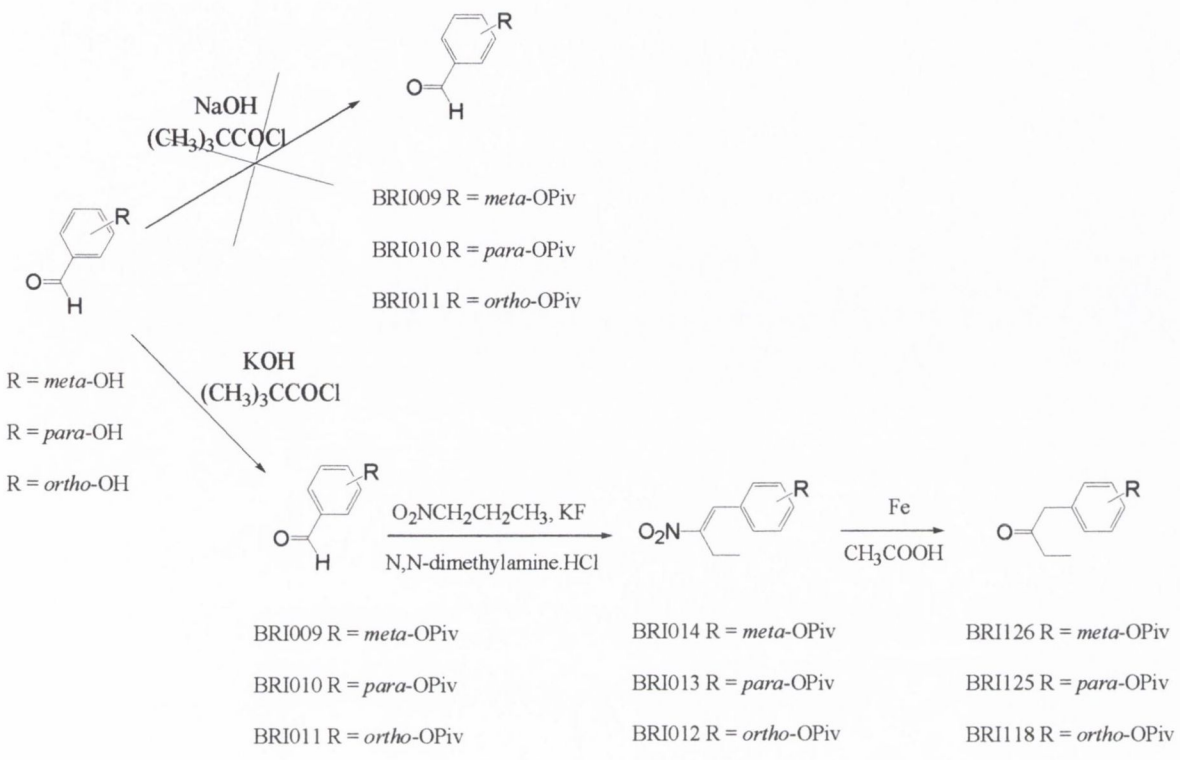
This section covers the synthesis of pivaloyl and methoxy substituted analogues (Figure 3.8). Pivaloyl substituted products[204] were of interest as they are regarded as prodrug esters[141] of parent phenolic compounds which would be slowly hydrolysed *in-vivo* by plasma esterases or chemically. It was necessary to first synthesise the pivaloyl protected ketones and proceed with the coupling of these to either 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) or 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) and then final basic side chain addition step to complete the synthesis.

3.9.1. Ketone formation

Compound	Yield %	IRυ_{max} (film cm^{-1}) Aldehyde carbonyl	IRυ_{max} (film cm^{-1}) Pivaloyl carbonyl
BRI009[201]	**10**	**1715 (C=O)**	**1735 (C=O)**
BRI010[202]	**28**	**1716 (C=O)**	**1740 (C=O)**
BRI011[203]	**17**	**1718 (C=O)**	**1743 (C=O)**

Table 3.19: Yield and infrared data for compounds BRI009, BRI010 and BRI011

3.9.1.1. Synthesis of *ortho*, *meta* and *para*-pivaloyl protected benzaldehydes



Scheme 3.26 Pivaloyl substituted aldehyde synthesis

The general method for pivaloyl protection of hydroxy groups involves the use of pyridine, as discussed in chapter two, or sodium hydride and dimethylformamide (DMF). Both of these methods produce a product that requires much purification, as pyridine and DMF are difficult to remove. A new simpler and cleaner method for pivaloylation was developed in chapter 2 and was used in this section for the pivaloylation of aldehydes. The synthesis of *meta*-pivaloyloxybenzaldehyde (BRI009)[201], *para*-pivaloyloxybenzaldehyde (BRI010)[202] and *ortho*-pivaloyloxybenzaldehyde (BRI011)[203] (Scheme 3.26) were first attempted by stirring 3-hydroxybenzaldehyde [16], 4-hydroxybenzaldehyde [15] and 2-hydroxybenzaldehyde [17] separately with sodium hydroxide in acetone to produce anions. Trimethylacetyl chloride was then added to each solution and left stirring overnight. The reactions produced the products in very low yields; *meta*-pivaloyloxybenzaldehyde (BRI009) (6%), *para*-pivaloyloxybenzaldehyde (BRI010)[202] (5%) and *ortho*-pivaloyloxybenzaldehyde (BRI011) (7.3%). The reaction was repeated for *meta*-pivaloyloxybenzaldehyde (BRI009) this time using potassium hydroxide and stirring in acetone for 1 hour with 3-hydroxybenzaldehyde [16] then treated with trimethylacetyl

chloride. The yield was still very low (4.5%). The reaction was attempted again but this time 3-hydroxybenzaldehyde [16] was stirred with potassium hydroxide (0.01M) in acetone for 3 hours and then the trimethylacetyl chloride was added. The reaction was left stirring overnight. The product was yellow oil produced in a 10% yield. Synthesis of *para*-pivaloyloxybenzaldehyde (BRI010) and *ortho*-pivaloyloxybenzaldehyde (BRI011) were repeated using this same method and both yields increased to 28% and 17%. It can be assumed from the success of the reaction due to the change in reaction conditions that the base is a very important factor as it determines the extent of anion formation, which then leads to pivaloylation with trimethylacetyl chloride. In this case it appears that potassium hydroxide is a more efficient at removing hydrogen atoms from hydroxy groups and therefore ion formation than sodium hydroxide.

As seen in Table 3.19 above there are two carbonyl signals for each compound BRI009, BRI010 and BRI011 in the infrared spectra indicating the existence of an aldehyde structure plus the presence of a pivaloyl-protecting group ester. The ^{1}H NMR spectrum for *meta*-pivaloyloxybenzaldehyde (BRI009) and each of the other compounds confirms the presence of a pivaloyl group by a singlet at δ1.40 that integrates for nine protons. The ^{13}C NMR spectrum of BRI009 also has signals that represent the pivaloyl-protecting group. Signals at 26.61ppm, 38.69ppm and also a carbonyl signal at 176.30ppm all verify the presence of a pivaloyl group.

3.9.1.2. Nitrostyrene synthesis from pivaloyl protected benzaldehydes[191, 194]

Compound	Yield %	IRυ_{max} (film cm^{-1})	IRυ_{max} (film cm^{-1}) Pivaloyl carbonyl	^{13}C NMR ppm ($CDCl_3$)
BRI014	**33**	**1652 (C=C)**	**1726 (C=O)**	**150.90 (C-NO_2)**
BRI013	**52**	**1651 (C=C)**	**1732 (C=O)**	**151.87 (C-NO_2)**
BRI012	**76**	**1646 (C=C)**	**1729 (C=O)**	**153.64 (C-NO_2)**

Table 3.20: Yield and infrared data for compounds BRI014, BRI013 and BRI012

The synthesis of 1-(-2-nitro-but-1-enyl)-2-pivaloyloxy-benzene (BRI012), 1-(3-pivaloyloxyphenyl)-2-nitrobut-1-ene (BRI014) and 1-(2-Nitrobutyl-1-enyl)-4-pivaloyloxy-

benzene (BRI013) (Scheme 3.26) were attempted using the reaction carousel by placing each of the starting materials; 2-pivaloyloxybenzaldehyde (BRI011), 3-pivaloyloxybenzaldehyde (BRI009) and 4-pivaloyloxy-benzaldehyde (BRI010) in separate reaction vessels with nitropropane, potassium fluoride and N,N-dimethyamine.HCl in toluene and leaving to reflux for one week. The reactions for 1-(-2-nitro-but-1-enyl)-2-pivaloyloxy-benzene (BRI012), 1-(3-pivaloyloxyphenyl)-2-nitrobut-1-ene (BRI014) were unsuccessful but a low yield of 1-(2-nitrobutyl-1-enyl)-4-pivaloyloxy-benzene (BRI013) was obtained. The synthesis of each of the compounds was attempted again using the same reagents but this time reacting them in a round-bottomed flask with a Dean-Stark condenser attached. The reactions were refluxed at 115°C for 36 hours. The reactions were all successful with good yields as seen in Table 3.20.

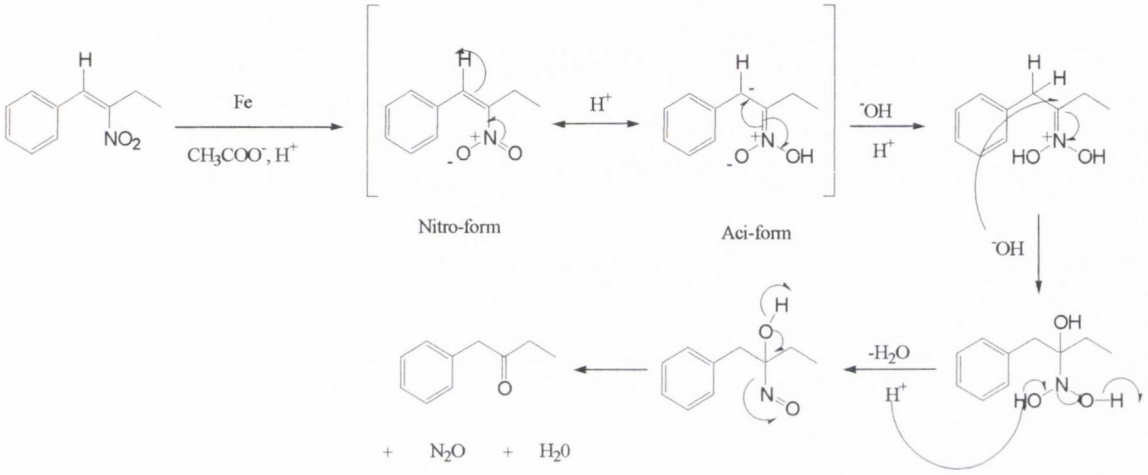
On examination of the infrared spectrum of each of the compounds the presence of a pivaloyl ester group is obvious with the presence of a carbonyl signal. Table 3.20 gives details of the infrared spectra results. Also it is clear that the aldehyde carbonyl signal has disappeared due to the reaction indicating the success of the nitrostyrene synthesis. Some new signals are present in the ^{1}H NMR spectrum of 1-(-2-nitro-but-1-enyl)-2-pivaloyloxy-benzene (BRI012) e.g. triplet at δ1.28 that integrates for three protons and a quartet at δ2.86 that integrates for two protons both of which combined represent the ethyl group of the product. The ^{13}C NMR spectrum supports the presence of this new group by signals at 21.96ppm and 32.61ppm. As shown in Table 3.20 above the presence of a nitro group is confirmed by a signal at 153.64ppm.

3.9.1.3. Reduction of nitrostyrenes to ketones BRI126, BRI125 and BRI118[193]

Compound	Yield %	IRυ_{max} (film cm^{-1}) Aldehyde carbonyl	IRυ_{max} (film cm^{-1}) Pivaloyl ester carbonyl
BRI126	**100**	**1715 (C=O)**	**1735 (C=O)**
BRI125	**100**	**1716 (C=O)**	**1740 (C=O)**
BRI118	**100**	**1718 (C=O)**	**1743 (C=O)**

Table 3.21: Yield and infrared data for compounds BRI126, BRI125 and BRI118

The final step in the synthesis of the required ketones is the reduction of the appropriate nitrostyrenes. 2,2-Dimethylpropionic acid 2-(2'-oxo-butyl)-phenyl ester (BRI118), 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (BRI125) and 2,2-dimethylpropionic acid 3-(2'-oxo-butyl)-phenyl ester (BRI126) (Scheme 3.26) were synthesised by slow addition of 1-(-2-nitro-but-1-enyl)-2-pivaloyloxy-benzene (BRI012), 1-(2-nitrobutyl-1-enyl)-4-pivaloyloxy-benzene (BRI013) and 1-(3-pivaloyloxyphenyl)-2-nitrobut-1-ene (BRI014) each to heated suspensions of iron powder in glacial acetic acid and continued heating at 100°C for 2 hours. These reactions were very successful producing excellent yields as listed in Table 3.21.



Scheme 3.27 Mechanism for reduction of nitrostyrene to ketone

Nitrostyrene compounds resonate between the Nitro-form and Aci-form[205] as seen in Scheme 3.27 above. The presence of glacial acetic acid donates protons to the nitrostyrene resonating compound stabilising the hydroxy substitutents. Reduction[192] of this structure leads to removal of the nitro and hydroxy groups and produces the ketone. Comparison of Table 3.21 and Table 3.20 infrared spectra results leads to the observation that a new carbonyl has appeared in the product structures due to the reduction reaction performed on the nitrostyrenes. In the ^{1}H NMR spectrum of 2,2-dimethylpropionic acid 2-(2'-oxo-butyl)-phenyl ester (BRI118), as with the spectra of 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (BRI125) and 2,2-dimethylpropionic acid 3-(2'-oxo-butyl)-phenyl ester (BRI126), the presence of a new singlet at δ3.58 is observed which integrates for two protons therefore representing the newly formed CH_2 group. In the ^{13}C NMR spectrum this

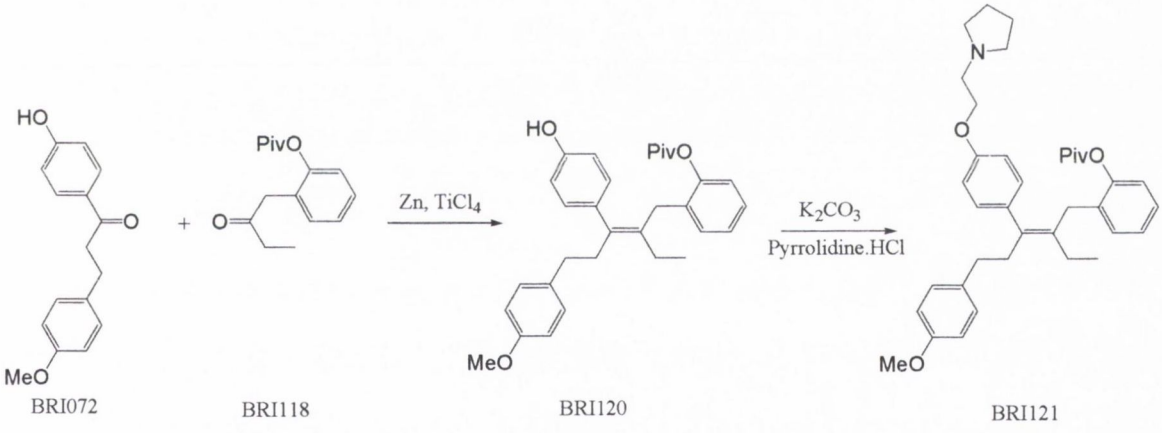
same CH_2 group is observed as a signal at 44.23ppm. This combined with the presence of a new carbonyl signal at 207.45ppm confirms the reduction of the nitrostyrene to a ketone.

3.9.2. Synthesis of pivaloyl substituted analogues

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI121	**37**	**1611 (C=C)**	**1:1**
BRI148	**32**	**1607 (C=C)**	**1.5:1**
BRI155	**10**	**1609 (C=C)**	**1:1**

Table 3.22: Yield and infrared data for compounds BRI121 and BRI148

3.9.2.1. Synthesis of 2,2-dimethylpropionic acid 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI121)



Scheme 3.28 Pivaloyl substituted analogue synthesis

3.9.2.1.1. Synthesis of 2,2-dimethylpropionic acid 2-[2-ethyl-3-(4-hydroxyphenyl)-5-(4-methoxyphenyl)-pent-2-enyl]-phenyl ester (BRI120)

The coupling of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) and 2,2-dimethylpropionic acid 2-(2'-oxo-butyl)-phenyl ester (BRI118) (Scheme 3.28) was performed using the general method described in section 3.3.1. 2,2-Dimethylpropionic acid 2-[2-ethyl-3-(4-hydroxyphenyl)-5-(4-methoxyphenyl)-pent-2-enyl]-phenyl ester (BRI120)

was synthesised in a 44% yield in a 30:1 Z:E isomeric ratio. The Z isomer was assigned to the downfield methoxy and benzyl signals in the ^{1}H NMR discussed below therefore all downfield signals were assigned to the Z-isomer. The E-isomer, though almost not visible, was assigned to all the minor, upfield signals.

The infrared spectrum contains a broad signal at $\nu 3390 cm^{-1}$ that indicates the presence of a hydroxy group in the structure. A signal at $\nu 1731 cm^{-1}$, is also observed which represents the carbonyl of the pivaloyl ester protecting group. A signal at $\nu 1610 cm^{-1}$ confirms the formation of a C=C and therefore the success of the coupling reaction.

The ^{1}H NMR spectrum of the ester BRI120 can be compared to the ^{1}H NMR spectrum of the phenol BRI119 in section 3.8.2 as they both have the same general type of structures. All the expected signals for these structures are present in the ^{1}H NMR spectrum. The major difference is the presence of a large singlet at $\delta 1.38$ that integrates for nine protons indicating that it represents the protons of the pivaloyl-protecting group. The ^{13}C NMR spectrum supports the presence of this pivaloyl group by the signals at 26.15ppm and 33.15ppm and a carbonyl signal at 191.00ppm.

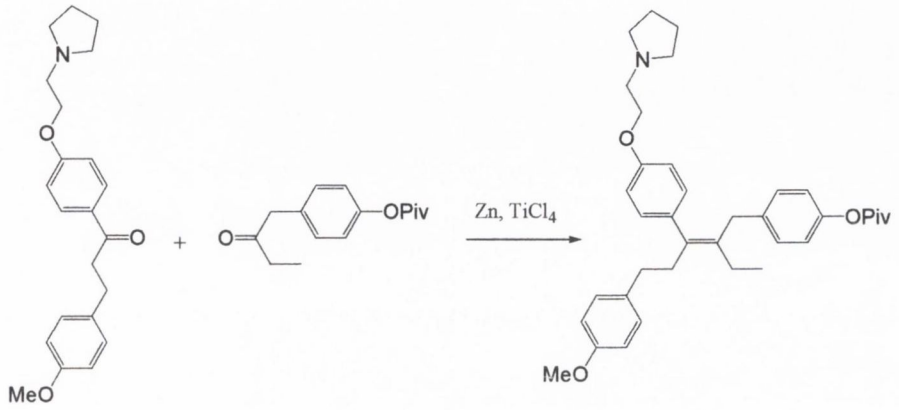
3.9.2.1.2. Addition of a basic side chain to the pivaloyloxy coupled compound

The basic side group addition[119] of pyrrolidine to the ester BRI120 was performed using the method described in section 3.2.6.5 (Scheme 3.28) to afford the product 2,2-dimethylpropionic acid 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI121).

The infrared spectrum of BRI121 no longer contains a broad hydroxy band, which may be due to the successful addition of a basic side chain. The pivaloyl carbonyl signal and the C=C double bond signals remain at $\nu 1747 cm^{-1}$ and $\nu 1611\ cm^{-1}$. The ^{1}H NMR spectrum of BRI121 is the main source of identification for the presence of a basic side chains with signals at $\delta 1.78$ and $\delta 2.72$ that represent the protons H-3'''', H-5'''' and H-2'''', H-6'''' of the pyrrolidine ring. The remaining basic side chain protons appear as signals at $\delta 2.95$ and $\delta 4.11$. The ^{13}C NMR spectrum contains the usual signals expected of this type of compound. The new signals at 29.25ppm and 54.07ppm represent the carbons of the pyrrolidine side chain ring C-2'''', C-3'''' and C-1'''', C-4''''. The remaining carbons of

the basic side chain C-8 and C-9 are found as signals at 54.15ppm and 65.58ppm. This clearly indicates that the addition of the basic side chain was successful.

3.9.2.2. Synthesis of 2,2-dimethylpropionic acid 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI148)



BRI145 BRI125 BRI148

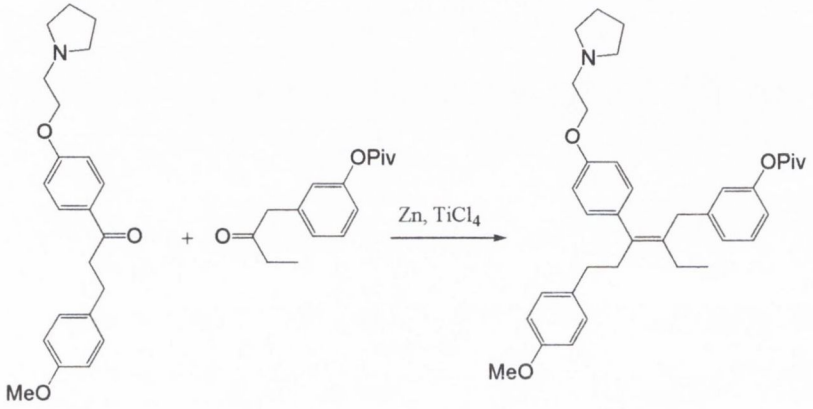
Scheme 3.29 Pivaloyl substituted analogue synthesis

The McMurry coupling of the alkylated dihydrochalcone 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) and 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (BRI125) (Scheme 3.29) was investigated using the reaction conditions from section 3.3.1 to afford the product (32%). Because the basic side chain addition had been performed previously on the hydroxy substituted ketone this coupling produced the final product in one step.

The infrared spectrum of BRI148 shows the existence of a carbonyl group and a C=C double bond by the signals at $\nu 1748 cm^{-1}$ and $\nu 1607 cm^{-1}$. The ^{1}H NMR spectrum of BRI148 contains all the general signals of this type of structure together with the pyrrolidine side chain signals. A slight difference from BRI121 is noted though in the positions of the basic side group signals. H-3'''', H-5'''' and H-2'''', H-6'''' of the pyrrolidine ring appear to be shifted slightly downfield appearing at δ2.05 and δ3.24 which must be due to the *para*-position of the pivaloyl ester protecting group as that is the only difference between the two compounds. The protons of the aliphatic chain of the basic side group also experience a downfield shift to δ3.34 and δ4.40. The ^{13}C NMR spectrum verifies the presence the

pivaloyl by the carbonyl signal at 207.6ppm. High resolution mass spectrometry for this compound affords the molecular ion M+1 570.3597 ($C_{37}H_{48}O_4$) in 100% abundance (calculated M+1 = 570.3583).

3.9.3. Synthesis of 2,2-dimethylpropionic acid 3-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI155)



BRI145 BRI126 BRI155

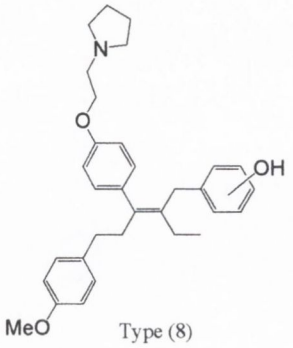
Scheme 3.30 Pivaloyl substituted analogue synthesis

The coupling of 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) (Scheme 3.30) and 2,2-dimethylpropionic acid 3-(2'-oxo-butyl)-phenyl ester (BRI126) was performed employing the method of McMurry coupling reaction from section 3.3.1 to afford the product (10%). Because the basic side chain addition had been performed previously on the hydroxy substituted ketone this coupling produced the final product in one step.

The infrared spectrum of BRI155 shows the existence of a carbonyl group and a C=C double bond by the signals at ν1755cm^{-1} and ν1609cm^{-1}. The ^{1}H NMR spectrum of BRI155 contains all the general signals of this type of structure together with the pyrrolidine side chain signals. H-3'''', H-5'''' and H-2'''', H-6'''' of the pyrrolidine ring appear to be shifted slightly downfield appearing at δ2.04 and δ2.85 which must be due to the *meta*-position of the pivaloyl ester protecting group. The protons of the aliphatic chain of the basic side group also experience a downfield shift to δ3.67 and δ4.24. The ^{13}C NMR spectrum verifies the presence the pivaloyl by the carbonyl signal at 176.69ppm.

3.10. Hydroxy substituted Analogues

3.10.1. Analogues Type 8



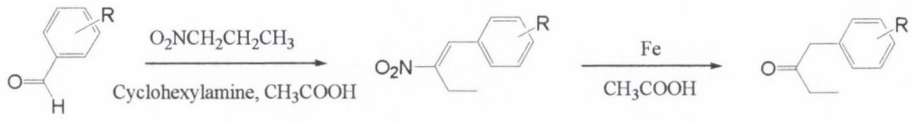
Type (8)

Figure 3.9 Analogues Type 8

As mentioned many times before it is a well-known fact that the introduction of a phenolic group into the structure of a good anti-estrogen generally increases the ER binding efficacy of the analogue. So in this section the synthesis of hydroxy substituted analogues (Figure 3.9) is pursued via couplings of hydroxy substituted ketones, deprotections and depivaloylations.

To synthesis hydroxy substituted analogues the simplest approach was to synthesise hydroxy substituted 1-phenyl-2-butanones and proceed with the coupling of these ketones to 1,3-diphenylpropan-1-ones.

3.10.1.1. Synthesis of hydroxy nitrostyrenes



R = H	BRI024 R = H	
R = *meta*-OH	BRI025 R = *meta*-OH	BRI129 R = *meta*-OH
R = *para*-OH	BRI026 R = *para*-OH	BRI128 R = *para*-OH
R = *ortho*-OH	BRI027 R = *ortho*-OH	BRI127 R = *ortho*-OH

Scheme 3.31 Hydroxy substituted ketone synthesis

Compound	Yield %	IRυ_{max} (film cm^{-1}) Aldehyde carbonyl	IRυ_{max} (film cm^{-1}) Hydroxy group	^{13}C NMR ppm ($CDCl_3$)
BRI024[206]	**40**	**1652 (C=C)**	**---------**	**152.90 (C-NO_2)**
BRI025[207]	**8.3**	**1645 (C=C)**	**3386 (OH)**	**150.70 (C-NO_2)**
BRI026	**11**	**1651 (C=C)**	**3394 (OH)**	**145.00 (C-NO_2)**
BRI027	**61**	**1655 (C=C)**	**3421 (OH)**	**148.70 (C-NO_2)**

Table 3.23: Yield and infrared data for compounds BRI024, BRI025, BRI026 and BRI027

A new preparation for nitrostyrenes was attempted for these hydroxy substituted compounds. Synthesis of 1-(2-nitrobutyl-1-enyl)-benzene (BRI024), 1-(-2-nitro-but-1-enyl)-4-hydroxy-benzene (BRI025)[207], 1-(3-hydroxyphenyl)-2-nitrobut-1-ene (BRI026) and 1-(-2-Nitro-but-1-enyl)-2-hydroxy-benzene (BRI027) was achieved by refluxing the corresponding hydroxybenzaldehydes as seen in Scheme 3.31 with nitropropane, cyclohexylamine and glacial acetic acid in a round bottom flask at 150°C for one day. This reaction produced yellow crystals in each case the yields of which are listed in Table 3.23 above.

On examination of the infrared spectra (Table 3.23) of each of the newly synthesised nitrostyrenes the presence a C=C double bond signal and the disappearance of the aldehyde carbonyl signal suggests the reaction was successful. The ^{1}H NMR spectrum of 1-(2-nitrobutyl-1-enyl)-benzene (BRI024)[206] presents a triplet at δ1.28 (J=7.54Hz) that integrates for three protons and a quartet at δ2.86 (J=7.28Hz) that integrates for two protons both of which combined represent an ethyl group. The aromatic protons appear in the region δ7.43 to δ8.04. Signals in the ^{13}C NMR spectrum at 11.98ppm and 20.25ppm also confirm the presence of an ethyl group. Table 3.23 lists the signals that represent the C-NO_2 groups in the ^{13}C NMR spectra of each of the compounds.

3.10.1.2. Reduction of nitrostyrenes to ketones[193]

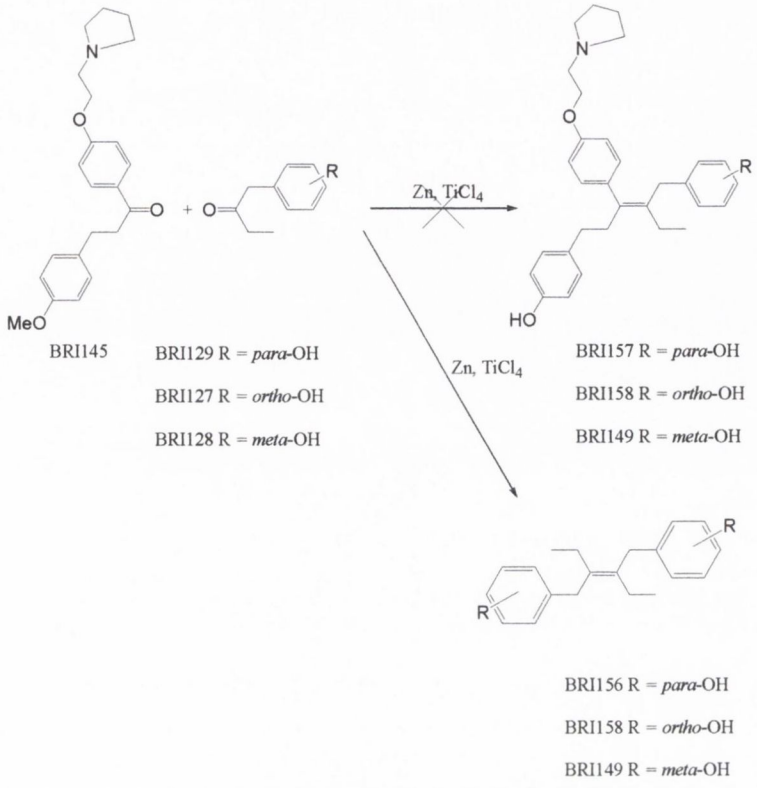
Compound	Yield %	IRυ_{max} (film cm^{-1}) Aldehyde carbonyl	IRυ_{max} (film cm^{-1}) Hydroxy group
BRI127[208]	**100**	**1720 (C=O)**	**3370 (OH)**
BRI128	**100**	**1703 (C=O)**	**3379 (OH)**
BRI129[209]	**100**	**1698 (C=O)**	**3386 (OH)**

Table 3.24: Yield and infrared data for compounds BRI127, BRI128 and BRI129

The reduction of the nitrostyrenes to ketones was performed according to the method described in section 3.9.1.3. Synthesis of 1'-(2-hydroxyphenyl)-butan-2'-one (BRI127)[208], 1'-(3-hydroxyphenyl)-butan-2'-one (BRI128) and 1'-(4-hydroxyphenyl)-butan-2'-one (BRI129)[209] (Scheme 3.31) was achieved by the addition of the starting nitrostyrenes; BRI025, BRI026 and BRI027 produced earlier to vessels containing iron powder suspended in glacial acetic acid at 100°C. These reactions produced the ketones successfully as colourless oils.

The synthesis of these ketones can initially be confirmed by the presence of carbonyl signals in the infrared spectrum as seen in Table 3.24 above. Comparison of the infrared spectra of the starting material in Table 3.23 and of the product in Table 3.24 its clear that the C=C double bond has disappeared and a new carbonyl group has appeared suggesting the reduction of the nitrostyrenes to ketones was successful. The ^{1}H NMR spectrum for 1'-(2-hydroxyphenyl)-butan-2'-one (BRI127) displays the usual characteristic ethyl group signals. A new signal present at δ3.66 that integrates for two protons represents a new CH_2 group formed from the reduction of the C=C double bond. The ^{13}C NMR spectrum also presents a new signal at 28.62ppm that verifies the presence of this new CH_2 group. The ketone carbonyl is confirmed by the presence of a carbonyl signal at 206.1ppm in the ^{13}C NMR spectrum.

3.10.2. Attempted McMurry coupling of hydroxy substituted ketones to 1,3-diphenylpropan-1-ones



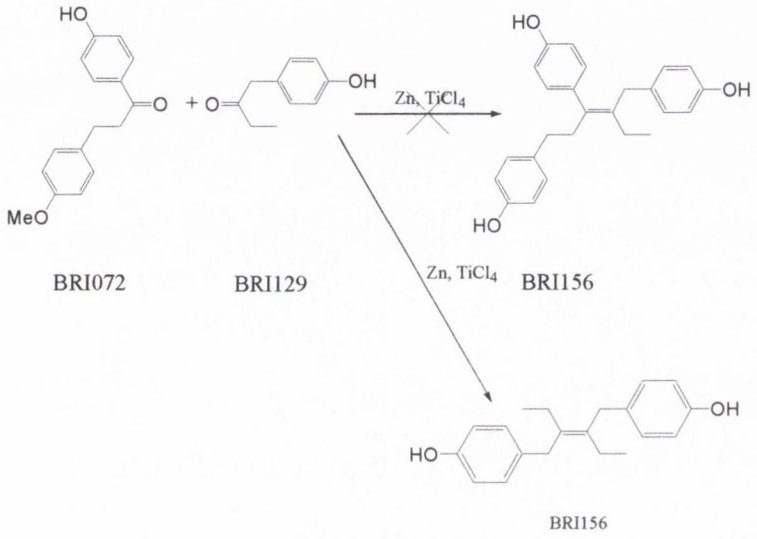
Scheme 3.32 Attempted synthesis of hydroxy substituted coupled compounds

3.10.2.1. Attempted synthesis of 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI157)

A McMurry coupling between 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) and 1'-(4-hydroxyphenyl)-butan-2'-one (BRI129) (Scheme 3.32) using the general coupling method described in section 3.3.2 was attempted. A very impure oil sample was recovered from the reaction, which after purification, only self-coupled hydroxy1-phenyl-2-butanone (BRI157) and starting material were identified as products of the reaction. The coupling was attempted between 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) and 1'-(2-hydroxyphenyl)-butan-2'-one (BRI127) and 1'-(3-hydroxyphenyl)-butan-2'-one (BRI128) in an attempt to synthesise 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI158) and 1-[2-(4-{2-(3-methoxy-benzyl)-1-[2-(4-hydroxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI149) and once again only the

self-coupled hydroxy-1-phenyl-2-butanones were recovered as the *cis*-isomers from the reactions.

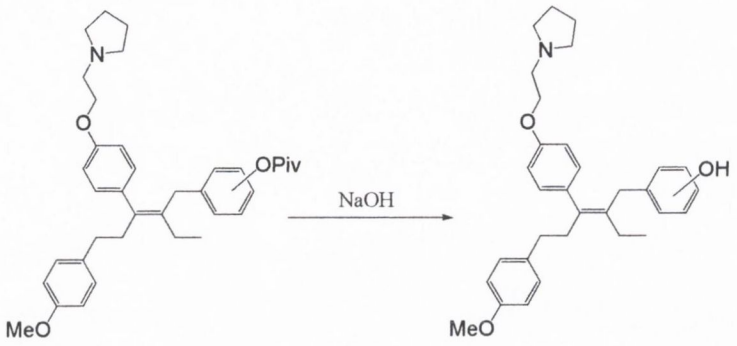
3.10.2.2. Attempted synthesis of 4-{2-(4-methoxy-benzyl)-1-[2-(4-hydroxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI156)



Scheme 3.33 Attempted synthesis of hydroxy substituted coupled compounds

Assuming that the polarity of the basic side chain on the 1,3-diphenylpropan-1-one may have been the problem with the previous reactions, an alternative coupling between 1'-(4-hydroxyphenyl)-butan-2'-one (BRI129) and 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (Scheme 3.33) was attempted. Unfortunately only self-coupled hydroxy1-phenyl-2-butanone (BRI157) and starting material were once again retrieved from the reaction. These reactions were abandoned and the synthesis of these hydroxy substituted analogues was pursued in other ways.

3.10.3. Synthesis of analogue Type 8



BRI148 *para*-OPiv

BRI121 *ortho*-OPiv

BRI157 *para*-OH

BRI158 *ortho*-OH

Scheme 3.34 Synthesis of hydroxy substituted coupled compounds

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI158	**33**	**1608 (C=C)**	**1:1**
BRI157	**21**	**1610 (C=C)**	**1.5:1**

Table 3.25: Yield and infrared data for compounds BRI158 and BRI157

After the failure of the methoxy deprotection reactions the removal of pivaloyl ester protecting groups was examined. The reagent required for the depivaloylations was sodium hydroxide and it was hoped that this would not be as destructive to the starting material as boron trifluoride-dimethyl sulphide.

3.10.3.1. Synthesis of 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI158)[139]

2,2-Dimethylpropionic acid 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI148) (Scheme 3.34) was depivaloylated using the method described above. The product BRI158 was obtained in 33% yield.

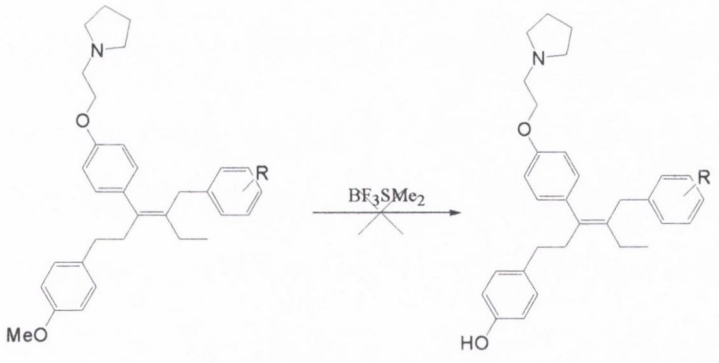
The infrared spectrum of the product was examined and compared with that of the starting material. It was observed that a broad hydroxy band was now present due to the reaction

suggesting the pivaloylation had occurred. Also the carbonyl signal of the pivaloyl group had dissappeared. Further analysis of the product structure was performed by examination of the ^{1}H NMR spectrum. All signals characteristic of the expected product were present. It was found that the pivaloyl singlet, present in the spectrum of the starting material, was absent. The ^{13}C NMR spectrum was also absent signals that represented the pivaloyl ester protecting group. This indicated that the desired product BRI158 had been synthesised.

3.10.3.2. Synthesis of 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI157)[139]

A depivaloylation was performed on 2,2-dimethylpropionic acid 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI121) (Scheme 3.34). The ^{1}H NMR spectroscopic examination of BRI157 revealed all necessary signals were present except for those representing the pivaloyl ester protecting group. The synthesis of BRI157 was therefore successful.

3.10.4. Attempted synthesis of hydroxy substituted analogues via deprotections



BRI133 R = *ortho*-OMe / BRI158 R = *ortho*-OH

BRI134 R = *meta*-OMe

BRI135 R = *para*-OMe / BRI157 R = *para*-OH

BRI164 R = *ortho*-OH

BRI152 R = *meta*-OH

BRI184 R = *para*-OH

Scheme 3.35 Attempted synthesis of hydroxy substituted coupled compounds

After the failure of the McMurry coupling of hydroxy 1-phenyl-2-butanones and 1,3-diphenylpropan-1-ones the only other logical means of obtaining the required hydroxy

analogues was via the deprotection of a hydroxy group. The decision was made to attempt to remove the methoxy group as this had been very successful in chapter two and could be performed on some of the methoxy substituted analogues already synthesised. Deprotections involving the removal of methoxy groups were discussed in detail in chapter two using various different reagents such as; pyridine, sodium ethanethiolate[125] and boron trifluoride-dimethyl sulphide. The deprotections that used pyridine and sodium ethanethiolate required excessive heat and therefore due to the fragility of the basic side chain these reagents were not used. Boron trifluoride-dimethyl sulphide reacted at room temperature and was assumed to be a suitable reagent to use on these types of structures as similar compounds were successfully deprotected in chapter two.

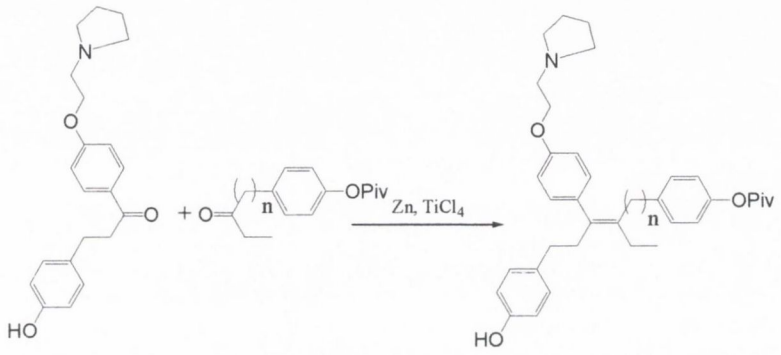
3.10.4.1. Attempted synthesis of BRI164, BRI152 and BRI184 via the McMurry coupling reaction

The deprotection of both 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI158) and 1-[2-(4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI133) were attempted in order to synthesise 2-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI164) (Scheme 3.35). Boron trifluoride-dimethyl sulphide was added dropwise over 30 min to BRI158 and BRI133 each in separate reacting vessels in dichloromethane and left stirring for 12 hours and not overnight as normal due to the fact that the reagent appears to degrade the compound as no starting material was recovered from any of the previous reactions. One examination of the ^{1}H NMR spectra of the fractions of the chromatographed deprotected BRI133 it appears that the methoxy groups are removed which is what was expected. Further analysis noted the loss of some aromatic protons despite the presence of all other relevant signals. This suggested the destruction of the starting material by the reagent. The chromatographic identification of the products produced by the deprotection of BRI158 produced spectroscopic data.

The deprotection of 1-[2-(4-{2-(3-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI134) and 1-[2-(4-{2-(4-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI135) were then attempted. When no product was recovered from these reactions a deprotection was attempted on 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-

pent-2-enyl}-phenol (BRI157) (Scheme 3.35) in the hope that the presence of only one methoxy group should allow for easier and faster deprotection and therefore less time for destruction of the starting material. No product or starting material was recovered from this reaction. The failure of these deprotections initiated another search to a means of synthesising these hydroxy substituted analogues.

3.10.5. Analogues Type 9



BRI177

BRI125 n = 1

BRI176 n = 0

BRI181 n = 1

BRI130 n = 0

Scheme 3.36 Synthesis of hydroxy substituted coupled compounds

The plan here was to obtain analogues with pivaloyl protected hydroxy groups then depivaloylate in order to obtain phenolic analogues in the hope that this would increase the affinity for the ER ligand binding domain.

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI130	**43**	**1606 (C=C)**	**Z only**
BRI181	**80**	**1607 (C=C)**	**1:1**

Table 3.26: Yield and infrared data for compounds BRI130 and BRI181

3.10.5.1. Synthesis of 2,2-dimethylpropionic acid 4-{1-ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl ester (BRI130)[139]

3-(4-Hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI177) and 4-pivaloyloxypropiophenone (BRI176) (Scheme 3.36) were coupled via the McMurry reaction described in section 3.3.1.

Identification was achieved by first observing the infrared spectrum. A broad band at $\nu 3290 cm^{-1}$ indicated the presence of a hydroxy group. The pivaloyl group was found as a carbonyl signal at $\nu 1747 cm^{-1}$. Proof that the actual coupling reaction had taken place was C=C double bond signal at $\nu 1606 cm^{-1}$. In the ^{1}H NMR spectrum a triplet found at δ0.88 integrating for three protons combined with the presence of a quartet at δ2.41 that integrated for two protons verified the existence of an ethylene group in the structure of the product. The pivaloyl ester protecting group appeared as a large singlet signal at δ1.32 integrating for nine protons. Pyrrolidine ring protons H-2'''', H-6'''' and H-3'''', H-5''''were observed as signals at δ1.88 and δ2.82. The remaining side chain proton signals were found at δ3.0 and δ4.10. The butylene proton signals were found as triplets at δ2.51 and δ2.75 each integrating for two protons. All the aromatic protons were found downfield in the region δ6.57 to δ6.98. The ^{13}C NMR spectrum supported the presence of these protons with confirmation of the presence of the corresponding carbons of these protons. A carbonyl signal at 191ppm verified the existence of the pivaloyl group.

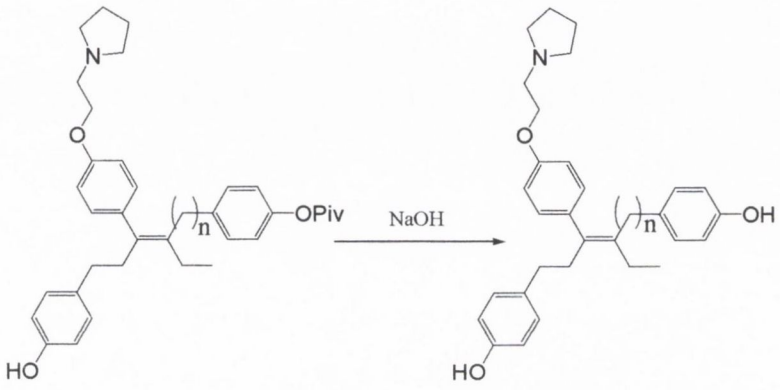
3.10.5.2. Synthesis of 2,2-dimethylpropionic acid 4-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI181)[139]

A McMurry coupling between 3-(4-hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI177) and 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (BRI125) (Scheme 3.36) was carried out according to the reaction in section 3.10.4.1 and the product obtained (80%).

Identification data of the product proved the structure to be very similar to that of BRI130 synthesised in section 3.10.4.1. The only difference between the two analogues was the

presence of an additional CH_2 group in the product ester (BRI181), which was confirmed by the signal δ3.24 in the ^{1}HNMR spectrum. The ^{13}C NMR spectrum also confirmed the presence of this extra CH_2 group by a signal at 29.25ppm.

3.10.6. Analogue Type 10



BRI181 n = 1

BRI130 n = 0

BRI184 n = 1

BRI185 n = 0

Scheme 3.37 Synthesis of hydroxy substituted coupled compounds

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric ratios Z:E
BRI184	**42**	**1609 (C=C)**	**3:1**
BRI185	**31**	**1609 (C=C)**	**3:1**

Table 3.27: Yield and infrared data for compounds BRI184 and BRI185

3.10.6.1. Synthesis of 4-{1-ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (BRI185)[139]

Depivaloylation was attempted on ester (BRI130) (Scheme 3.37) as outlined above. The oil produced was examined spectroscopically. The infrared spectrum showed the absence of the usual pivaloyl carbonyl signal and this fact combined with the absence of the pivaloyl signal in the ^{1}H NMR spectrum indicated the depivaloylation had taken place. The ^{13}C NMR spectrum also verified the deprotection by the absence of pivaloyl carbon signals, especially the ester carbonyl signal that generally occurs around 190.00ppm.

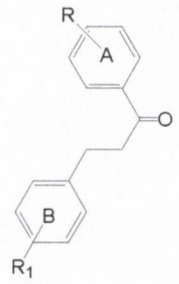
3.10.6.2. Synthesis of 4-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI184)[139]

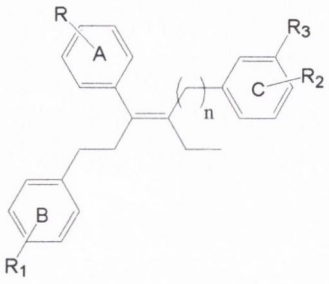
A deprotection was performed on 4-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI181) (Scheme 3.37) using the method descibed above. The oil produced was identified firstly by infrared spectroscopy. The absence of the carbonyl signal indicated the loss of a pivaloyl group. The ^{1}H NMR spectrum contained an extra CH_2 group when compared with 4-{1-ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (BRI185). As can be seen in Table 3.27 above both BRI184 and BRI185 have the same isomeric ratios for E and Z isomers. Comparing this result with Table 3.26 containing the isomeric ratios of the products BRI181 and BRI130 it can be concluded that the depivaloylation reaction caused an increase in the isomeric ratios of E and Z to occur.

3.11. Summary of analogue series 1-10

In conclusion, a new series of novel antiestrogens with increased flexibility were developed, as shown in Figure 3.10 below. They were prepared via the McMurry coupling of newly synthesised dihydrochalcones and substituted ketones. The purpose here was to investigate the flexibility tolerance of the estrogen receptor towards novel antiestrogens. Flexibility was introduced between Ring A and Ring B by means of a propylene group. Various substitutents were positioned around Ring A, B and C as was the pyrrolidine side chain.

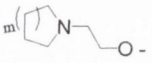
Biochemical evaluation and estrogen receptor binding tests were performed on these compounds and the results are discussed in chapter 4.





Type (1) R = side chain, R_1 = OMe, OH, H

R = OMe, OH, H, R_1= side chain

Side chain = 

Type (2) n = 0, R = OMe, R1 = side chain, R3 = H

Type (3) n = 0, R = side chain (m = 1, 2), R1 = OM_e, R2 = $_H$, R3 = H

Type (4) n = 1, 0, R = side ch_ain, R1 = $_O$Me, R2 = $_O$Me, R3 = $_{H\,/}$ CH3

Type (5) n = 0, R = OMe, R1 = OMe, R2 = side $_c$hain, R3 = H

Type (6) n = 1, R = sid_e chain, R_1 = OMe, R_2 = OMe, R3 = H

Type (7) n = 1, R = $s_i$de chain, R1 = OMe, $_R$2 = OPiv, R3 = H

Type (8) n = 1 , R = side $chai_n$, R1 = O_Me, R2 = OH, R3 = H

Type (9) n = 1, 0, R = side $c_h$ain, R1 = $_O$H, R2 = OPiv, R3 = H

Type (10) n = 1, 0, R = side chain, R_1 = OH, R2 = OH, R3 = H

Figure 3.10 General ring assignment for analogues Type 1-10

4. Biochemical studies of Tamoxifen Analogues

4.1. Introduction

Statistics state that one in eleven Irish women are likely to develop breast cancer at some stage in their lives. So it's obvious how serious the situation is. This could mean that if left undetected and untreated one in eleven Irish women would die from breast cancer. Thankfully due to new detection techniques, new treatments (radiological and surgical) and of course hormone chemotherapy (including tamoxifen and anastrozole) the number of women dying from breast cancer is on the decline. The worrying fact is that the primary mitogen for breast cancer is thought to be estrogen, a naturally occurring vital hormone in the female body. In order to reduce the risk of developing breast cancer in all women and thus reduce the death rate one would have to deprive the body of all estrogen (exogenous and endogenous). It is virtually impossible to remove all exogenous estrogen from the body as the sources vary widely from food products, containers for food, plastics, chemicals, to blood serum and hormone replacement therapies for menopausal women. As for the endogenous estrogens, it is possible to cut off the supply of the more significant ones like estradiol but not without some serious side effects. Only those women at high risk of developing breast cancer are advised to consider treatment with tamoxifen as not every woman is in danger and the side effects they would suffer may have been in vain. So once again we're left with a situation of regular check-ups in the hope of catching the cancer, if it's present, before it's too advanced to treat with any degree of success[1, 4].

The MCF-7 breast cancer cell line[213] is commonly used in evaluating new compounds for antiproliferative activity. It has been shown that when comparing different cell lines response to tamoxifen their response varies according to estrogen receptor content. The proliferation of is readily inhibited when treated with tamoxifen as they contain high levels of estrogen receptors. Therefore these cells have been used for *in-vitro* testing, with tamoxifen and other anti-estrogen compounds, with great success.

The purpose of biochemical evaluation of these synthesised compounds is to compare their potential ability as treatments for breast cancer to that of tamoxifen. Tamoxifen is a competitive inhibitor where it binds to the estrogen receptor therefore blocking any estradiol from binding. It then translocates to the nuclei where the complex prevents cell proliferation. The ability of tamoxifen to do this was tested on MCF-7 cells[210]

using MTT and LDH assays and the results were used as references to which the novel tamoxifen analogues assay results could be compared.

Biochemical studies were carried out on novel tamoxifen analogues using the MCF-7[213] cells (breast cancer cells) and Ishikawa cells[211] (endometrial cells). These studies examined the inhibition of proliferation (MTT) in the cells when treated with these compounds in comparison to tamoxifen. Also the cytotoxicity (LDH) of the compounds was tested using the MCF-7 cells. An alkaline phosphatase assay was used to measure the estrogenic effects of analogues on uterine cells (Ishikawa cells[211]). Estrogen receptor competitor ligand-binding assays were used to determine the ability of the analogues to displace estradiol from the ER and to measure their affinity for ER-α and ER-β.

The alkaline phosphatase (AlkP) assay [212, 214] was used to assess the alkaline phosphatase enzyme activity in estrogen sensitive cells. Estradiol induces AlkP in Ishikawa cells (endometrial), as does tamoxifen (but to a much lesser extent than estradiol). All the same stimulation of the endometrial cells can lead to the development of uterine cancer in tamoxifen breast cancer patients and so it is perferable that new breast cancer treatments do not stimulate AlkP in Ishikawa cell. This is a widely used assay developed by R.Hochberg *et al.*[212], for the determination of the estrogenic character of a novel compounds. It allows the monitoring of large numbers of samples, is easily performed and produces highly accurate and reproducible results. R. Hochberg *et al.* report the optimum AlkP for estradiol in Ishikawa cells to be 1nM and report the testing of many potential antiestrogens. This assay was used in this chapter to measure the estrogenicity of synthesised analogues and also assess their ability to compete with estradiol for the ER.

As discussed in Chapter 1 all ligands[215] bind exclusively to the C-terminal LBD that contains the Activation Function (AF-2). Binding to this domain by an agonist results in AF-2 activity, while the binding of an antagonist does not. In a study by Shiau *et al*[18]., it was shown that when ER-α had bound the TAM metabolite, OHT, a potent anti-estrogen, helix 12 is prevented from being positioned over the LBD by the OHT side chain (see chapter 1). The alternate packing arrangement that takes place permits the helix 12 to reach the static region of the AF-2 surface and mimic bound activator.

Apoptosis[216] or programmed cell death is an essential physiological process required for normal development and maintenance of tissue homeostasis. Apoptosis describes the orchestrated collapse of a cell, staging membrane blebbing, cell shrinkage, protein fragmentation, chromatin condensation and DNA degradation followed by rapid engulfment of corpses by neighbouring cells, thereby preventing inflammation of the surrounding tissue. It is by far the most predominant form of cell death, where the cell swells and bursts releasing its contents in an unregulated manner and eliciting a local immune response. Many chemotherapeutic drugs are cytotoxic to their target cells, through the induction of apoptosis. Research into the mechanism by which tamoxifen induces apoptosis is ongoing. The process of apoptosis is dependent in most cases on the activation of the caspase casade (intracellular cysteine proteases that cleave various cytoplasmic structural and nuclear proteins). The cleavage of the various substrates contributes to the typical morphological and biochemical features in apoptosis in both ER-positive and ER-negative human breast cancer cells by activating the caspase pathway[217]. Apoptosis can also be induced by proteases such as serine granzymes A and B, which activate caspases triggering apoptosis[218] or calpins (calcium activated proteases). In a study by Zhang *et al.* [219], OHT was shown to induce apoptosis in MCF-7 cells[213] and in contrast no apoptosis was induced when the cells were treated with estradiol.

This chapter will provide the experimental details (methods and materials) of the assays performed on the synthesised analogues and will discuss the results obtained for the analogues and compare to tamoxifen.

4.2. Materials and methods for biochemical analysis

4.2.1. Materials

The full names and addresses of the sources listed below are given at the end of the list.

Materials	Supplier
Activated charcoal	Sigma
DMSO	Sigma

Eagles minimum essential medium	Sigma
β-estradiol	Sigma
ER-α Competitor Assay, Beacon kit	Panvera
ER-β Competitor Assay, Beacon kit	Panvera
Estradiol (E_2)	Sigma
Foetal calf serum (FCS)	Sigma
Foetal bovine serum (FBS)	Sigma
Gentamycin	Sigma
Glutamine	Gibco
Hams F-12:DMEM medium	Sigma
4-Hydroxytamoxifen (OHT)	Sigma
Ishikawa cells	E.C.A.C.C.
L-glutamine	Sigma
MCF-7 cells	E.C.A.C.C.
MTT and LDH assay kits	Promega
Non-essential amino acid medium	Richard Hochberg
p-Nitrophenyl phosphate	Sigma
Penicillin/Streptomycin	Sigma
Pipettes (sterile)	Grenier
Propidinium Iodide	Sigma
Tamoxifen OHT	Sigma
Tissue culture flasks	Grenier
Trypsin	Sigma

All compounds from this thesis used in these tests are described in Chapter 2 and 3 i.e. tamoxifen-like (flexible) analogues. Some related compounds[214] synthesised by Dr.T.O'Sullivan, were prepared in the Pharmaceutical Chemistry Department, TCD. All other reagents were of analytical grade, where possible and were obtained from Sigma.

4.2.2. Addresses of suppliers

European Collection of Animal Cell Cultures (E.C.A.C.C.), PHLS, Centre for Applied

British Drugs House (BDH) Chemicals Ltd., c/o Lennox Chemicals, J.K.Kennedy Drive, Dublin 12, Ireland.

Gibco, Life Technologies Ltd., 3 Fountain Drive, Inchinnan Bussiness Park, Paisley, PA4 9RF, UK.

Grenier GMBH., Maybachstrasse 2, 72636 Frickenhausen, Germany.

Microbiology and Research, Porton Down, Salisbury, SP40JG, UK.

Panvera, 501 Charmany Drive, Madison, WI 53719, USA.

Promega Corporation, c/o Medical Supply Co., Santry Hall Ind. Est., Santry, Dublin 9, Ireland.

Sigma Chemical Co. Ltd., Fancy Road, Pool, Dorset, UK.

Sterlin, Bibby, Sterlin Ltd., Stone, Staffordshire, UK.

Riedel de Haen AG, c/o R.B. Chemicals Ltd., Hoecht House, Cookstown Industrial Estate, Tallaght, Dublin 24, Ireland.

4.2.3. Growth and maintenance of cells

4.2.3.1. Growth and maintenance of MCF-7 (breast cancer) cell lines

MCF-7 cells, a human breast adenocarcinoma cell line, cloned from a 69 year old female Caucasian, (Soule et al.,)[210] were grown as monolayer cultures at 37°C, under a humidified atmosphere of 95% O_2, 5% CO_2 in 75cm^2 flasks containing Eagles Modified Essential Medium (EMEM), with 10% foetal calf serum (FCS), 1% Non Essential Amino Acid Medium (NEAAM), 2mM L-glutamine and supplemented with 100µg/ml gentamycin (complete medium).

Cells were harvested and reseeded after reaching confluence (once weekly) by first washing with serum-free EMEM (10ml). They were then removed from the surface of the flask by a 1 minute exposure to trypsin (2ml). The cells were then sedimented by centrifugation at 600 x g for 5 minute and the pellet was resuspended in 1ml of complete medium. Cell numbers were counted using a haemocytometer. An aliquot of cells (1.5 x 10^6 cells) was seeded in 75cm^2 flasks in 20ml complete medium.

4.2.3.2. Growth and maintenance of Ishikawa (endometrial) cells

Ishikawa cells, a human endometrial cancer cell line, were grown as monolayer cultures at 37°C, under a humidified atmosphere of 95% O_2, 5% CO_2 in 75cm^2 flasks containing Eagles Minimum Essential Medium (EMEM) containing 10% foetal bovine serum (FBS) and supplemented with 2mM L-glutamine, and 100µg/ml gentamycin.

Cells were harvested and reseeded after reaching confluence (once weekly) by first washing with serum-free EMEM (10ml). They were then removed from the surface of the flask by a 1 minute exposure to trypsin (2ml). The cells were then sedimented by centrifugation at 600 x g for 5 minute and the pellet was resuspended in 1ml of complete medium. Cell numbers were counted using a haemocytometer. An aliquot of cells (1.5 x 10^6 cells) was seeded in 75cm^2 flasks in 20ml complete medium.

4.2.3.3. Cryopreservation of cells

All cells were grown to a state of subconfluency, were harvested and counted as described (Section 4.2.3.1). The cells (5 x 10^6) were centrifuged and resuspended in a mixture of 90% FCS / 10% dimethylsulphoxide (DMSO)[220]. The 1ml aliquots were transferred to a 1.5ml cryotube and placed at –20°C for 4hours. The cryotube was then transferred to –70°C for 2 hours, before storage in a liquid nitrogen vessel at –180°C.

When required, an aliquot of cells was removed from the liquid nitrogen vessel rapidly thawed and resuspended in 10ml of complete medium. This cell suspension was centrifuged at 600 x g for 5 minutes, the medium (containing DMSO) discarded and the pellet resuspended in complete medium. Cells were then seeded in tissue culture flasks as previously described (Sections 4.2.2).

4.2.4. Assessment of antiproliferative / cytotoxic effects of novel compounds

4.2.4.1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay

The MTT assay was performed by a modification of the method of Mosann[221]. 3-(4,5

-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), a tetrazolium salt that is yellow in colour, was taken up only by *meta*bolically active cells. Once in the cells it is cleaved by mitochondrial dehydrogenases to form a formazan dye, which appears as purple crystals. These crystals were dissolved by the addition of DMSO and the resulting purple coloured solution was measured spectrophotometrically.

MCF-7 cells were cultured as described in section 4.2.3.1, at 2.5 x 10^4 cells per well in 200µl of complete medium in a 96-well plate at 37°C leaving the outer wells with 200µl non-supplemented medium in order to prevent the evaporation of medium from the treated wells. Only 48 wells were required to perform the assay in triplicate on a tamoxifen analogue therefore the remaining 48 wells could be used for treatment with tamoxifen for comparison purposes. After 24 hours the cells were treated either with a vehicle (0.1% ethanol) or a range of concentrations of the synthesised compounds (final concentration 0.1nM to 50µM) and the cells were incubated for a further 72 hours. Following the incubation period, 50µl aliquots were removed from each well and transferred to a new 96-well plate and left aside for use in the LDH assay.

The plate was inverted to remove all medium from the wells and immersed in PBS solution and then emptied again. 50µl of an MTT solution (final concentration 1mg/ml) was then added to each well. The cells were then incubated in the dark for 2-3 hours at 37°C. Once the blue/purple formazan crystals had formed 200µl of DMSO was added to each well and the well contents were triturated to ensure the crystals were completely dissolved. The absorbance was read at 570nm in a Dynatech MR5000 plate reader and cell viability expressed as a percent of control. IC_{50} values were calculated for each compound and compared to that of tamoxifen.

4.2.4.2. Lactate dehydrogenase (LDH) assay

Cytotoxicity[222] of the compounds on the cells was assayed through the use of a Promega LDH assay kit. These studies allowed an evaluation of the extent of cell death induced by the compounds and in combination with the MTT assay facilitated distinction between the cytostatic and cytotoxic activities of the compounds.

MCF-7 cells were cultured as described as in section 4.2.4.1 and seeded down at a density of 1.5 x 10^4 cells per well with 200µl complete medium in a 96-well plate at 37°C. After 24 hours the cells were then treated with varying concentrations of the synthesised compounds and incubated for a further 72 hours.

A Blank was produced by lysing the cells by adding 10µl of lysis (10% Triton X-100) solution to each well 45 minutes before starting the assay. This gave 100% lysis control, which was necessary for each assay performed in order to calculate the percentage cytotoxicity. From this the data could be presented as percentage cell lysis compared to control versus concentration of compound.

To assay for LDH activity an aliquot (50µl) of each sample was transferred in triplicate to another 96-well plate. To this 50µl of substrate mix from the LDH assay kit (lyophilised diaphorase, lactate and NAD^+) was added and the plate was placed in the dark at room temperature for 30 minutes. After this period, 50µl of stop solution was added to each well before reading the plate absorbance at 490nm using a Dynatech MR5000 plate reader.

4.2.5. Alkaline phosphatase assay

This assay measures estrogen stimulation of alkaline phosphatase enzyme activity (AlkP), EC 3.1.3.1, by the Ishikawa line[211] of human endometrial adenocarcinoma cells grown in 96-well microtiter plates. It does so by using a chromogenic substrate that binds to the enzyme producing a yellow colour. When cells were treated with an estrogenic compound the greater the intensity of the yellow colour correlates with the extent of alkaline phosphatase activity in the cells and therefore the estrogenic character of that compound. The assay was monitored at 405nm and the IC_{50} values calculated for each compound and compared to that of estradiol and tamoxifen.

Ishikawa cells were maintained as described in section 4.2.2.3. 24 Hours before the start of the assay, near confluent cells were changed to an estrogen-free basal medium (EFBM): a 1:1 mixture of phenol red-free Ham's F-12 and Dulbecco's Modified Eagle's Medium, all of the supplements listed in section 4.2.2.3 and 5% calf serum stripped of endogenous estrogens with dextran coated charcoal (see section 4.5.1). On

the day of the experiment, cells were harvested with trypsin (see section 4.2.2.3) and plated in 96-well flat-bottomed microtiter plate in 50µl of EFBM at a density of 2.5 x 10^4 cells per well. Test compounds were added to the wells via 100µl of EFBM bringing the total volume of each well to 150µl of EFBM. This introduced a dilution factor of 1 in 3 (50µl/150µl total well volume) therefore the concentration of a test compound before addition to the cells was 3 times (3nM in 50µl EFBM, 0.3% EtOH) the desired final concentration (1nM, 150µl medium, 0.1% EtOH in well). The ethanol (EtOH) content of each well was maintained at or less than 0.1% in order to eradicate any error caused by variability of any effect that EtOH may have on the cells. The assay was performed in quadruplicate and when the cells had adhered to the bottom of the wells (5-24 hours after harvesting) they were treated. The cells were treated with varying concentrations of estradiol in order to obtain the concentration at which maximum stimulation of alkaline phosphatase activity occurs. The IC_{50} value is reported in the results section 4.4.

For the testing of tamoxifen analogues the cells were treated with a vehicle 100µl of medium (EFBM 0.1% EtOH), estradiol 1nM (0.1%EtOH), a range of concentrations of the analogue (0.01nM-1µM final concentrations) (0.1% EtOH) and a range of concentrations of the analogue with 1nM estradiol (0.1%EtOH) The remaining cells were treated with the same varying concentrations of the selected analogue (0.1% EtOH) in 50µl of medium (EFBM) plus 1nM estradiol (final concentration) in 50µl of medium (EFBM) bringing the total volume of the wells to 150µl.

After treatment the plate was kept at 37°C for 4 days in an incubator and on the fourth day the assay was performed. The microtiter plate was inverted and the growth medium removed with a brisk shake of the wrist. The plate was then immersed gently in PBS solution (concentration 1mg/ml) and removed from the container and immersion repeated. The PBS solution was shaken out and the inverted plate blotted gently on a paper towel. The cover of the plate was replaced and the plate stored at –80°C for at least 15 minutes followed by thawing at room temperature for 5-10 min. The plate was then placed on ice and 50 µl of ice-cold solution containing 5mM p-nitrophenyl phosphate, 0.24 mM $MgCl_2$, and 1M diethanolamine (pH 9.8) was added. The plate was then warmed to room temperature (time zero), and the yellow colour, from the production of p-nitrophenol, allowed to develop. The absorbance was read at

405nm in a Dynatech MR5000 plate reader until maximally stimulated cells showed an absorbance of about 1.2 with non treated cells showing an absorbance of about 0.2.

4.2.5.1. 1:1 Preparation of phenol red-free Ham's F-12 and Dulbecco's Modified Eagle's Medium mixture

In order to prepare the 1:1 mixture of phenol red-free Ham's F-12 and Dulbecco's Modified Eagle's phenol free medium it was necessary to strip calf serum (CS) of its endogenous estrogens with using dextran coated charcoal. Firstly 2.5g of activated charcoal and 0.25g of dextran (dextran only, no saline or sulphate form) were stirred in 100ml of deionised water for 15 minutes. The resulting solution was poured into two large sterilins (50ml each) and centrifuged at 1000 x g for 10 minutes to achieve a pellet. The supernatant was then poured off and 50ml of calf serum was added to each pellet and shaken well. Both 50ml preparations were left to stand for 15 minutes after which they were centrifuged at 1200 x g for 15 minutes. The calf serum was transferred to a new container without disturbing the pellet and centrifuged again at 1200 x g for 15 minutes. The newly stripped calf serum was then transferred in a sterile atmosphere to a sterile container via a 0.45um sterile filter.

Once the serum had being stripped of its endogenous estrogens the estrogen free medium could be prepared. To 93ml of Ham's F-12:Dulbecco's Modified Eagle's 1:1 phenol free medium 1ml of gentamycin (100μg/ml), 1ml of L-glutamine (2mM) and 5ml of stripped calf serum (5%) were added.

4.2.6. Estrogen Receptor (ER) Competitor Assay

4.2.6.1. Assay Theory

The ER Competitor assay kits[224] use insect expressed, full length, untagged human ERs and a novel, tight binding, fluorescent estrogen ligand (*E). ER was added to the fluorescent estrogen ligand to form an ER/*E complex. This complex was then added to individual test compounds in individual tubes. If the test compounds did not compete with *E for binding to the ER, then the ER/*E complex would remain intact. The ER/*E complex, as a large molecule, rotated little during the excitation state and

therefore, had high polarisation values. When *E was released due to competition by a test compound, it as a small molecule rotated rapidly during the excitation state, and upon emission, had a low polarisation value. Thus, the change in polarisation value in the presence of test compound was used to determine the relative affinity of the test compounds for the ER.

4.2.6.2. Assay procedure

ER-α and ER-β fluorescence polarization-based competitor assay[224] kits were obtained from Panvera. The recombinant ER and the fluorescent estrogen ligand were removed from the –80°C freezer and thawed on ice for one-hour prior to use. The fluorescent estrogen (2nM) was added to the ER (30nM for ER-α and 20nM for ER-β) and screening buffer (100nM potassium phosphate (pH 7.4), 100μg/ml BGG, 0.02% NaN_3) was added to make up to a final volume that was dependent on the number of tubes used (number of tubes (e.g.50) x volume of complex in each tube (50μl) = total volume (e.g. 2500μl)).

Test compound (1μl, concentration range 1nM to 100μM) was added to 49μl screening buffer in each borosilicate tube (6mm diameter). To this, 50 μl of the fluorescent estrogen/ER complex was added to make up a final volume of 100μl and final concentration range for the test compound – 0.01nM to 1μM. A vehicle control contained 1% of ethanol. A negative control contained 50μl screening buffer and 50μl fluorescent estrogen/ER complex. This negative control was used to determine the polarization value when no competitor was present (theoretical maximum polarization). The tubes were incubated in the dark at room temperature for 2 hours and were mixed by shaking on a plate shaker. The polarisation instrument with 485nm excitation and 530nm emission interference filters. For ER-α and ER-β, graphs of polarization (mP) versus concentration were drawn using Prism software from Graphpad.

4.2.7. Flow cytometry

4.2.7.1. Sample fixing

The MCF-7 cells[210] were seeded at a density of 8 x 10^5 cells/25cm^2 flask in 5ml of complete medium. After 24 hours, when the cells were in the exponential phase of growth, they were treated with 50µl (concentration range spanning 10-50µM) of TAM or the selected analogues. Control flasks were treated with an equal volume of ethanol.

Following incubation for up to 72 hours, the cells were washed three times with PBS, trypsinised and harvested. The cell pellet was resuspended in 1ml ice-cold PBS and centrifuged at 500xg for 5 minutes and the supernatant was removed. PBS (200µl) was added and the pellet was resuspended followed by addition of 1800µl ice-cold 70% ethanol. The fixed samples were ready for staining and analysis after 30 minutes at 4°C, or alternatively, could be stored at 4°C for up to one month.

4.2.7.2. Nuclear Staining of Cells with Propidium Iodide

The fixed samples were centrifuged at 500xg for 5 minutes and the PBS/ethanol supernatant was removed. PBS (400µl) was used to gently resuspend the pellet, which was delicate following fixing. The resuspended pellet was transferred to a fluorescence-activated cell sorter (FACS) [216] tube. RNase A (25 µl of 1mg/ml stock) and propidium iodide (75µl of 1 mg/ml stock) was added to the samples. Following vortexing, the samples were incubated in the dark at 37°C for 30 minutes.

4.2.7.3. FACS analysis

The samples were read at 488nm using FACScalibur flow cytometer from Becton Dickinson. The FACS data for 10,000 cells was analysed using the Macintosh-based application Cellquest and the data was stored as frequency histograms.

4.3. Results and discussion

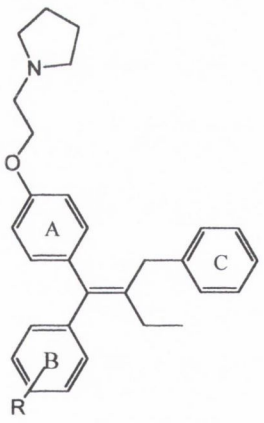
4.3.1. Introduction

A selective estrogen receptor modulator (SERM) acts as a partial estrogen antagonist in breast tissue and an agonist in other tissue such as uterus. Trans configurations of the compound tend to have anti-estrogenic activity while the *cis* conformations display estrogenic activity in assays of inhibition of human breast cancer cell proliferation. The antagonist activity occurs through inhibition of the ER in breast tissue. Tamoxifen has been shown to act not only as an antagonist but also as an agonist in humans, as evidenced by the increased incidence of endometrial cancer in tamoxifen patients.

4.3.2. Antiproliferative and cyctotoxicity profiles

MCF-7 cells were tested using tamoxifen (as reference control) and the novel synthesised analogues.

4.3.2.1.Results for Analogues series 1 (Chapter 2) and tamoxifen



R = *para*-OH BRI049
R = *ortho*-OH BRI106
R = meta-OH BRI045
R = *para*-OPiv BRI037
R = *meta*-OPiv BRI038
R = *ortho*-OPiv BRI044
R = *para*-OMe BRI040
R = *meta*-OMe BRI042
R = *ortho*-OMe BRI041

Figure 4.1 Pivaloyloxy, methoxy and hydroxy substituted analogues (chapter 2)

These analogues shown in Figure 4.1 above contain a pyrrolidine side chain on Ring A and various substitutents in either *ortho, meta* or *para*-positions on Ring B while Ring C remains unsubstituted. These compounds were tested for antiproliferative activity in MCF-7 cells and their IC_{50} values are recorded in Table 4.1 below. Tamoxifen has an

IC_{50} value for the MTT assay of 4.56μm, to which the analogue IC_{50} values were compared to aid the selection of the best analogues for further testing.

Analogue series 1	Antiproliferative Activity in MCF-7 cells, IC_{50} value (μM)
BRI037	**5.9 ± 6.6**
BRI042	**7.5 ± 7.5**
BRI049	**0.2 ± 8.5**
BRI038	**23.7 ± 9.1**
BRI044	**29.0 ± 1.1**
BRI160	**5.1 ± 1.4**
BRI045	**1.5 ± 0.1**
BRI106	**16.5 ± 3.9**
BRI040	**35.9 ± 4.8**
BRI041	**12.1 ± 3.4**
Tamoxifen	**4.5 ± 9.4**

Table 4.1 Antiproliferative effects of Methoxy, pivaloyl and hydroxy substituted analogues on MCF-7 cells. IC_{50} values: the concentration required to inhibit 50% of MCF-7 growth. Values represent the mean ± S.E.M (error values x 10^{-6}) for three experiments performed in triplicate.

In Table 4.1 above the blue section represents the analogues that halt the proliferation of MCF-7 cells to the same or a greater extent than tamoxifen and were thus selected to undergo more testing. It was observed by comparison of the results to the IC_{50} value of tamoxifen that a few compounds exhibited a greater antiproliferative effect than tamoxifen and therefore were considered for further testing. Compounds BRI037 and BRI042 display slightly higher IC_{50} values than tamoxifen and so they were also selected for further testing. As an example Figure 4.2 below, illustrates inhibition of proliferation and induction of cytotoxicity for compound BRI037.

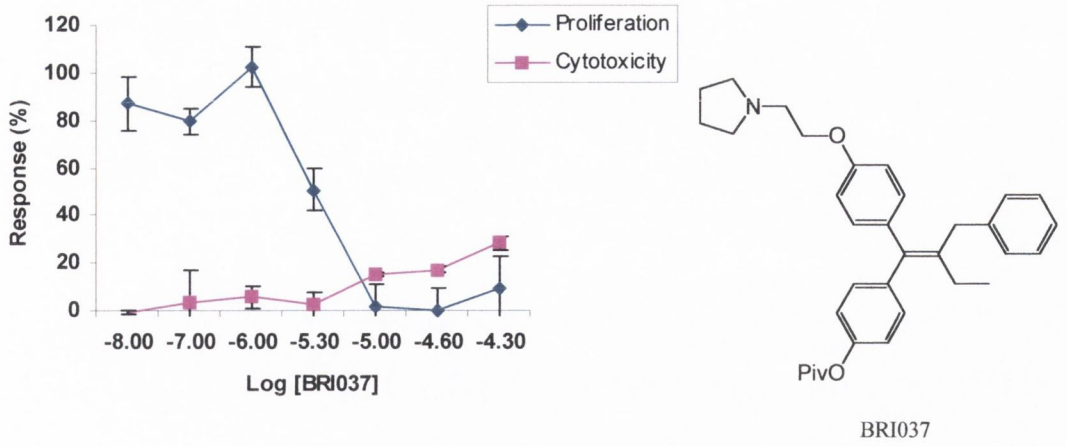


Figure 4.2 Compound (BRI037) inhibited proliferation and induced cytotoxicity of MCF-7 cells

This is a representative graph for analogues series 1. MCF-7 cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for 24 hours. After this period, various concentrations (0.1nM-50μM) of (BRI037) were added and the cells were left for a further 72 hours. Determination of cell proliferation was carried out using the MTT assay described in section 4.2.3.1 and the cytotoxicity was evaluated using the LDH assay as described in section 4.2.3.2. Figure 4.2 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

4.3.2.2.Results for Analogues series 2 and 3 (Type 1 and 2) (Chapter 2)

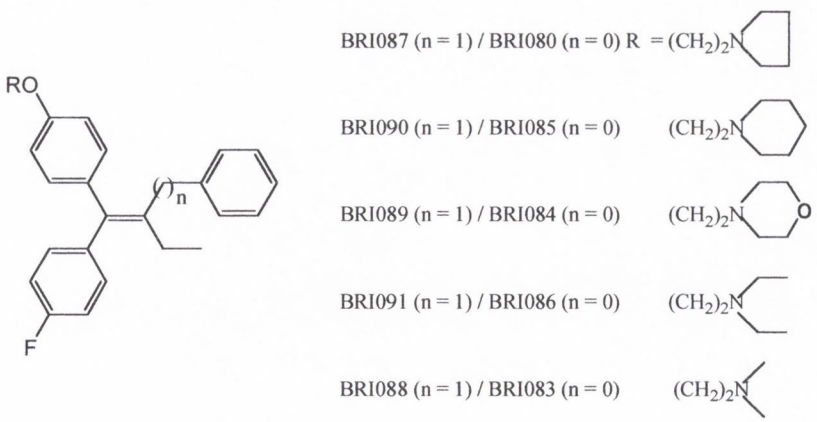


Figure 4.3 Fluoro-substituted analogues (Chapter 2)

These analogues shown in Figure 4.3 above contain fluoro-substitutents on Ring B in the *para*-position plus various side chains in the *para*-positions on Ring A while Ring C remains unsubstituted. Type 2 of these analogues are flexible and are expected to perform better than non-flexible Type 1 analogues due to their ability to adopt different positions when binding to the ER. These compounds were tested for antiproliferative activity in MCF-7 cells and their IC_{50} values are recorded in Table 4.1 below for comparison with tamoxifen.

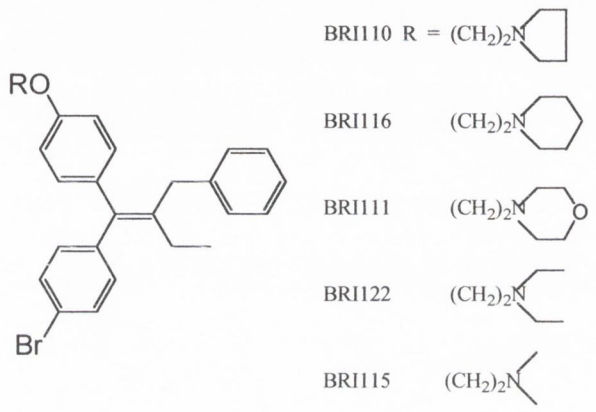


Figure 4.4 Bromo-substituted analogues (Chapter 2)

The analogues displayed in Figure 4.4 above are flexible and contain Bromo-substitutents on Ring B in the *para*-position plus various side chains in the *para*-positions on Ring A while Ring C remains unsubstituted. These compounds were tested for antiproliferative activity in MCF-7 cells and their IC_{50} values are recorded in Table 4.2 below for comparison with tamoxifen.

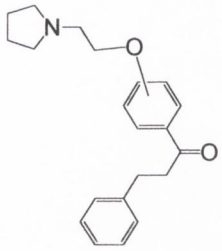
Figure 4.3 and 4.4 display the structures of the analogues reported in Table 4.2. Table 4.2 displays the antiproliferative effect of analogues series 2 and 3 on MCF-7 cells and these results were compared to the antiproliferative tamoxifen IC_{50} value (4.5μM). Two compounds, BRI080 and BRI116 compare closely with tamoxifens action on the MCF-7 cells and was being considered for further testing. The remaining analogues in Table 4.2 were not pursued as their IC_{50} values were not comparable to tamoxifen.

Analogue Series 2 and 3	Antiproliferative Activity in MCF-7 cells, IC_{50} value (μM)
BRI080	**1.94 ± 1.2**
BRI083	**36.2 ± 2.6**
BRI084	**33.9 ± 3.1**
BRI085	**42.4 ± 7.2**
BRI086	**28.5 ± 6.4**
BRI087	**20.5 ± 3.2**
BRI088	**18.1 ± 1.3**
BRI089	**25.3 ± 4.4**
BRI090	**31.3 ± 5.1**
BRI091	**14.4 ± 8.2**
BRI110	**14.0 ± 1.1**
BRI116	**6.3 ± 3.3**
BRI111	**27.8 ± 4.7**
BRI122	**9.54 ± 5.2**
BRI115	**10.6 ± 2.4**

Table 4.2 Antiproliferative effects of fluoro and bromo-substituted analogues. IC_{50} values: the concentration required to inhibit 50% of MCF-7 growth. Values represent the mean ± S.E.M (error values x 10^{-6}) for three experiments performed in triplicate.

4.3.2.3.Results for analogues series 1-10 (Chapter 3) and related compounds[214]

4.3.2.3.1. Results for analogues series 1



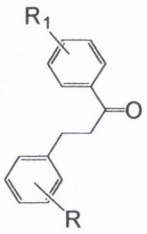
Type 1.1

BRI165 *ortho*-side chain

BRI173 *meta*-side chain

BRI172 *para*-side chain

side chain = OCH_2CH_2N



Type 1.2

BRI068 R = *ortho*-OMe, R_1 = side chain

BRI166 R = *meta*-OMe, R_1 = side chain

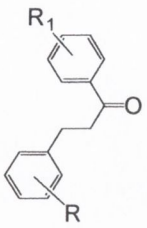
BRI145 R = *para*-OMe, R_1 = side chain

BRI167 R_1 = *ortho*-OMe, R = side chain

BRI136 R_1 = *meta*-OMe, R = side chain

BRI168 R_1 = *para*-OMe, R = side chain

side chain = OCH_2CH_2N



Type 1.3

BRI183 R_1= *ortho*-OH, R = side chain

BRI177 R = *para*-OH, R_1= side chain

BRI178 R = *meta*-OH, R_1= side chain

side chain = OCH_2CH_2N

Figure 4.5 Analogues Type 1 (1,3-diphenylpropan-1-ones) (Chapter 3)

These analogues are side chain modified dihydrochalcones (Analogue series 1) with various substitutions on both rings as seen in Figure 4.5 below. Previous research[184] on compounds structurally related to Analogues series 1 were tested for the treatment of malaria. These dihydrochalcones were then used to synthesise highly flexible tamoxifen analogues (Analogue series 2-10) as seen in Figure 4.6.

Table 4.3 displays IC_{50} values representing the antiproliferative effect of analogues series 1 Type 1.1 on MCF-7 cells and allowed their comparison with tamoxifen (4.5μM). When compared with tamoxifen it was clear that all the compounds had higher IC_{50} and were therefore not considered for further testing.

Analogue series 1 Type 1.1	Antiproliferative Activity in MCF-7 cells, IC_{50} value (μM)
BRI165	**12.2 ± 1.9**
BRI173	**39.9 ± 2.2**
BRI172	**32.4 ± 2.4**
BRI167	**32.9 ± 4.8**
BRI068	**29.9 ± 2.7**
BRI166	**32.2 ± 0.7**
BRI145	**35.5 ± 1.2**
BRI136	**24.3 ± 2.4**
BRI183	**32.5 ± 3.6**
BRI177	**29.8 ± 6.1**
BRI178	**29.1 ± 2.7**
BRI168	**14.3 ± 1.8**

Table 4.3 Antiproliferative effects of analogues Type 1 (Chapter 3) on MCF-7 cells. IC_{50} values: the concentration required to inhibit 50% of MCF-7 growth. Values represent the mean ± S.E.M (error values x 10^{-6}) for two experiments performed in triplicate.

4.3.2.3.2. Results for analogues series 2-10

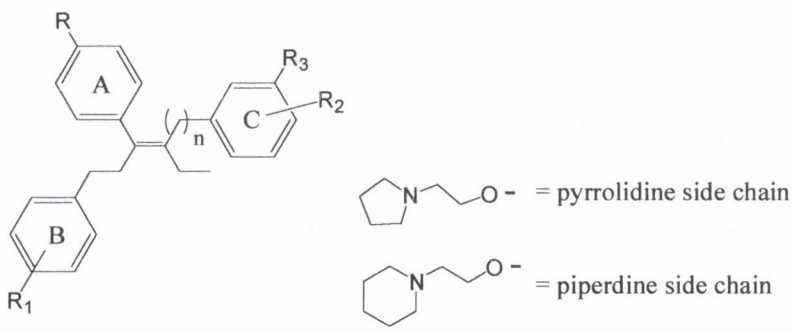


Figure 4.6 Structures of Analogues series 2-10 (Chapter 3)

The related and flexible analogues displayed in Figure 4.7 were synthesised to investigate the effect of Ring C substitution on the ER binding affinity of the analogues. This combined with flexibility and substitutents on all three Rings. Table 4.5 shows the IC_{50} values for the compounds and allows their comparison with tamoxifen.

Compound No.	n	R_1	R_2	R_3	Analogues series Type (2-10)	Antiproliferative Activity in MCF-7 cells, IC_{50} value (μM)
BRI182	0	side chain	H	H	R = *ortho*-OMe	6.0 ± 0.3
BRI179	0	side chain	H	H	R = *meta*-OMe	14.0 ± 2.2
BRI109	0	side chain	H	H	R = *para*-OMe	29.7 ± 1.8
BRI101	0	*para*-OMe	H	H	R = *para*-pyrrolidine side chain	14.0 ± 1.2
BRI096	0	*meta*-OMe	H	H	R = *para*-piperidine side chain	11.8 ± 1.1
BRI093	0	*meta*-OMe	H	H	R = *para*-pyrrolidine side chain	4.4 ± 1.2
BRI162	0	*para*-OMe	*para*-OMe	H	R = side chain	11.4 ± 1.0
BRI180	1	*para*-OMe	*para*-OMe	CH_3	R = side chain	5.7 ± 0.1
BRI169	0	*para*-OMe	side chain	H	R = *para*-OMe	7.1 ± 0.4
BRI144	0	*para*-OMe	side chain	H	R = *meta*-OMe	6.9 ± 0.1
BRI138	0	*para*-OMe	side chain	H	R = *ortho*-OMe	12.2 ± 0.8
BRI135	1	*para*-OMe	*para*-OMe	H	R = side chain	19.5 ± 0.1
BRI134	1	*para*-OMe	*meta*-OMe	H	R = side chain	16.0 ± 1.3
BRI133	1	*para*-OMe	*ortho*-OMe	H	R = side chain	27.8 ± 1.3
BRI148	1	*para*-OMe	*para*-OPiv	H	R = side chain	6.5 ± 0.4
BRI121	1	*para*-OMe	*ortho*-OPiv	H	R = side chain	8.1 ± 1.2
BRI155	1	*para*-OMe	*meta*-OPiv	H	R = side chain	7.2 ± 1.2
BRI157	1	*para*-OMe	*para*-OH	H	R = side chain	6.9 ± 2.2
BRI158	1	*para*-OMe	*ortho*-OH	H	R = side chain	4.6 ± 1.2
BRI181	1	OH	OPiv	H	R =Side chain	6.2 ± 1.3
BRI130	0	OH	OPiv	H	R =Side chain	5.4 ± 1.0
BRI184	1	OH	OH	H	R =Side chain	10.5 ± 0.1
BRI185	0	OH	OH	H	R =Side chain	0.2 ± 0.1

Table 4.4 Structure and antiproliferative effects of Figure 4.6 Analogues on MCF-7 cells. IC_{50} values: the concentration required to inhibit 50% of MCF-7 growth. Values represent the mean ± S.E.M (error values x 10^{-6}) for two experiments performed in triplicate.

Table 4.4 displays IC_{50} values that represent the antiproliferative effect of the listed analogues on MCF-7 cells and allows their comparison with tamoxifen (4.5μM). According to Table 4.4 compounds BRI182, BRI093, BRI180, BRI144, BRI169, BRI148, BRI157, BRI158, BRI181 and BRI130 (see Figure 4.9 and Table 4.4) all display antiproliferative values close to that of tamoxifen and therefore could be selected for future testing as anti-estrogens. One excellent analogue, BRI185, displayed a much lower IC_{50} value than tamoxifen and therefore required more biochemical testing in order to determine its estrogenic or anti-estrogenic characteristics.

4.3.2.3.3. Results for flexible and rigid analogues

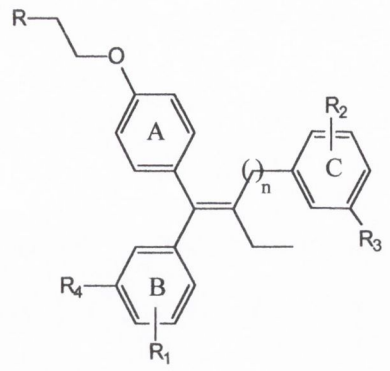


Figure 4.7 Related analogues flexible and rigid[214]

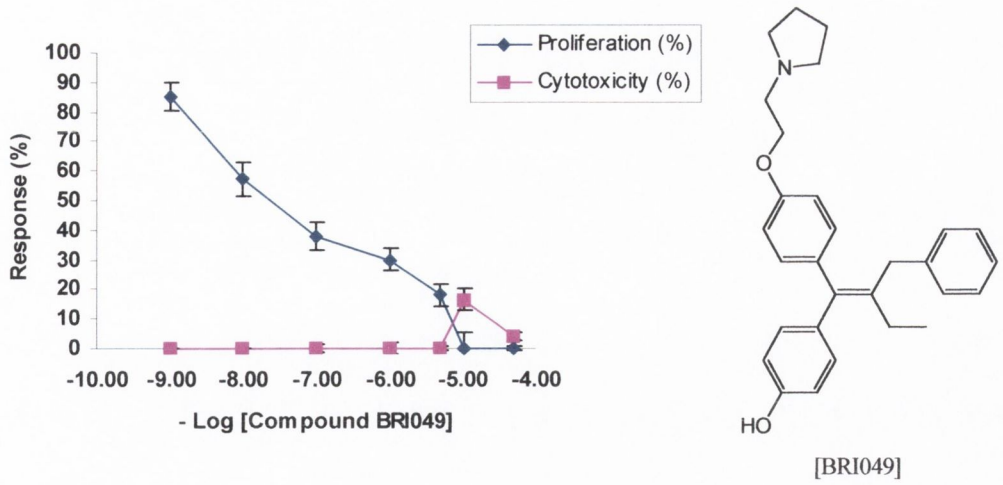
The related and flexible analogues displayed in Figure 4.7 were synthesised to further investigate the effect of Ring C substitution on the ER binding affinity of the analogues. This combined with flexibility and substitutents on all three Rings. Table 4.5 show the IC_{50} values for the compounds and allows their comparison with tamoxifen. These compounds are numbered using a new numbering system beginning with the prefix TSA.

Table 4.5 displays IC_{50} values that represent the antiproliferative effect of the analogues listed on MCF-7 cells and allow their comparison with tamoxifen (4.5μM). The analogues in the blue section of Table 4.5 above have antiproliferative values close to that of tamoxifen and therefore were selected to undergo further testing to assess their potential as anti-estrogens.

As examples Figure 4.8, 4.9 and 4.10 illustrate inhibition of proliferation and induction of cytotoxicity for analogues BRI049, TSB-3 and TSA-142.

Compound Code	R	R_1	R_2	R_3	R_4	N	Antiproliferative Activity in MCF-7 cells, IC_{50} value (μM)
Tamoxifen							4.6 ± 1.6
TSB-19	Piperidinyl	*p*-OH	H	H	H	1	1.6 ± 1.9
TSA-144	Pyrrolidinyl	*p*-OH	*m*-OMe	H	H	1	5.2 ± 1.8
TSB-21	Pyrrolidinonyl	*p*-OH	H	H	H	1	2.1 ± 2.7
TSB-20	N,N-Dimethyl	*p*-OH	H	H	H	1	1.6 ± 1.3
TSA-142	Pyrrolidinyl	*p*-OH	*p*-OPiv	H	H	1	1.1 ± 3.6
TSB-3	Pyrrolidinyl	*p*-OH	*o*-OPiv	H	H	1	0.7 ± 1.4
BRI049	Pyrrolidinyl	*p*-OH	H	H	H	1	0.2 ± 0.1
TSA-48	N,N-Dimethy	*p*-OH	*p*-OH	H	H	1	8.0 ± 0.6
TSA-60	Pyrrolidinyl	$OCON(Me)_2$	H	H	H	1	4.4 ± 0.2
TSA-150	Pyrrolidinyl	*p*-OH	*o*-OMe	H	H	1	4.9 ± 3.8
TSB-8	Pyrrolidinyl	*p*-OH	*p*-OMe	H	H	1	0.9 ± 2.5
TSA-51	Pyrrolidinoyl	*p*-OH	*p*-OH	H	H	1	9.4 ± 3.4
TSA-39	Pyrrolidinyl	*p*-OH	*p*-OH	H	H	1	45.9 ± 8.3
TSA-25	Pyrrolidinyl	*p*-OMe	*p*-OMe	H	H	1	36.0 ± 2.6
TSA-26	Pyrrolidinyl	*p*-OMe	*p*-OMe	CH_3	CH_3	1	12.0 ± 4.3
TSA-40	Pyrrolidinyl	*p*-OCOMe	H	H	H	1	32.0 ± 4.1
TSA-53	Piperidinyl	*p*-OH	*p*-OH	H	H	1	30.0 ± 6.2
TSA-56	Morpholinyl	*p*-OH	*p*-OH	H	H	1	35.0 ± 2.3
TSA-59	Pyrrolidinyl	*p*-OCOMe	*p*-OCOMe	H	H	1	10.6 ± 0.6
TSB-22	Morpholinyl	*p*-OH	H	H	H	1	27.0 ± 1.1
TSB-11	Pyrrolidinyl	*p*-OH	*o*-OH	H	H	1	20.0 ± 1.7
TSB-14	Pyrrolidinyl	*p*-OH	*p*-OH	H	H	1	14.5 ± 0.9
TSA-143	Pyrrolidinyl	*p*-OH	*p*-OPiv	H	H	1	14.7 ± 1.8
TSB-33	Pyrrolidinyl	*p*-OCONH*Ph*	H	H	H	1	39.0 ± 0.1
TSB-32	Pyrrolidinyl	*p*-OCONHEt	H	H	H	1	6.5 ± 0.7
TSB-31	N,N-Dimethyl	*p*-OH	H	H	H	1	15.0 ± 2.1
TSB-12	N,N-Dimethyl	*p*-OH	*m*-OH	H	H	0	31 ± 3.2

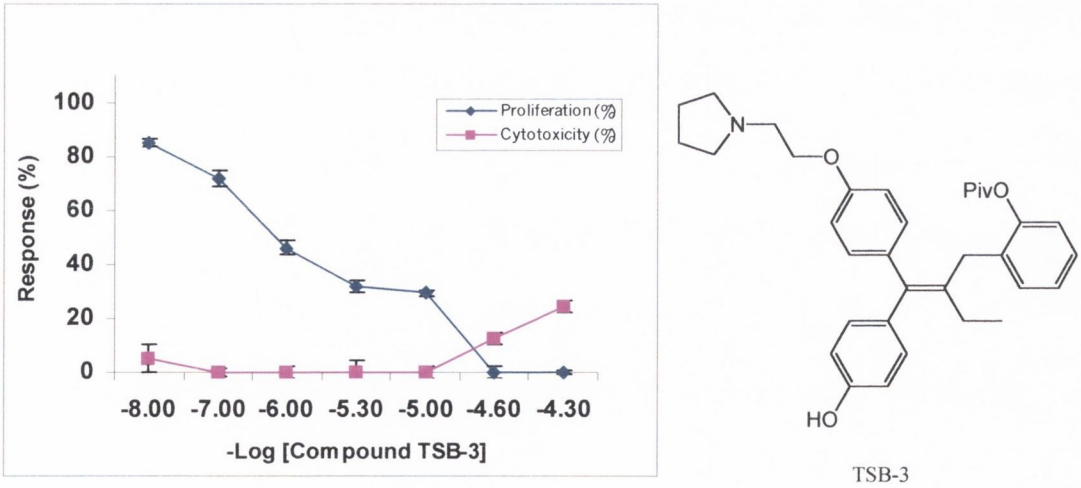
Table 4.5 Structures and antiproliferative effects of related analogues on MCF-7 cells. These related compounds were prepared in the Pharmaceutical Chemistry Laboratory, Trinity College Dublin by Dr.T.O'Sullivan. In the present work, they were evaluated for antiproliferative activity. IC_{50} values: the concentration required to inhibit 50% of MCF-7 growth. Values represent the mean ± S.E.M (error values x 10^{-6}) for two experiments performed in triplicate.



[BRI049]

Figure 4.8 Compound (BRI049) inhibited proliferation and induced cytotoxicity of MCF-7 cells

MCF-7 cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for 24 hours. After this period, various concentrations (0.1nM-50μM) of (BRI049) were added and the cells were left for a further 72 hours. Determination of cell proliferation was carried out using the MTT assay described in section 4.2.3.1 and the cytotoxicity was evaluated using the LDH assay as described in section 4.2.3.2. Figure 4.8 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates that the error was smaller than the size of the symbol

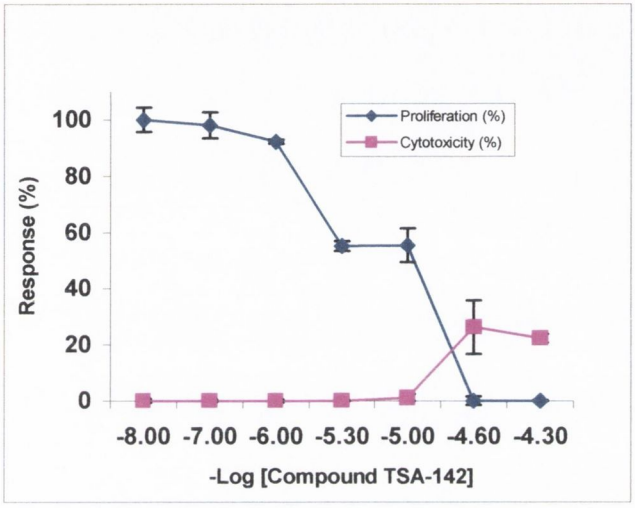


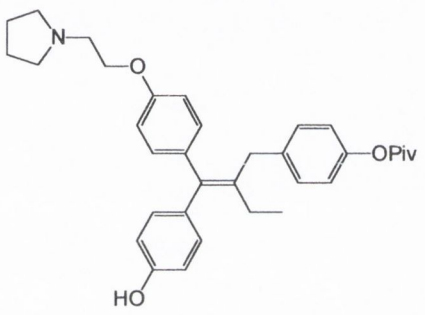
TSB-3

Figure 4.9 Compound (TSB-3) inhibited proliferation and induced cytotoxicity of MCF-7 cells

MCF-7 cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for 24 hours. After this period, various

concentrations (0.1nM-50μM) of (TSB-3) were added and the cells were left for a further 72 hours. Determination of cell proliferation was carried out using the MTT assay described in section 4.2.3.1 and the cytotoxicity was evaluated using the LDH assay as described in section 4.2.3.2. Figure 4.9 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.





TSA-142

Figure 4.10 Compound (TSA-142) inhibited proliferation and induced cytotoxicity of MCF-7 cells

MCF-7 cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for 24 hours. After this period, various concentrations (0.1nM-50μM) of (TSA-142) were added and the cells were left for a further 72 hours. Determination of cell proliferation was carried out using the MTT assay described in section 4.2.3.1 and the cytotoxicity was evaluated using the LDH assay as described in section 4.2.3.2. Figure 4.10 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

4.3.3. Conclusion

Examination of the antiproliferative and cyotoxicity profiles documented for the novel Tamoxifen analogues in Tables 4.1-4.5 indicates that many of the compounds exhibit antiproliferative activity comparable to that of tamoxifen. In order to proceed 15

compounds were selected for further testing. The compounds selected are displayed in Tables 4.1 and 4.5 (blue section) and are all very similar in structure or contain the same substitutents in the same positions therefore suggesting an affinity of these particular type of structures[225] for the estrogen receptor. Compounds such as TSB-33, and TSB-3 have a pyrrolidinyl sidechain and a hydroxy substitutent in common and both exhibit antiproliferative activity comparable to tamoxifen (see Table 4.5).

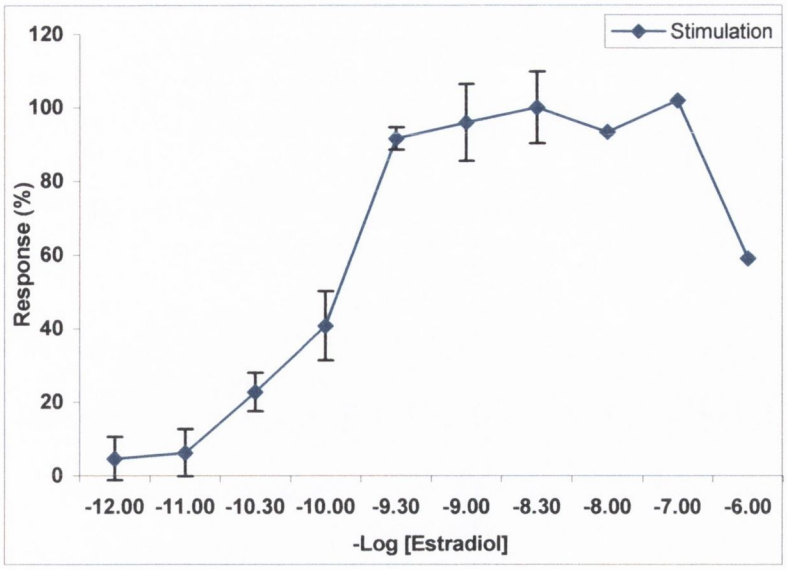
4.3.4. Alkaline phosphatase assay results

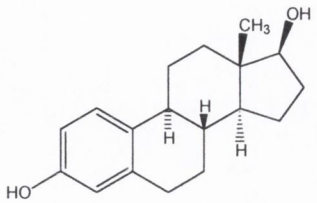
The alkaline phosphatase assay was performed as a means of measuring the estrogenic character of the analogues selected from Tables 4.1 and 4.5 (blue section) and to assess their ability to compete with estradiol for the ER.

A batch of Ishikawa cells[211] were obtained from Prof. R. Hochberg who developed the alkaline phosphatase assay in Yale University Connecticut and the assay was performed as described in section 4.2.6.

The cells were cultured as described in section 4.2.2.3 and the medium was changed 24 hours before the experiment was initiated. The cells were trypsinised and seeded at $2.5x10^4$ cells per well (in 100µl) in Hams F-12/dulbeccos 1:1 phenol free medium (with stripped calf serum) in 96-well microtiter plates (greiner bio-one, cellstar). The Ishikawa cells were then treated with and without 1nM estradiol for 4 days. According to R. Hochberg *et al.*[212], three days produce a significant stimulation and after 6 days there is more than a 20-fold stimulation therefore 4 days is adequate for the assay. AlkP activity is consistently low in the control wells, approximately 0.1.

After four days the cells were treated as described in section 4.2.5 and the assay was performed. Generally 20 minutes to one hour was a sufficient length of time to wait for development of the yellow colour before reading.





Estradiol

Figure 4.11 Estradiol induced proliferation of Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.001nM-1μM) of estradiol were added and the cells were left for a further 96 hours. Determination of cell proliferation was carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.11 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

Figure 4.11 displays the estrogenic effects of estradiol on AlkP in Ishikawa cells. Previous reports have demonstrated that on treatment of Ishikawa cells with various concentrations of estradiol optimum alkaline phosphatase activity[212] is achieved at 1nM (1 x 10^{-9}). The results presented in Figure 4.11 are in agreement with these previous reports with an IC_{50} value of 1.7 x 10^{-9} thus validating the assay.

For testing the analogues the plate was sectioned off into three as shown in Figure 4.12 for treatment with; ethanol (control 0.1%), estradiol 1nM plus concentrations of selected analogue (0.1nM-1μM) (ethanol 0.1%), concentrations of selected analogue (0.1nM-1μM) (ethanol 0.1%).

Figure 4.12 below is an example of a successful alkaline phosphatase assay for a potential anti-estrogen with similar results to tamoxifen. The white section shows no

stimulation of alkaline phosphatase activity as expected as there was no estradiol present in these wells. The yellow section, which was treated with 1nM estradiol, exhibits optimum stimulation. The blue section shows very little stimulation and therefore the analogue can be described as weakly estrogenic. Finally treatment of the Ishikawa cells in the red section with both estradiol and the analogue appears to suggest a blocking of the effect of estradiol by the analogue starting at a concentration of 100nM, which would confirm this analogue as a good anti-estrogen.

B	E_2 1nM	0.1nM	1nM	10nM	100nM	1μm	0.1nM + E_2 1nM	1nM + E_2 1nM	10nM + E_2 1nM	100nM + E_2 1nM	1μm + E_2 1nM
0.15	0.99	0.34	0.2	0.2	0.2	0.14	1.03	1.01	0.98	0.38	0.2
0.11	1.09	0.32	0.3	0.19	0.1	0.13	1.05	1.0	0.88	0.3	0.19
0.14	1.07	0.36	0.3	0.18	0.2	0.14	1.10	0.99	0.90	0.31	0.23
0.12	1.01	0.33	0.2	0.22	0.2	0.15	1.09	1.0	0.96	0.34	0.24

Figure 4.12 Alkaline phosphatase assay performed on Ishikawa cells

White section = control (0.1 % ethanol)
Yellow section = 1nM estradiol (100% proliferation)
Blue section = treated with analogue (concentrations 0.1nM-1μM)
Red section = treated with analogue (concentrations 0.1nM-1μM) and 1nM estradiol

The potential anti-estrogens that had been selected after examination of the MTT and LDH assay results were then tested with the Ishikawa cell line according to the method described in section 4.2.6 and the results are recorded in this section.

Figures 4.13 to 4.20 are representative of the estrogenic and anti-estrogenic AlkP activities (in Ishikawa cells) of the analogues displayed in Table 4.6.

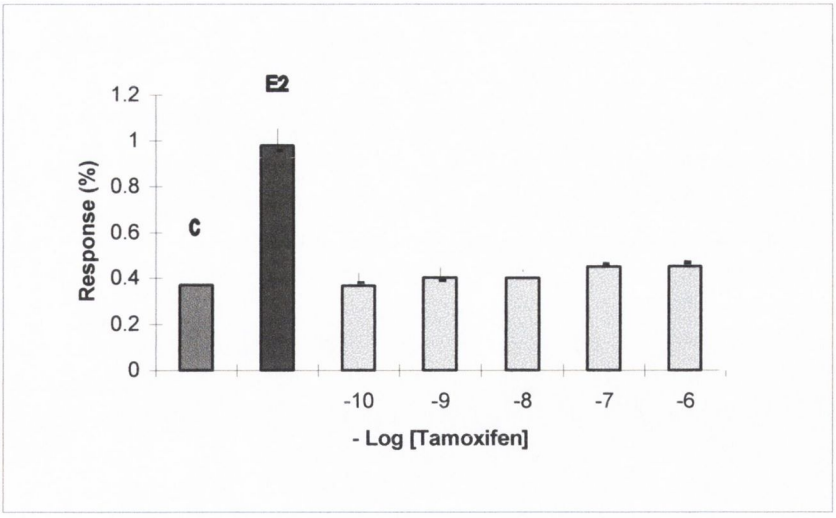
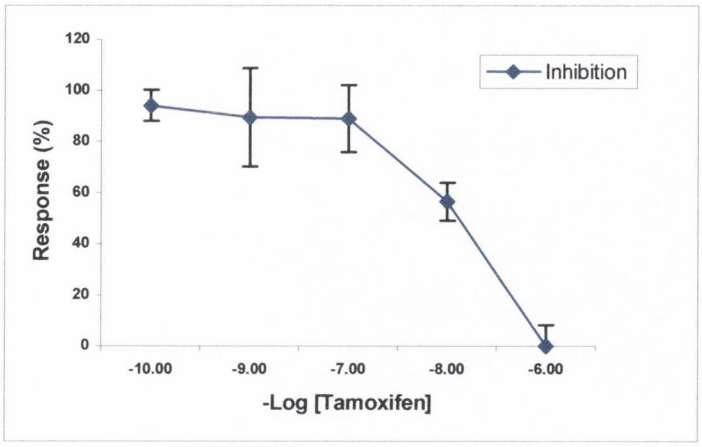
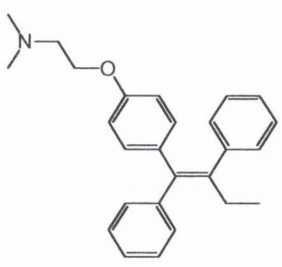


Figure 4.13 Tamoxifen induced proliferation of Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.1nM-1μM) of tamoxifen were added combined with 1nM estradiol were added and the cells were left for a further 96 hours. Determination of cell proliferation was carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.13 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

As can seen in Figure 4.13 TAM has an estrogenic effect on the Ishikawa cells (4%, Table 4.6). This is known side effect for TAM patients and is also reported by R.Hochberg *et al.* [212], the developers of the alkaline phosphatase assay.



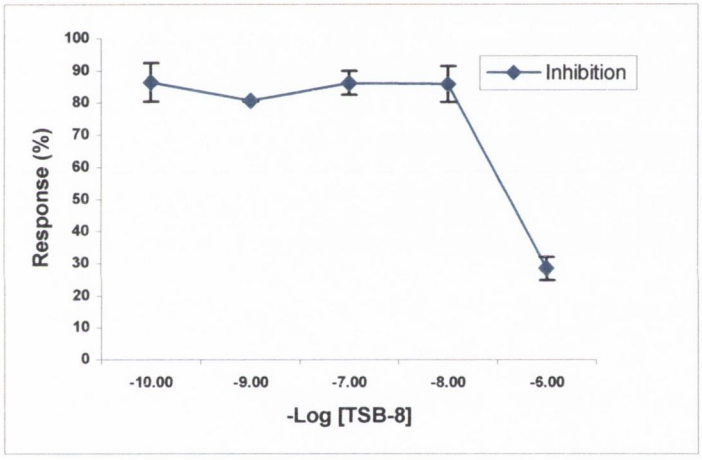


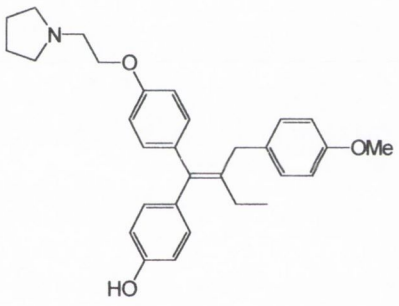
Tamoxifen

Figure 4.14 Tamoxifen inhibition of estradiol induced proliferation of Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.1nM-1μM) of tamoxifen were added combined with 1nM estradiol and the cells were left for a further 96 hours. Determination of cell proliferation was carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.14 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

Figure 4.14 displays TAM as an estrogen antagonist where it reduces the estradiol stimulated AlkP with an IC_{50} value of 0.17μM. TAM, while antagonistic at high concentrations was 100-fold less active than OHT as seen in Table 4.6 below.





TSB-8

Figure 4.15 Compound TSB-8 inhibition of estradiol stimulation of Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.1nM-1μM) of TSB-8 combined with 1nM of estradiol were added and the cells were left for a further 96 hours. Determination of cell proliferation was carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.15 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

Figure 4.15 represents an IC_{50} value of 1.15μM for TSB-8, which is 10-fold less active than TAM. Figure 4.16 displays an estrogenic stimulation of 11% which when

compared with TAM it is more estrogenic. Despite these results the analogue was still considered for further testing as the differences between results for TSB-8 and TAM results were not extreme.

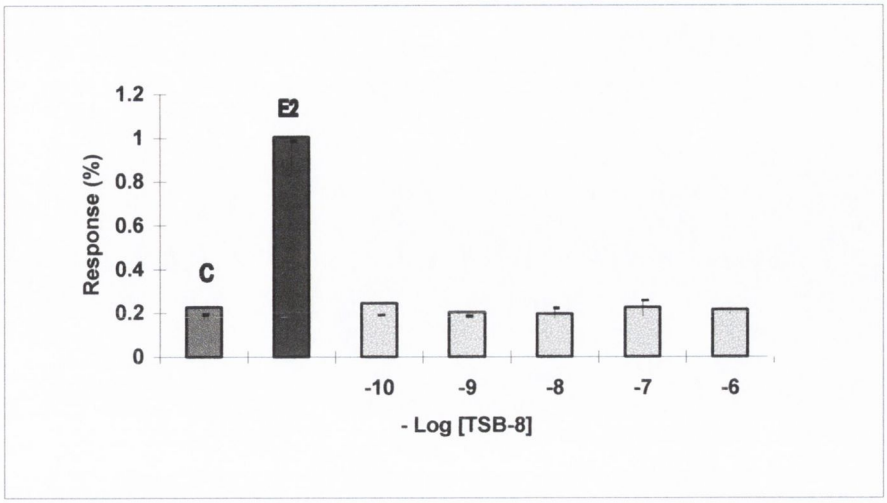
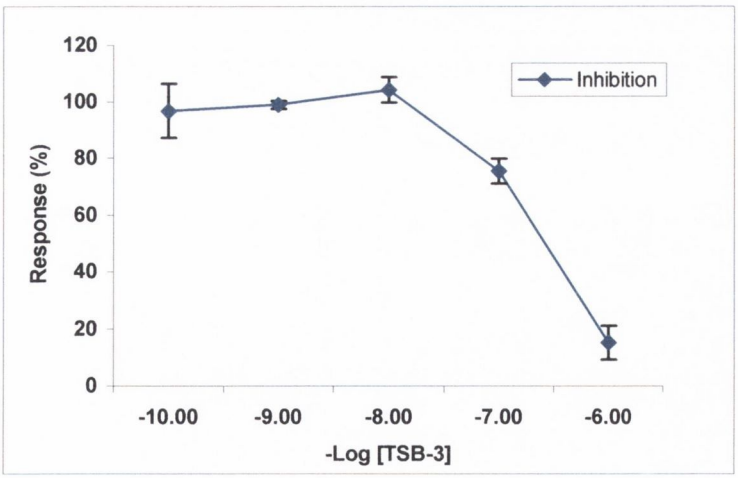
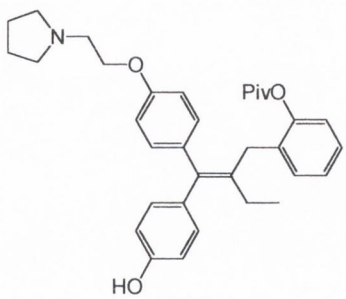


Figure 4.16 Compound TSB-8 induced proliferation of Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.1nM-1μM) of TSB-8 were added combined with 1nM estradiol was added and the cells were left for a further 96 hours. Determination of cell proliferation was carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.16 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.





TSB-3

Figure 4.17 Compound TSB-3 inhibition of estradiol stimulation in Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.1nM-1μM) of tamoxifen were added combined with 1nM estradiol

was added and the cells were left for a further 96 hours. Determination of cell proliferation was carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.17 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

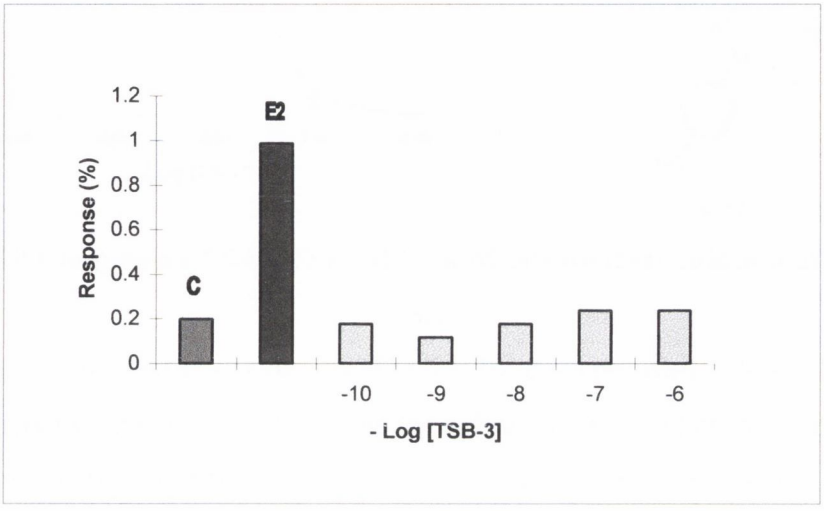
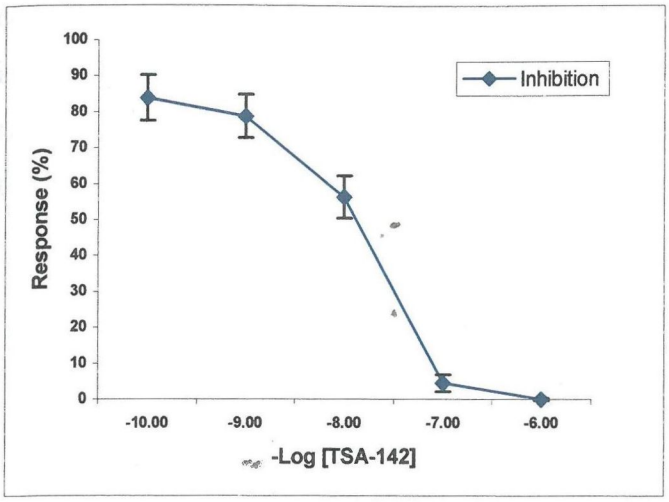


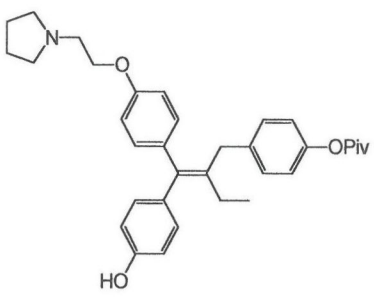
Figure 4.18 Compound TSB-3 induced proliferation of Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.1nM-1μM) of TSB-3, combined with 1nM estradiol, were added and the cells were left for a further 96 hours. Determination of cell proliferation was carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.18 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

Figure 4.18 displays a low estrogenic characteristic for TSB-3 of 2.5%, which is lower than TAM (4%) and therefore this analogue was considered for further testing as this analogue has potential as an anti-estrogen.

Figure 4.19 and Figure 4.20 represent TSA-142, which has an IC_{50} value of 0.01μM and is therefore 10-fold more antagonistic to estradiol in Ishikawa cells than TAM. Also it has a low estrogenic stimulation of 5%. This analogue has great potential as an anti-estrogen.





TSA-142

Figure 4.19 Compound TSA-142 inhibition of estradiol stimulation in Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.1nM-1μM) of TSA-142 combined with 1nM of estradiol were added and the cells were left for a further 96 hours. Determination of cell proliferation was carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.19 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

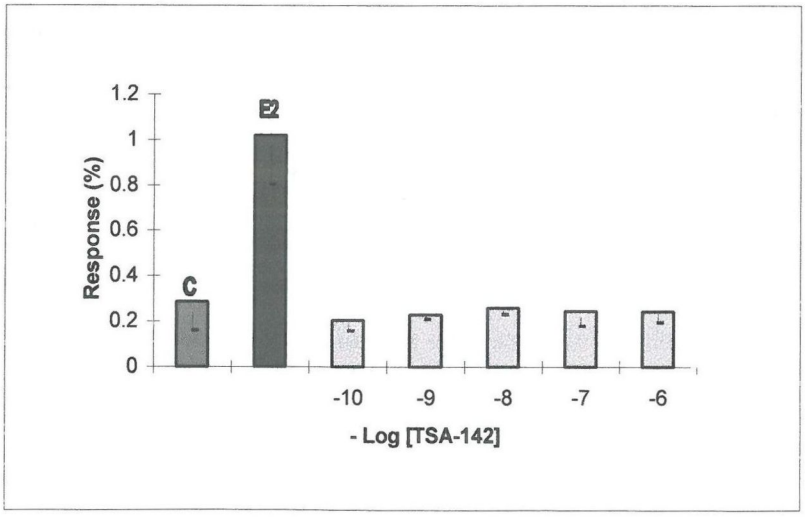


Figure 4.20 Compound TSA-142 induced proliferation of Ishikawa cells

Ishikawa cells were seeded at a density of 2.5 x 10^4 cells per well in 96-well plates and allowed adhere to the surface of the wells for at least 5 hours. After this period, various concentrations (0.1nM-1M) of TSA-142 combined with 1nM of estradiol were added and the cells were left for a further 96 hours. Determination of cell proliferation was

carried out using the alkaline phosphatase assay described in section 4.2.6. Figure 4.20 is representative of an experiment that was carried out three times. Each point represents the mean ± S.E.M. of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

Compound Code	Antiproliferative Activity in MCF-7 cells, IC_{50} value (μM)	% Cell death in MCF-7 Cells	Anti Estrogenic Activity in Ishikawa cells IC_{50} value (μM)	% Estrogenic effect in Ishikawa cells
TSB-19	1.6	0 @ 10μm	0.04 ± 0.0	0.0
TSA-144	5.2	16.3 @ 10μm	0.23 ± 0.2	4.0
TSB-21	2.1	0 @ 10μm	0.08 ± 0.0	7.0
TSB-20	1.6	0 @ 10μm	0.06 ± 0.0	6.0
TSA-142	1.1	20 @ 10μm	0.01 ± 0.0	5.0
TSB-3	0.7	12 @ 10μm	0.21 ± 0.1	2.5
BRI049	0.17	0 @ 10μm	0.21 ± 0.1	11.0
TSA-48	8.0	11 @ 5μm	4.86 ± 0.0	0.0
TSA-60	4.4	2.3 @ 5μm	50.94 ± 0.5	1.0
TSA-150	4.9	13 @ 10μm	0.14 ± 0.2	10
TSB-8	0.9	18 @ 5μm	1.15 ± 0.1	4.0
TSA-51	9.4	18.2 @10μm	2.34 ± 0.1	1.0
BRI037	5.9	13.3 @ 5μm	1.40 ± 0.3	7.0
BRI042	7.5	14.8 @ 10μm	0.11 ± 0.4	4.0
TSA-39	45.9	0.8 @ 10μm	0.24 ± 0.1	2.0
Tamoxifen	3.8	24 @ 10μm	0.17 ± 0.0	4.0
Hydroxy tamoxifen		0 @ 10μm	0.01 ± 0.0	0.0

Table 4.6 Combined assay results for MCF-7 and Ishikawa cells. IC_{50} values: the concentration required to inhibit 50% of Ishikawa growth. Values for Anti-Estrogenic Activity, induced by analogues displayed in Table 4.6 above, represent the mean ± S.E.M (error values x 10^{-6}) for three experiments performed in triplicate.

The results of the remaining compounds are displayed in Table 4.6 above. All fifteen of the alkaline phosphatase assayed analogues were comparable either agonistically or antagonistically to TAM. The results show that none of the selected compounds exhibit any significant agonistic estradiol like activity in the Ishikawa cells. As for antagonistic effects, one compound, TSA-142 was of particular interest as it showed high inhibition

of estradiol in the Ishikawa cells and therefore was considered to be the most potent of the analogues. Once again a selection of the most potent analogues was made and these compounds were tested further. TSB-19, TSB-21, TSB-20, TSA-142, TSA-150 and BRI042 all exhibit IC50 values lower than TAM and similar to OHT. Bri049 is more interesting as it's a potent antagonist in MCF-7 cells and is less estrogenic in Ishikawa cells that TAM.

All of the analogues displayed in Table 4.6 above were considered as potential anti-estrogens and therefore were all were selected for further testing to measure ER ligand-binding.

4.3.5. Ligand estrogen receptor binding assay results

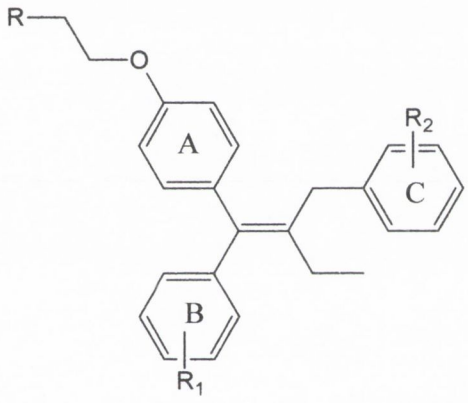


Figure 4.21 Basic Nucleus of the selected Analogues

There is a strong dependence of anti-tumor effect and estradiol-receptor affinity on the number and position of substituents on the aromatic rings of the 1,1,2-triphenylbut-1-enes. Relative binding affinity (RBA) research values show that a substitution on Ring B, particularly in the *para*-position, (thus corresponding to the C_3OH of Estradiol), is necessary for a high binding affinity to the estrogen receptor. *In-vivo* Tamoxifen is converted into *para*-Hydroxytamoxifen, and is therefore a *meta*bolite with greater activity than the parent compound. Substitution on ring C causes an increase in receptor affinity. Tamoxifen and many other anti-estrogenic triphenylethylenes display substitution of a basic side chain on ring A. The basic side chain has been postulated to interact with a region of the receptor different from those with which steroidal

estrogens or diethylstilbestrol normally interact. In order to gain more knowledge about the anti-estrogenic characters of the selected analogues ER ligand-binding is a good way to go and considering the factors discussed above that are characteristic of good anti-estrogens it was expected that the selected analogues would perform well in the ligand-binding assay.

The ligand-binding assay was performed according to the method described in section 4.2.7. There are two estrogen receptors in this assay; ER-α and ER-β and they represent the estrogen receptors involved in the development of hormone induced breast cancer. As discussed in chapter one the α-receptor is thought to be involved in the growth of breast cancer tumors by activation with estrogen. It is also believed that a compound that binds well to the α-receptor can compete with estrogen for the receptor and therefore halt the growth of a tumor by blocking the estrogenic action under the assumption that it is not itself estrogenic. As for the β-receptor, in many cases if a compound binds well to this receptor, it does not bind well to the α and vice versa.

The results are combined in Table 4.7 below to give a better overall view of each of the analogues anti-estrogenic qualities. They show that the majority of the compounds tested favour the α receptor over the β each to different extents.

In the ER fluorescence polarisation assay, the IC50 values for TAM and OHT binding to ER-α and ER-β were considered to be simlar to that quoted in the Panvera ER α/β Competitor Assay protocol (Table 4.7).

Compound Code	Antiproliferative Activity in MCF-7 cells, IC_{50} value (μM)	% Cell death in MCF-7 Cells	Anti Estrogenic Activity in Ishikawa cells IC_{50} value (μM)	% Estrogenic effect in Ishikawa cells	ER Binding assay IC_{50} (μM) for	
					α	β
TSB-19	1.6	0 @ 10μm	0.04	0.0	0.12 ± 0.77	1.01 ± 0.97
TSB-8	0.9	16.3 @ 10μm	1.15	4.0	0.04 ± 0.69	0.51 ± 0.78
TSB-21	2.1	0 @ 10μm	0.08	7.0	0.08 ± 0.11	0.46 ± 0.11
TSB-20	1.6	0 @ 10μm	0.06	6.0	0.11 ± 0.14	0.89 ± 0.13
TSA-142	1.1	20 @ 10μm	0.01	5.0	0.09 ± 0.24	0.69 ± 0.22
TSB-3	0.7	12 @ 10μm	0.21	2.5	0.08 ± 0.18	1.38 ± 0.23
TSA-60	4.4	0 @ 10μm	50.94	1.0	0.12 ± 0.34	1.49 ± 0.47
TSA-150	4.9	11 @ 5μm	0.14	10.0	0.73	0.52
TSA-51	9.4	2.3 @ 5μm	2.34	1.0	0.02	0.15
TSA-144	5.2	13 @ 10μm	0.23	0.0	0.22	0.37
BRI049	0.17	18 @ 5μm	0.21	11.0	0.02	0.16
TSA-48	8.0	18.2 @10μm	4.86	0.0	0.14	0.83
BRI037	5.9	13.3 @ 5μm	1.40	7.0	0.81	168.0
BRI042	7.5	14.8 @ 10μm	0.11	4.0	1.74	3.99
TSA-39	45.9	0.8 @ 10μm	120.00	2.0	0.59	9.24
Tamoxifen	3.8	24 @ 10μm	0.17	4.0	0.07	0.17
Hydroxy tamoxifen		0 @ 10μm	0.01	0.0	0.04	0.02

Table 4.7 Combined results of assays for selected compounds. Competition assay for ER-β and ER-α using a human recombinant ER-α and ER-β and a fluorescent estrogen. IC_{50} values: the concentration of competitor that results in a half maximum shift in polarisation equals the IC_{50} of the competitor. Displacement of the fluorescent estrogen with increasing concentrations of competitor results in a lowering of polarisation values. This is a measure of the relative affinity of the competitor for the ER-β. Values in the blue section of the Table represent the mean ± S.E.M (error values x 10^{-6}) for three experiments performed in triplicate. Values for the remaining compounds in the Table were the result one experiment performed in triplicate.

Examination of the ligand-binding assay results combined with all previous assay results in Table 4.7 aided the selection of the potential anti-estrogens, in order to which analogues would be subjected to further testing. In this case all of the analogues were chosen, as they all appeared to bind very well with the ER-α. Although some

compounds clearly favoured one receptor over the other i.e. TSB-3 had a high affinity for the α-receptor and was highly selective for it over the ER-β compound. TSA-142 also showed an affinity towards the ER-α but appeared to be slightly more selective for the ER-β.

TSA-142 was found to bind to the ER-α with similar affinity to TAM, IC_{50} values of 90nM and 70nM respectively. TSA-142 interestingly has a greater affinity for ER-α than TAM with an ER-β binding IC_{50} value of 700nM whereas TAM binds to the ER-β with greater affinity than TSA-142 with an IC_{50} value of 170nM. Figure 4.22 displays the ER-β binding with TSA-142 for comparison with TAM, OHT.

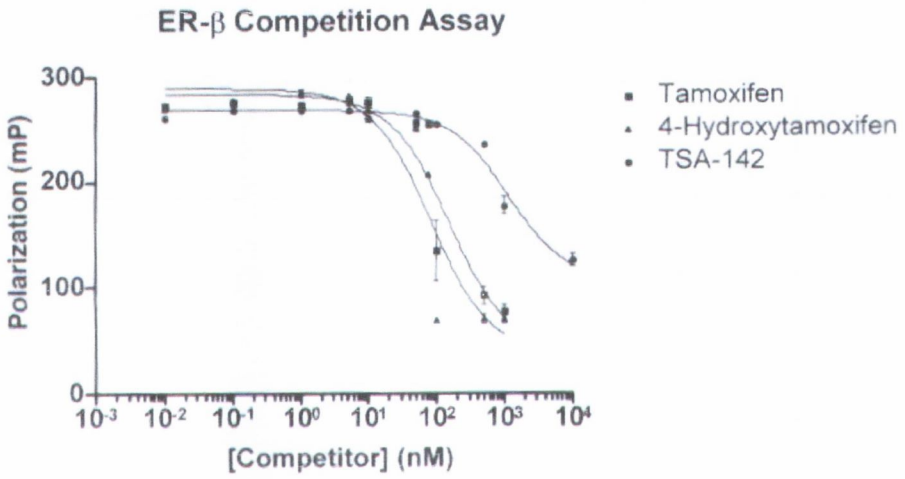


Figure 4.22 Competition assay for ER-β using a recombinant ER-β and a fluorescent estrogen. Displacement of the fluorescent estrogen with increasing concentrations of competitor results in a lowering of polarisation values. The concentration of competitor that results in a half maximum shift in polarisation equals the IC_{50} of the competitor. This is a measure of the relative affinity of the competitor for the ER-β. The IC_{50} values are displayed in Table below

Figure 4.22 displayed above is representative of the graphs produced for ER-β. Analysis of the results in Table 4.7 lead to the selection of analogues (blue section of Table 4.7) for in depth apoptotic analysis as these compounds exhibited the best ER-α binding values.

4.3.6. Results for induction of apoptosis in MCF-7 cells through FACS analysis

Flow Cytometric Analysis was performed as a means of statistically quantifying the extent of apoptosis[216] induced by TAM and the analogues displayed in Table 4.8 below. The fluorescent dye, PI interchelates with the DNA and hence, the amount of fluorescence measured per cell is proportional to the DNA content. Cells in the G_1 phase of the cell cycle have one copy of DNA and therefore, show a characteristic fluorescence pattern. Cells in G_2/M, which have two copies of the DNA in their nucleus, show double the fluorescence of G_1 populations of cells. Cells in the S-phase are actively synthesising DNA, and therefore, exhibit an intermediate amount of fluorescence. In apoptotic cells where the DNA is fragmented and packaged into apoptotic bodies, there is less fluorescence emitted. These cells show a pre-G_1 peak. The percentage of cells within this pre-G_1 is a direct measure of apoptosis[216].

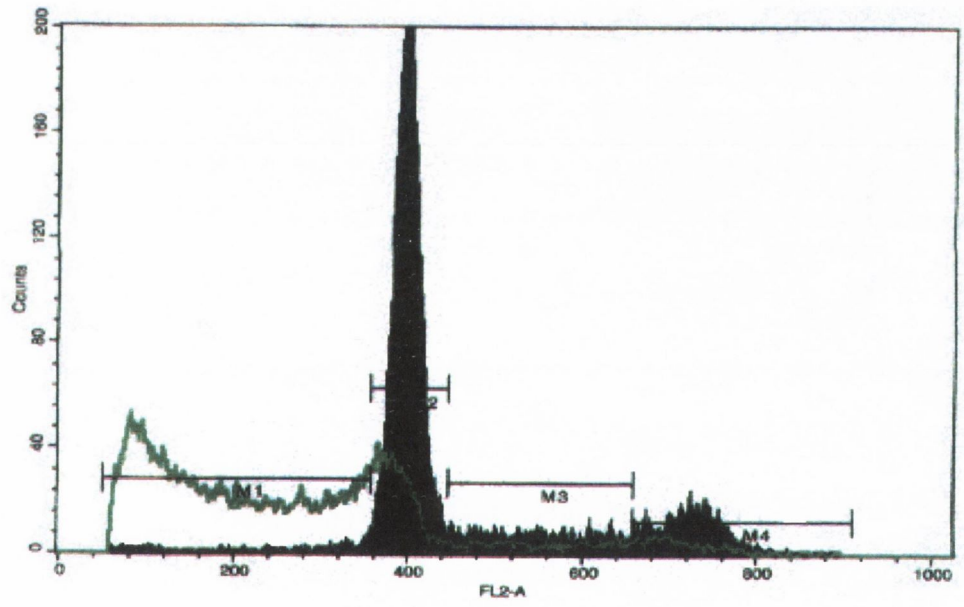


Figure 4.23 A standard FACS profile representing an ethanol treated control sample of cells. The parameters shown, M1, M2, M3, and M4, define the cell cycle phases, pre-G1, G1, S and G2/M, respectively. The overlay (green) illustrates an apoptotic cell population (cells treated with 25μM TAM for 48 hours), in which the cells are being forced out of all stages of the cell cycle and into the pre-G1 peak. The y-axis represents the number of cells (in total 10,000 cells were counted), while the axis represents the intensity of PI fluorescence. These settings were maintained throughout all sample analysis.

The MCF-7 cells were cultured and treated, as described in section 4.2.8, with ethanol (control 0.1%), tamoxifen and the fifteen compounds selected as displayed in Table 4.8 below.

Compound Code	Sub- G1	G1	S	G2/M
Control	5.24	88.1	1.59	5.21
Tamoxifen	20.48	56.00	14.80	9.42
TSB-19	15.02	65.69	9.01	11.10
TSA-144	21.1	58.41	13.11	9.45
TSB-21	30.35	59.83	4.11	6.64
TSB-20	14.49	76.55	3.08	6.46
TSA-142	18.34	63.96	10.90	7.16
TSB-3	18.45	68.38	7.20	6.81
BRI049	10.75	65.38	12.66	11.72
TSA-48	7.60	66.67	13.36	13.00
TSA-60	72.20	20.26	6.62	2.16
TSA-150	13.18	62.46	13.06	12.59
TSB-8	22.15	69.64	2.48	6.04
TSA-51	4.18	73.81	9.16	13.71
BRI037	2.21	65.97	13.50	18.44
BRI042	8.34	69.57	12.75	9.63
TSA-39	8.27	82.25	2.62	7.10

Table 4.8 Compounds selected for FACS analysis. Values for FACS analysis on MCF-7 cells treated for 24 hours with 50μM of the analogues displayed in Table 4.8 above, represent the mean ± S.E.M (error values x 10^{-6}) for three experiments performed in triplicate.

Tamoxifen induces apoptosis[216] as indicated by an increase in sub-G_1. This effect can be observed by the mapping of MCF-7 cells[213] in G_1 phase by morphology of prematurely condensed chromosomes clearly demonstrates that Tamoxifen-exposed cells accumulate in early G_1, thus provoking a transition delay which results in proliferation decrease. It is also known to decrease the local production of Insulin-like growth factor I (IGF-I) by surrounding tissues (IGF-I is a *para*crine growth factor for the breast cancer cell). There are several other hypothetical mechanisms of action of Tamoxifen that may not be related to action through the ER on the basis of basic laboratory studies. In fact experimental evidence has demonstrated that Tamoxifen might exert its anti-tumor effect through a variety of biologic mechanisms unrelated to its binding to tumor ER. These include a biologic response activity with a modulation

by inhibition of natural killer activity, inhibition of protein kinase C, a decrease in insulin-like growth factor levels, and antiangiogenic activity.

The results shown in Table 4.8 above show some clear differences between some of the analogues that may account for the different structures exhibiting similar anti-estrogenic characteristics in the previous. For comparison with previous results of each analogue all the results are combined in Table 4.9 below. One compound in particular TSA-60 previously appeared to have similar anti-estrogenic characteristics to the other analogues but with the FACS analysis it can be seen that its high activity was due to its apoptotic activity in the MCF-7 cells (Majority of cells are in the sub G1 phase).

Compound Code	Anti proliferative Activity in MCF-7 cells, IC_{50} value (μM)	% Cell death in MCF-7 Cells	Anti Estrogenic Activity in Ishikawa cells IC_{50} value (μM)	% Estrogenic effect in Ishikawa cells	ER Binding assay IC_{50} (μM)		% apoptosis (24 hrs) (50μM)
					α	β	
TSB-19	1.6	0 @ 10μm	0.04	0	0.12	1.01	15.02 ± 0.00
TSB-8	0.9	16.3 @ 10μm	1.15	4.0	0.04	0.51	22.15 ± 0.00
TSB-21	2.1	0 @ 10μm	0.08	7.0	0.08	0.46	30.35 ± 0.00
TSB-20	1.6	0 @ 10μm	0.06	6.0	0.11	0.89	14.49 ± 0.00
TSA-142	1.1	20 @ 10μm	0.01	5.0	0.09	0.69	18.34 ± 0.00
TSB-3	0.7	12 @ 10μm	0.21	2.5	0.08	1.38	18.45 ± 0.00
TSA-60	4.4	0 @ 10μm	50.94	1.0	0.12	1.49	72.20 ± 0.00
TSA-150	4.9	11 @ 5μm	0.14	10.0	0.73	0.52	13.18 ± 0.00
TSA-51	9.4	2.3 @ 5μm	2.34	1.0	0.02	0.15	4.18 ± 0.00
TSA-144	5.2	13 @ 10μm	0.23	0	0.22	0.37	21.10 ± 0.00
BRI049	0.17	18 @ 5μm	0.21	11.0	0.02	0.16	10.75 ± 0.00
TSA-48	8.0	18.2 @10μm	4.86	0	0.14	0.83	7.60 ± 0.00
BRI037	5.9	13.3 @ 5μm	1.40	7.0	0.81	168	2.21 ± 0.00
BRI042	7.5	14.8 @ 10μm	0.11	4.0	1.74	3.99	8.34 ± 0.00
TSA-39	45.9	0.8 @ 10μm	120.00	2.0	0.59	9.24	8.27 ± 0.00
Tamoxifen	3.8	24 @ 10μm	0.17	4.0	0.07	0.17	20.48 ± 0.00
Hydroxy tamoxifen		0 @ 10μm	0.01	0	0.04	0.02	5.24 ± 0.00

Table 4.9 Combined results of selected compounds for further testing. Values for FACS analysis on MCF-7 cells treated for 24 hours with 50μM of the analogues in the blue section of Table 4.9 above, represent the mean ± S.E.M (error values x

10^{-6}) for three experiments performed in triplicate. The remaining values for FACS analysis represent one experiment performed in triplicate.

The analogues in the blue section of Table 4.9 are of particular interest and can be developed for further testing.

4.3.7. Lipophilicity of selected analogues

Lipophilicity[226] of the selected analogues was calculated in order to predict the metabolic fate of the analogues and is calculated as Clog P An optimum value Clog P for drugs is estimated to be 4.2. lipophilic defines a compound soluble in lipids i.e. body fats therefore highly lipophilic compounds reside longer in the body than weakly lipophilic compounds. If a compound is weakly lipophilic[227] then it hydrophilic and is more likely to dissolve in water and this would lead to a faster rate of excretion from the body.

Compound Code	C log P Compound Lipophilicity values
TSB-19	7.78
TSB-21	6.03
TSB-20	6.35
TSA-142	7.81
TSB-3	7.81
TSA-60	7.72
TSB-8	7.14
Tamoxifen	6.64
4-Hydroxy tamoxifen	5.97

Table 4.10 C log P values for analogues

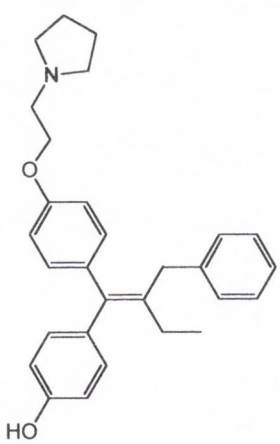
As can be seen in Table 4.10 above there is very little difference between the values displayed for the selected analogues and tamoxifen and hydroxytamoxifen. It can be concluded then that the compounds that have lipophilic values close to that of tamoxifen. Compounds TSB-21 and TSB-20 appear to be the closet in lipophilc values to tamoxifen and therefore could be developed for further testing.

4.4. Discussion

The development of novel SERMs should be viewed in the context of two ER subtypes, ER-α and ER-β, which have differing affinities and responsiveness to various anti-estrogens, and differing tissue distribution and effectiveness at gene regulatory sites. TSA-142, with its ER-α selectivity and efficacy in inducing apoptosis in the MCF-7 cell line, has the potential for future studies with the aim of the eventual safe, clinical use of this compound in the prevention and/or treatment of breast cancer. futher studies into the mechanisms of cyctotoxic action of this compound and other TSA compounds, will aid in a fuller understanding of breast tumour cells, the elucidation of the apoptotic pathway and may prove invaluable in chemotherapeutic drug research.

4.5. Computational Modeling

To gain insight into the mode of binding of the second generation compounds a brief computational docking study was undertaken using compound BRI049 (Figure 4.24), which is the most potent ligand of the series prepared.



[BRI049]

Figure 4.24 Structure of BRI049

The crystal structure used in the docking studies was that obtained from the cocrystallisation of ERα with 4-hydroxytamoxifen (1c) (OHT) as found in the PDB database (reference code 3ERT)[39]. After removal of 4-hydroxytamoxifen from the ligand binding site, FlexX was used to provide thirty putative docking poses for compound BRI049, from which the top ranked binding mode scored by the DrugScore

function was selected. The docked geometry for compound BRI049 in comparison to the experimental binding mode for OHT(1c) is illustrated in Figure 4.24.

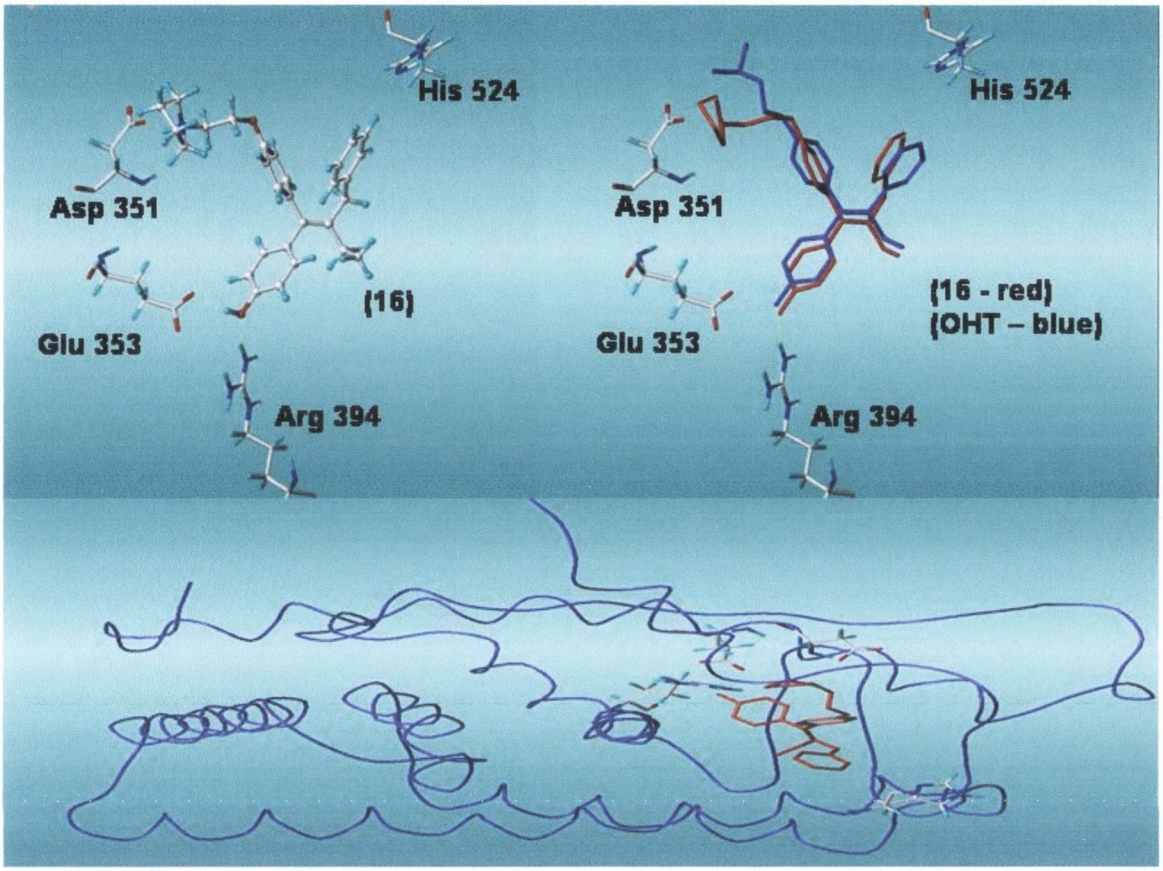


Figure 4.25 FlexX docked structure for BRI049

Top Left - BRI049 in the context of the ER active site, hydrogen bonding to Arg394, Glu353 and Asp 351. Top Right – docked mode for BRI049 overlaid with experimental binding mode of 4-hydroxytamoxifen (OHT). Bottom – BRI049 orientation in the human ER alpha ligand binding domain.

It is evident that incorporation of the additional methylene spacing group, while facilitating extra flexibility in the structure, also allows the compounds to adopt the required arrangement for liganding in an established antiestrogenic mode. The overlap of the docked geometries for BRI049 and OHT is high, differing only in the areas of the structure where the benzylic methylene is introduced, and in the orientation of the basic side chain, which in compound BRI049 is better oriented for hydrogen bonding interactions with Asp 351 in the active site than the OHT basic nitrogen orientation.

4.5.1. Ligand protein contacts

To quantify the relative interactions predicted for the ligand BRI049 in comparison to the experimental results observed for 4-hydroxytamoxifen and raloxifene, a simple ligand-protein contact (LPC) analysis was carried out, referring to the following specific residues: Glu 353 and Arg 394 (which are known to be crucial in the binding of Ring B of the ligands to the active site), His 524, (known to be an important estrogenic residue in the binding of diethylstilbestrol and estradiol) and Asp 351which is well recognized as an important antiestrogenic residue associated with the binding of the basic side chain nitrogen.

Table 4.11 illustrates the key predicted LPC data for the compound BRI049 with the specific residues chosen together with the residue contacts calculated from the existing crystal structures of 4-hydroxytamoxifen and raloxifene. It can be seen that the flexible ligands series as exemplified by compound BRI049 are predicted be accommodated in the receptor LBD in a very similar orientation to the known antiestrogenic compounds, relative to the residues Asp 351, Arg 394 and Glu 353. The primary divergence from receptor interactions associated with known ER ligands occurs in the region of His 524, where the flexibility of benzylic portion of compound BRI049 orients ring C of the structure differently. Interaction with His 524 is not essential for potency, but may increase receptor binding affinity, and the potential interactions of our scaffold with this receptor will be investigated in future work.

Ligand	Residue	Distance[d]	Surface[e]	HB[f]
RAL[a]	351 Asp	2.7	30.9	+
OHT[b]	351 Asp	3.2	29.6	+
BRI049[c]	351 Asp	2.7	24.7	+
RAL[a]	353 Glu	2.4	32.7	+
OHT[b]	353 Glu	2.4	34.0	+
BRI049[c]	353 Glu	2.9	30.8	+
RAL[a]	394 Arg	3.0	22.0	+
OHT[b]	394 Arg	3.0	23.0	+
BRI049[c]	394 Arg	2.9	23.6	+
RAL[a]	524 His[g]	3.0	22.0	+
OHT[b]	524 His	3.0	23.0	-
BRI049[c]	524 His	3.5	9.4	-

Table 4.11 Summary of key Protein-Ligand contacts

Table 4.11 details:

[a]RAL : raloxifene(2), from PBD 1ERR[47]

[b]OHT : 4-hydroxytamoxifen(1c), from PDB 3ERT[39]

[c]BRI049 : from FlexX docked structure above

[d]Distance - nearest distance (Angstoms) between atoms of the ligand and the residue

[e]Surf - contact surface area (Angstroms^2) between the ligand and the residue

[f]HB - hydrophilic-hydrophilic contact (hydrogen bond)

[g] - Hydrogen bonding to His 524 is a key feature in molecules with an estrogenic core and is not predicted for OHT or BRI049 as they do not possess a second hydrogen bonding feature on ring C, as found in Raloxifene.

4.6. Conclusion

Many series of analogues were prepared and analysed in this chapter in order to assess their potentials as treatments for breast cancer. Novel compounds possessing an ether or alcohol function (BRI049) on the Rings B or C appeared to have lower IC_{50} values than other analogues such as the methoxy substituted analogues.

The dihydrochalcones did not possess results comparable to that of tamoxifen but when they were coupled with ketones to form the highly flexible tamoxifen analogues their IC^{50} values increased 100 fold.

Many other analogues (see Table 4.4) had similar IC_{50} values to tamoxifen but were not pursued for further testing. Therefore future work will focus on the development of these analogues.

5. Experimental Section

5.1. Experimental Data

5.1.1. General experimental Details

Melting points were measured on a Gallenkamp apparatus and are uncorrected. Infra red (IR) spectra were recorded on a Perkin Elmer FT-IR Paragon 1000 spectrometer. Band positions are given in cm^{-1}. Solid samples were analysed by KBr disc, while oils were analysed as neat films on NaCl plates. ^{1}H, ^{13}C and ^{19}F Nuclear magnetic resonance (NMR) spectra were recorded at 20°C on a Brucker DPX 400 spectrometer (400.13MHz ^{1}H; 100.61MHz ^{13}C; 376.47MHz ^{19}F) in $CDCl_3$ (internal standard tetramethylsilane (TMS)). For $CDCl_3$, ^{1}H NMR spectra were assigned relative to the TMS peak at 0.00δ and ^{13}C NMR spectra were assigned relative to the centre peak of the $CDCl_3$ triplet at 77.00ppm. ^{19}F NMR spectra were not calibrated. Coupling constants are reported in Hertz. For ^{1}H NMR assignments, chemical shifts are reported: shift value (number of protons, description of absorption (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (J), where applicable, proton assignment). Low resolution mass spectra (LRMS) were acquired on a Hewlett-Packard 5973 MSD GC-MS system in electron impact mode. High resolution molecular ion determinations (HRMS) were acquired on micro mass spectrometer (EI mode) at the Department of Chemistry, Trinity College Dublin. R_f values are quoted for thin layer chromatography on silica gel Merck F-254 plates. Flash column chromatography was carried out on a Merck Kieselgel 60F254 (platter 20cm x 20cm x 2mm). Compounds were visually detected with UV at 254nm.

5.2. General experimental data

5.2.1. General method - Pivaloylation of hydroxy substituted benzophenones

The hydroxy substituted benzophenone (0.0093M) was stirred with trimethylacetyl chloride (0.0093M) and potassium hydroxide in acetone (30ml) under gentle heat overnight. The solution was diluted with water (50ml) and washed with dichloromethane (3 x 100ml), which removed any dipivaloylated product that may have been produced. The aqueous layer was then basified with sodium hydroxide and extracted with

dichloromethane. The organic layers were combined, dried (sodium sulphate) and the solvent removed *in vacuo*. The product was purified by flash chromatography.

5.2.2. Synthesis of 4-hydroxy-4'-pivaloyloxybenzophenone (BRI002)[140]

The reaction was performed many times with varying conditions in order to optimise the reaction as discussed in section 2.6.2.2 of chapter 2. Under the optimised reaction conditions 4,4'-dihydroxybenzophenone [1] (0.0093M), trimethylacetyl chloride (0.0093M) and potassium hydroxide (0.0093M), in acetone (30ml) were stirred overnight under gentle heat. The solution was reduced under reduced pressure and the resulting mixture was dissolved in water (50ml) and washed with dichloromethane (3 x 100ml) to remove the unreacted starting material. The aqueous phase was then basified and the products extracted with dichloromethane (3 x 100ml). The dichloromethane layers were then combined and removed *in vacuo* and the monopivaloyloxy and dipivaloyloxybenzophenones were separated by flash chromatography (eluant: DCM:Hexane 19:1). Yellow crystals were obtained from dichloromethane (40%), (m.p. 175°C), [lit. m.p. 172.5-175 °C], (R_f 0.25 dichloromethane:ethyl acetate 19:1). IR ν_{max} (KBr) 3458, 2976 (CHs), 1735 (C=O of pivaloyl), 1640 (C=O), 1605 (C=C), 1592 (CH_2), cm^{-1}. ^1H NMR δ($CDCl_3$) 1.37 (9H, s, $COCCH_3$), 6.60 (1H, s, OH), 6.85-6.88 (2H, d, H-3', H-5'), 7.14-7.16 (2H, d, H-3'', H-5''), 7.7-7.73 (2H, d, H-2', H-6'), 7.73-7.76 (2H, d, H-2'', H-6''). ^{13}C NMR δ($CDCl_3$) 27.08 ($(CH_3)_3$), 39.26 ($C(CH_3)_3$), 115.29 (C-3', C-5'), 121.41 (C-3'', C-5''), 129.82 (C-1', C-1''), 131.36(C-2', C-6'), 132.87 (C-2'', C-6''), 154.10 (C-4''),160.38 (C-4'), 179 (C=O, OPiv), 195.02 (C=O).

5.2.2.1. Synthesis of 2-pivaloyloxy-4'-methoxybenzophenone (BRI020)[229]

Many attempts were made, following general method 5.2.1 and varying reaction conditions, to synthesise 2-pivaloyloxy-4'-methoxybenzophenone (BRI020). A final attempt involved the stirring of 2-hydroxy-4'-methoxybenzophenone (BRI018) (0.004M) in acetone (20ml) at room temperature with potassium hydroxide (0.0356M) for 3 hours. Trimethylacetyl chloride (0.0248M) was added and the reaction was left stirring overnight. The TLC seemed to suggest that some product had formed. The reaction mixture was poured into water (50ml) and the water was basified using sodium hydroxide. The product was extracted using dichloromethane (3 x 100ml). The dichloromethane layers were

combined, dried (sodium sulphate) and removed under reduced pressure. The product was purified by flash chromatography (eluant: DCM:ethyl acetate 19:1) and a yellow oil (40%) were obtained. (R_f 0.4 hexane:diethyl ether 5:1). IR ν_{max} (film) 2926 (CHs), 1748 (C=O of pivaloyl), 1653 (C=O), 1586 (CH_2), cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.07 (9H, s, OPiv), 3.84 (3H, s, OCH_3), 6.9-6.92 (2H, d, H-3', H-5'), 7.15-7.17 (1H, d, H-6''), 7.28-7.32 (1H, t, H-4''), 7.45-7.50 (2H, t, H-3'', H-5''), 7.79 (2H, d, H-2', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 26.45 ($(CH_3)_3$), 31.09 ($C(CH_3)_3$), 54.89 (OCH_3), 113.16 (C-3', C-5'), 122.36 (C-3''), 124.98 (C-5''), 129.06 (C-6''), 129.81 (C-1''), 130.68 (C-2', C-6'), 131.83 (C-4''), 147.90 (C-2''), 163.35 (C-1'), 175.70 (C=O, OPiv),192.70 (C=O).

5.2.3. Synthesis of 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028)[119]

5.2.3.1. Attempted synthesis of 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028)[119]

This reaction was first attempted in the same reaction vessel as the reaction for the formation of the starting material. 3,4'-Dimethoxybenzophenone (BRI006) was refluxed with pyridine (50ml) for two hours. Assuming 3,4'-dihydroxybenzophenone (BRI016) (0.0093M expected product) was synthesised, trimethylacetyl chloride (0.0093M) was added to the reaction and refluxed for another 2 hours. None of the desired product was recovered.

5.2.3.2. Synthesis of 4-hydroxy-3'-pivaloyloxybenzophenone (BRI028)[119]

4,3'-Dihydroxybenzophenone (BRI016) (0.003M) was stirred in a round bottom flask (placed on ice) for one hour in N,N-dimethylformamide (30ml) with sodium hydride (0.003M), (60% in oil) while timethylacetylchloride (0.003M) was added dropwise. When addition was complete the reaction was left stirring for one hour and then quenched slowly with water (50ml). The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (eluant: dichloromethane:ethyl acetate 19:1). 4-Hydroxy-3'-pivaloyloxybenzophenone (BRI028) was obtained as colourless crystals (50%) (m.p. 118°C), [lit. m.p. 117-119 °C]. (R_f 0.51 dichloromethane:ethyl acetate 19:1). IR ν_{max} (KBr) 3325 (OH), 2974 (CHs), 1757 (C=O of pivaloyl), 1644 (C=O), 1601 (C=C), 1574 (CH_2), cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.39 (9H, s, OPiv), 5.31 (1H, s, OH), 6.85-6.90 (2H, d,H-3', H-

5'), 7.28-7.30 (1H, d, H-4'), 7.49-7.51 (2H, d, H-5'', H-6''), 7.59 (H-2''), 7.77-7.81 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 27.08 (CH_3), 39.17 (($\underline{C}H_3)_3$), 115.30 (C-3', C-5'), 122.70 (C-2''), 125.17 (C-4''), 127.08 (C-6''), 129.23 (C-5''), 129.40 (C-1'), 132.90 (C-2', C-6'), 139.54 (C-1''), 150.90 (C-3''), 160.60 (C-4'), 177.43 (C=O, OPiv), 194.92 (C=O).

5.3. Methylation – General method

The hydroxybenzophenone (0.0093M) and potassium hydroxide (0.0186M) were placed in a round-bottomed flask and stirred in DMSO (dimethyl sulphoxide) (30ml) to form the anion. Methyl iodide (0.01116M) was added to the reaction and the reaction was left stirring overnight. The basic solution was then neutralised using 10% hydrochloric acid, which caused the product to precipitate. The product was filtered via sintered glass funnel and purified by flash chromatography.

5.3.1. Synthesis of 4-methoxy-4'-fluorobenzophenone (BRI003)[153]

4-Methoxy-4'-fluorobenzophenone (BRI003) (0.0093M) was synthesised following the general method 5.3 by stirring 4-hydroxy-4'-fluorobenzophenone [4] in a flask with potassium hydroxide (0.0116M) and DMSO (30ml) until a colour change was observed indicating anion formation. Then methyl iodide was added to the mixture and this was left stirring for 30 minutes. The mixture was then transferred to a beaker of water (50ml) and the precipitate was filtered. This produced a yield of yellow crystals (50%) which were washed with hexane, (m.p. 99°C), [lit. m.p. 97-98 °C]. (R_f 0.8 dichloromethane). IR ν_{max} (KBr) 3458, 2974 (CHs), 1641 (C=O), 1599 (C=C), 1500 (CH_2), cm^{-1}. ^{1}H NMR δ($CDCl_3$) 3.90 (3H, s, OCH_3), 6.98-7 (2H, d, C-3', H-5'), 7.15-7.18m (2H, t, H-3'', H-5''), 7.79-7.80 (4H, m, H-2', H-6', H-2'', H-6''). ^{13}C NMR δ($CDCl_3$) 55.02 (OCH_3), 113.18 (C-3', C-5'), 114.94 (C-3'', C-5''), 129.61 (C-1'), 131.90 (C-2', C-6', C-2'', C-6''), 134 (C-1''), 162.80 (C-4'), 163.34 (C-4''), 193.57 (C=O).

5.3.2. Synthesis of 4-hydroxy-4'-methoxybenzophenone (BRI004)[121]

4,4'-Dihydroxybenzophenone [1] (0.0093M) and potassium hydroxide (0.0186M) were reacted, according the general method 5.3, by stirring in DMSO (dimethyl sulphoxide) (30ml) to form the anion. Then methyl iodide (0.01116M) was added and the solution was

left stirring overnight. The basic solution was neutralised and the product was filtered via sintered glass funnel. The solid left in the funnel was a mixture of mono and dimethoxy protected benzophenone. This was separated by flash chromatography (eluant: dichloromethane). The product was obtained as colourless crystals from dichloromethane (40%), (m.p. 160°C), [lit. m.p. 151 °C]. (R_f 0.4 dichloromethane:ethyl acetate 19:1). IR ν_{max} (KBr) 3249 (OH), 2989 (CHs), 1617 (C=O), 1605 (C=C), 1511 (CH_2) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 3.90 (3H, s, OCH_3), 6.02 (1H, s, OH), 6.92-6.94 (2H, d, H-3', H-5'), 6.97-6.99 (2H, d, H-2', H-6'), 7.75-7.77 (2H, d, H-3'', H-5''), 7.8-7.83 (2H, d, H-2'', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 54.98 (OCH_3), 113 (C-3', C-5', C-3'', C-5''), 130 C-2', C-6'), 132.87 (C-2'', C-6''), 131.73 (C-1', C-1''), 162.39 (C-4', C-4''), 193.94 (C=O).

5.4. General method - synthesis of benzophenones via Friedel Crafts reaction

General Method for synthesis of methoxybenzophenones via Friedel Crafts reaction. Anisole [2] (0.005M) and tin (IV) chloride (0.005M) were stirred in a round bottom flask in tetrachloroethane (25ml). Anisoyl chloride (0.005M) in tetrachloroethane (25ml) was added dropwise to the mixture. This mixture was all placed on ice and left stirring for two days. Ice was then added to the solution and the product extracted with dichloromethane (2 x 100ml). The sample was dried over sodium sulphate and concentrated under reduced pressure. The tetrachloroethane was distilled off. The product generally crystallised from hexane:diethylether 1:1 but in some cases required chromatography.

5.4.1. Synthesis of 3,4'-dimethoxybenzophenone (BRI006)[127]

5.4.1.1. Attempted synthesis of 3,4'-dimethoxybenzophenone (BRI006)[127]

The synthesis of 3,4'-dimethoxybenzophenone (BRI006) was attempted by stirring anisole [2] (0.005M), $AlCl_3$ (0.005M) and *meta*-anisoyl chloride (0.005M) in 30ml of tetrachloroethane on ice for two days. Ice was added to the reaction and the product extracted with dichloromethane (2 x 100ml). The sample was dried over sodium sulphate and concentrated under reduced pressure. The mixture was then chromatographed on silica gel (eluant: dichloromethane:ethyl acetate 19:1). This produced a low yield of product (10%) and most of the starting material was recovered.

5.4.1.2. Synthesis of 3,4'-dimethoxybenzophenone (BRI006)[127]

The reaction was repeated by placing anisole [2] (0.005M), and *meta*-anisoyl chloride (0.005M) in the reaction vessel in 30ml of tetrachloroethane and stirring for 45 minutes. The $AlCl_3$ (0.005M) was then added and the reaction was left stirring for another 45 minutes. The reaction was chromatographed by thin layer chromatography and showed a faint presence of product and a strong presence for each of the starting materials.

5.4.1.3. Synthesis of 3,4'-dimethoxybenzophenone (BRI006)[127]

In the final attempt anisole [2] (0.005M) and tin (IV) chloride (0.005M) were stirred in a round bottom flask in 25ml of tetrachloroethane. *Meta*-anisoyl chloride (0.005M) in 25ml tetrachloroethane was added dropwise to the mixture. This mixture was all placed on ice and left stirring for two days. Ice was then added to the solution and the product extracted with dichloromethane. The sample was dried over sodium sulphate and concentrated under reduced pressure. The tetrachloroethane was distilled off. The product crystallised as light yellow crystals from dichloromethane (90%), (m.p. 49°C), [lit. m.p. 55 °C], (R_f 0.78 dichloromethane:ethyl acetate 19:1). IR ν_{max} (film) 3448, 2927 (CHs), 1648 (C=O), 1588 (CH_2), cm^{-1}. ^1H NMR δ($CDCl_3$) 3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.97-6.99 (2H, d, H-3', H-5'), 7.11-7.13 (1H, d, H-4''), 7.32 (2H, d, H-2'', H-3''), 7.38 (1H, s, H-6'') 7.84-7.87 (2H, d, H-2', H-6'), ^{13}C NMR δ($CDCl_3$) 55.23 (OCH_3), 56.08 (OCH_3), 113.11 (C-3', C-5'), 114.62 (C-2''), 118.90 (C-4''), 124.95 (C-6''),129.64 (C-5''), 130.70 (C-1'), 133.07 (C-2', C-6'),140.19 (C-1''), 161.06 (C-4'), 165.79 (C-3''),196.80 (C=O).

5.4.2. Synthesis of 2,4'-dimethoxybenzophenone (BRI008)[130, 128]

The synthesis of 2,4'-dimethoxybenzophenone (BRI008) was attempted as for 3,4'-dimethoxybenzophenone (BRI006) except aluminium chloride was used instead of tin chloride. Anisole [2] (0.005M) and aluminium chloride (0.005M) were stirred in 25ml of tetrachloroethane. O*rtho*-anisoyl chloride (0.005M), dissolved in 25ml of tetrachloroethane, was added dropwise to the mixture. This mixture was placed on ice and left stirring for two days. The reaction was quenched with dilute hydrochloric acid and ice mixture (50ml) and extracted with dichloromethane (3 x 50ml). The combined organic

layers were then washed with 10% sodium hydroxide solution and dried on sodium sulphate. The solvent was removed under reduced pressure and the product was purified on silica gel (eluant: dichloromethane). The reaction gave a very low yield (16.74%) so optimisation was required.

5.4.2.1. Synthesis of 2,4'-dimethoxybenzophenone (BRI008)[130, 128]

This time the *ortho*-anisoyl chloride (0.005M) was dissolved in tetrachloroethane (25ml) and added dropwise via a pressure equalising funnel to the reaction mixture of anisole [2] (0.005M) and aluminium chloride (0.005M) also in tetrachloroethane (25ml). The solvent was removed under reduced pressure and the product was purified on silica gel (eluant: dichloromethane). This did not increase the yield (9%) as expected.

5.4.2.2. Synthesis of 2,4'-dimethoxybenzophenone (BRI008)[130, 128]

Tin (IV) chloride (0.005M) was stirred with anisole [2] (0.005M) in tetrachloroethane (25ml), *ortho*-anisoyl chloride (0.005M) in tetrachloroethane (25ml) was added dropwise to the reaction and this was left stirring for two days on ice. Ice was then added to the solution and the product extracted with dichloromethane (3 x 100ml). The sample was dried over sodium sulphate and concentrated under reduced pressure. The tetrachloroethane was distilled off. The yellow product crystallised from dichloromethane (82%), (m.p. 99°C), [lit. m.p. 99 °C], (R_f 0.94 dichloromethane:ethyl acetate 19:1). IR ν_{max} (film) 3436, 2835 (CHs), 1646 (C=O), 1467 (CH_2), cm^{-1}. ^1H NMR δ($CDCl_3$) 3.76 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 6.92-6.94 (2H, d, H-3', H-5'), 7-7.07 (1H, m, H-4'', H-5''), 7.32-7.35 (2H, d, H-3''), 7.44-7.49 (1H, t, H-6'') 7.81-7.84 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 54.98 (OCH_3), 55.20 (OCH_3), 110.98 (C-3''), 113.03 (C-3', C-5', C-5''), 120 (C-1''), 128.76 (C-1'), 130.9(C-4''), 131.80 (C-2', C-6', C-2''), 156.60 (C-6''), 163.10 (C-4'), 193.02 (C=O)

5.4.3. Synthesis of 3-methyl-4,4'-dimethoxybenzophenone (BRI022)[230]

The synthesis of BRI022, 3-methyl-4,4'-dimethoxybenzophenone, was attempted as for BRI008. 2-Methylanisole (0.02M) and tin (IV) chloride (0.03M) were stirred in a round bottom flask in 25mL of tetrachloroethane (50ml). *Para*-anisoyl chloride (0.02M) in 25ml tetrachloroethane was added dropwise to the mixture. This mixture was all placed on ice

and left stirring for two days. Ice was then added to the solution and the product extracted with dichloromethane (2 x 100ml). The sample was dried over sodium sulphate and concentrated under reduced pressure. The tetrachloroethane was distilled off. The product crystallised from dichloromethane (66%), (m.p. 64°C), [lit. m.p. 65-66 °C], (R_f 0.82 dichloromethane). IR ν_{max} (KBr) 3448, 2943 (CHs), 1649 (C=O), 1511 (CH_2), cm^{-1}. ^1H NMR δ($CDCl_3$) 2.28 (3H, s, CH_3), 3.90 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.87-6.90 (1H, d, H-3''), 6.97-6.99 (2H, d, H-3', H-5'), 7.66-7.67 (2H, d, H-2'', H-6''), 7.8-7.82 (2H, d, H-2', H-6'), ^{13}C NMR δ($CDCl_3$) 15.73 (CH_3), 54.90 (OCH_3), 108.50 (C-3''), 112.95 (C-3', C-5', C-5''), 126.14 (C-6''), 129.60 (C-1'), 129.62 (C-2', C-6', C-2''), 132.08 (C-1'') 160.65 (C-4'), 162.30 (C-4''), 194.24 (C=O).

5.5. General method for selective deprotection of dimethoxybenzophenones

The methoxy protected benzophenone starting material (0.0062M) was selectively deprotected by dissolving it in N,N-dimethylformamide (30ml) and stirring it with sodium ethanethiolate (0.0093M) for 3 hours at 80°C. N,N-Dimethylformamide was distilled off and the product was washed with water and extracted using DCM (2 x 100ml) and then washed with sodium chloride solution (2 x 50ml). The organic layers were combined, dried (sodium sulphate) and the product was purified by flash chromatography. The reaction was observed by TLC to ensure no complete deprotection.

5.5.1. Synthesis of 4-hydroxy-3'-methoxybenzophenone (BRI015)[6]

5.5.1.1. Synthesis of 4-hydroxy-3'-methoxybenzophenone (BRI015)[6]

The reaction was attempted according to the general method 5.4. 4-Hydroxy-3'-methoxybenzophenone was synthesised from 3,4'-dimethoxybenzophenone (BRI006) (0.0062M) by dissolving it in N,N-dimethylformamide (30ml) and stirring with sodium ethanethiolate (0.0093M) for 3 hours at 80°C. The N,N-dimethylformamide was distilled off and the product was purified by flash chromatography (eluant: hexane:diethyl ether 50:50). 3-Methoxy-4'-hydroxybenzophenone (BRI015) was produced (43%) and the reaction was then optimised.

5.5.1.2. Synthesis of 3-methoxy-4'-hydroxybenzophenone (BRI015)[6]

The following reaction was performed according to the general method 5.4. 3,4'-Dimethoxybenzophenone (BRI006) (0.0062M) was stirred in N,N-dimethylformamide (30ml) and refluxed with sodium ethanethiolate (0.0093M) for 4 hours at 130°C. N,N-Dimethylformamide was distilled off and the product was washed with water (50ml) and extracted using DCM (2 x 100ml) and then washed with sodium chloride solution (2 x 50ml). The product was purified by flash chromatography (eluant: hexane:diethyl ether 50:50) and yielded the required product as brown crystals (67%), (m.p. 140°C), [lit. m.p. 141-142 °C], (R_f 0.55 dichloromethane:ethyl acetate 19:1). IR ν_{max} (KBr) 3335 (OH), 2958 (CHs), 1647 (C=O), 1597 (C=C), 1571 (CH_2), cm^{-1}. ^1H NMR δ($CDCl_3$) 3.87 (1H, s, OCH_3), 6.39 (1H, s, OH), 6.93-6.95 (2H, d, H-3', H-5'), 7.13-7.15 (1H, dd, H-4'') 7.32-7.33 (2H, s, H-2'', H-3''), 7.33-7.39 (1H, d, H-6''), 7.8-7.82 (2H, d, H-2', H-4'). ^{13}C NMR δ($CDCl_3$) 54.98-55.02 (OCH_3), 113.08 (C-3', C-5'), 113.76 (C-2''), 117.77 (C-4''), 121.93 (C-6''), 128.65 (C-5''), 129.70 (C-1'), 132.07 (C-2', C-6'), 139.19 (C-1''), 159.06 (C-4'), 162.79 (C-3''), 194.80 (C=O).

5.5.2. Synthesis of 3,4'-dihydroxybenzophenone (BRI016)[119]

5.5.2.1. Attempted synthesis of 3,4'-dihydroxybenzophenone (BRI016)[119]

This reaction was performed according to the general method 5.4 as an attempt at complete deprotection of 3,4'-dimethoxybenzophenone (BRI006) to produce 3,4'-dihydroxybenzophenone (BRI016). 3-Methoxy-4'-methoxybenzophenone (BRI006) (0.0062M) was dissolved in N,N-dimethylformamide (30ml) and stirred with sodium ethanethiolate (0.0093M) for 3 hours at 80°C. N,N-Dimethylformamide was distilled off and the product was washed with water and extracted using DCM (2 x 100ml) and then washed with sodium chloride solution (2 x 50ml). All the starting material was recovered by flash chromatography and so the reaction was attempted using a different procedure.

5.5.2.2. Synthesis of 3,4'-dihydroxybenzophenone (BRI016)[119]

3,4'-Dihydroxybenzophenone (BRI016) was synthesised by the complete deprotection of 3,4'-dimethoxybenzophenone (BRI006). 3,4'-Dimethoxybenzophenone (0.003M) was

refluxed at 220°C in a flask with an excess of pyridine-HCl for 30 minutes. The reaction mixture was washed with 150ml of a water, HCl (hydrochloric acid) and ice mixture (10ml concentrated HCl, 50g ice, 90ml of water) and extracted with ethyl acetate (2 x 100ml). The ethyl acetate extracts were combined, dried and concentrated under reduced pressure to afford colourless crystals (92%), (m.p. 211°C), [lit. m.p. 205-206 °C], (R_f 0.3 dichloromethane:ethyl acetate 19:1). IR ν_{max} (KBr) 3310 (OH), 2912 (CHs), 1653 (C=O), 1571 (CH_2), cm^{-1}. ^1H NMR δ ($CDCl_3$) 5.00 (OH), 5.00 (OH), 7.30 (2H, d, H-3', H-5'), 8.05 (2H, d, H-2'', H-4''), 8.50 (2H, d, H-6'', H-3''), 9.0 (2H, d, H-2', H-6'), ^{13}C NMR δ ($CDCl_3$) 113.90 (C-3', C-5'), 113.19 (C-6''), 114.95 (C-4''), 117.91 (C-2''), 121.99 (C-3''), 128.76 (C-1'), 132.50 (C-2', C-6'), 139.15 (C-1''), 156.07 (C-5''), 163 (C-4'), 195.61 (C=O).

5.5.3. Synthesis of 2-hydroxy-4'-methoxybenzophenone from 2,4'-dihydroxybenzophenone (BRI018) *via* Methylation.

5.5.3.1. Attempted synthesis of 2-hydroxy-4'-methoxybenzophenone (BRI018)[134]

2,4'-Dihydroxybenzophenone (BRI188) was obtained as a by-product from a deprotection reaction on 2,4'-dimethoxybenzophenone (BRI008) and so an attempt was made to selectively protect it to produce 2-hydroxy-4'-methoxybenzophenone (BRI018). 2,4'-Dihydroxybenzophenone (BRI188) (0.0011M) was dissolved in dry THF (20ml) and added slowly to a solution of sodium hydride (0.0132M) in dry THF (20ml) under nitrogen. The mixture was then heated at 60°C for 1 hour and 30 minutes. The mixture was allowed to cool and a solution of methyl iodide (0.0143M) in dry THF (20ml) was added slowly and the reaction was stirred for 2 hours. The mixture was then poured slowly into ice water (30ml) and extracted using diethyl ether (2 x 50ml). The ether was dried over sodium sulphate and reduced under reduced pressure. The reaction was unsuccessful as the product was completely protected 2,4'-dimethoxybenzophenone (BRI008) (89%).

5.5.3.2. Attempted synthesis of 2-hydroxy-4'-methoxybenzophenone (BRI018)[134]

This reaction was attempted following the general method 5.3. 2,4'-Dihydroxybenzophenone (BRI188) (0.0032M) was reacted with potassium hydroxide (0.00384M) and methyl iodide (0.0064M) for 2 hours in acetone (50ml). The reaction was also unsuccessful as TLC observed complete protection of the starting material as the product 2,4'-dimethoxybenzophenone (BRI008) (92%).

5.5.4. Synthesis of 4'-hydroxy-2-methoxybenzophenone (BRI019)[132] *via* Friedel Crafts reaction

5.5.4.1. Attempted synthesis of 4'-hydroxy-2-methoxybenzophenone (BRI019)[132]

An attempted synthesis of 4'-hydroxy-2-methoxybenzophenone (BRI019) was performed following the general method 5.4. Phenol (0.015M) and tin (IV) chloride (0.005M) were stirred in a round bottom flask in tetrachloroethane (25ml). *Ortho*-anisoyl chloride (0.015M) in tetrachloroethane (25ml) was added dropwise to the mixture. This mixture was all placed on ice and left stirring for two days. Ice was then added to the solution and the product extracted with dichloromethane (2 x 100ml) after basifying the solution. The sample was dried over sodium sulphate and concentrated under reduced pressure. This reaction was unsuccessful.

5.5.4.2. Attempted synthesis of 4'-hydroxy-2-methoxybenzophenone (BRI019)[132]

The reaction was attempted again but this time using polyphosphoric acid. Phenol (0.0105M) and ortho-anisoyl chloride (0.01M) were placed in a flask with polyphosphoric acid (20ml) and the mixture heated and refluxed at 100°C for 20 minutes. This reaction mixture was purified on silica gel (eluant: hexane:ethyl acetate 70:30) but no product was recovered.

5.5.5. Synthesis of 4'-hydroxy-2-methoxybenzophenone (BRI019) and by-products 2-hydroxy-4'-methoxybenzophenone (BRI018) and 2,4'-dihydroxybenzophenone (BRI88) *via* demethylation

5.5.5.1. Attempted synthesis of 4'-hydroxy-2-methoxybenzophenone (BRI019)[132]

The selective deprotection of 2,4'-dimethoxybenzophenone (BRI008) (0.002M) was performed, following the general method 5.4, by refluxing with sodium ethanethiolate (0.006M) in N,N-dimethylformamide (20ml) for 4 hours at 80°C. The N,N-dimethylformamide was distilled off and the product purified by flash column chromatography (eluant: dichloromethane:ethyl acetate 19:1). But only the completely deprotected product 2,4'-dihydroxybenzophenone (BRI188) was recovered (88%).

5.5.5.2. Attempted synthesis of 4'-hydroxy-2-methoxybenzophenone (BRI019)[132]

The reaction was attempted again with 2,4'-dimethoxybenzophenone (BRI008) (0.00826M) following the general method 5.4, by refluxing with sodium ethanethiolate (0.00826M) in N,N-dimethylformamide (30ml) for 3 hours at 80°C. Once again the majority of of product recovered was the completely deprotected product 2,4'-dihydroxybenzophenone (BRI188) (89%). A very low yield of 4-hydroxy-2'-methoxybenzophenone (BRI019) was obtained (4%).

5.5.5.3. Synthesis of 4'-hydroxy-2-methoxybenzophenone (BRI019)[132]

The selective deprotection of 2,4'-dimethoxybenzophenone (BRI008) (0.0025M) was performed, following the general method 5.4, by refluxing with sodium ethanethiolate (0.00826M) in N,N-dimethylformamide (30ml) for 2 hours at 80°C. The reaction was watched by thin layer chromatography until the expected spot started to develop on the TLC plate. The N,N-dimethylformamide was distilled off and the product was washed with water and extracted using dichloromethane (2 x 100ml) and then washed with sodium chloride solution (50ml). The product was purified by flash chromatography (eluant: hexane:diethyl ether 50:50) (R_f 0.17 Hexane:ethyl acetate 7:3) and crystals (26.3%) were

obtained, (m.p. 150°C), [lit. m.p. 152-153 °C], (R_f 0.25 hexane:diethyl ether 70:30). IR v_{max} (film) 358 (OH), 2926 (CHs), 1643 (C=O), 1576 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$)3.76 (3H, s, OCH_3), 5.68 (1H, s, OH), 6.86-6.88 (2H, d, -3', H-5'), 6.99-7.07 (2H, m, H-4'', H-5''), 7.33-7.35 (1H, d, H-3''), 7.45-7.46 (1H, t, H-6''), 7.77-7.79 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 55.15 (OCH_3), 110.92 (C-3''), 114.85 (C-3', C-5'), 119.88 (C-5''), 128.68 (C-6''), 130.95 (C-4''), 132.29 (C-2', C-6'), 161.55 (C-4'), 162.73 (C-2''), 195.25 (C=O).

2-Hydroxy-4'-methoxybenzophenone (BRI018)[134]

The by-product 2-hydroxy-4'-methoxybenzophenone (BRI018) was produced as clear crystals, (50%), (m.p. 53°C), [lit. m.p. 52-53 °C], (R_f 0.7 dichloromethane:ethyl acetate 19:1). IR v_{max} (film) 3408 (OH), 2896 (CHs), 1647 (C=O), 1569 (CH_2), cm^{-1}. ^1H NMR δ($CDCl_3$) 3.86 (3H, s, OCH_3), 6.87-6.91 (1H, t, H-5''), 6.9-7.02 (2H, d, H-3', H-5'), 7.07-7.09 (1H, d, H-3''), 7.48-7.52 (1H, t, H-4''), 7.64-7.66 (1H, d, H-2''), 7.72-7.74 (2H, d, H-2', H-6'), 11.94 (1H, s, OH). ^{13}C NMR δ($CDCl_3$) 55.04 (OCH_3), 113.23 (C-3', C-5'), 117.86 (C-3'', C-5''), 118.05 (C-1''), 128.20 (C-1'), 129.90 (C-2', C-6'), 132.81 (C-2''), 135.34 (C-4''), 162.49 (C-4'), 162.49 (C-6''), 199.54 (C=O).

2,4'-Dihydroxybenzophenone (BRI188)[135, 128]

A by-product from this reaction, 2'hydroxy-4'-hydroxybenzophenone (BRI188) was produced as clear crystals (20%), (m.p. 150°C), [lit. m.p. 150-151 °C], IR v_{max} (film) 3408 (OH), 2896 (CHs), 1647 (C=O), 1569 (CH_2), cm^{-1}. ^1H NMR δ($CDCl_3$) 5.39 (1H, s, OH), 6.9-6.96 (1H, t, H-3''), 6.94-6.97 (2H, d, H-3', H-5'), 7.08-7.10 (1H, d, H-5''), 7.52-7.60 (1H, t, H-4''), 7.63-7.66 (1H, d, H-2''), 7.69-7.71 (2H, d, H-2', H-6'), 11.95 (1H, s, OH). ^{13}C NMR δ($CDCl_3$) 122.87 (C-3', C-5'), 125.77 (C-3'', C-5''), 127.86 (C-1''), 129.65 (C-2'', C-6''), 134.82 (C-4', C-6'), 152.04 (C-1'), 176.22 (C-4''), 184.80 (C-2'), 187.93 (C=O). HRMS calculated for $C_{13}H_{10}O_3$ (M^++1) 215.0708, observed 215.0716.

5.5.6. Synthesis of 3-methyl-4-hydroxy-4'-methoxybenzophenone (BRI036)

The general method 5.4 for deprotecting hydroxy groups was applied here. 4,4'-Dimethoxy-3-methylbenzophenone (BRI022) (0.0389M) were dissolved in N,N-dimethylformamide (30ml) and stirred with sodium ethanethiolate (0.0117M) for 3 hours at

80°C. N,N-Dimethylformamide was distilled off and the product was washed with water and extracted using DCM (2 x 100ml) and then washed with sodium chloride solution (50ml). The product was purified by flash column chromatography (eluant: hexane:ethyl acetate 70:30). Colourless crystals were obtained (55%), (R_f 0.3 hexane:ethyl acetate 70:30) (m.p. 68°C). IR ν_{max} (KBr) 3448 (OH), 2943 (CHs), 1649 (C=O), 1511 (CH_2), cm^{-1}. ^1H NMR δ($CDCl_3$) 2.16 (3H, s, CH_3), 3.95 (3H, s, OCH_3), 5.42 (1H, s, OH), 6.90-6.92 (1H, d, H-3''), 6.93-6.95 (2H, d, H-3', H-5'), 7.56-7.58 (2H, dd, H-2'', H-6''), 7.75-7.77 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 15.74 (CH_3), 55.00 (OCH_3), 133.99 (C-3''), 114.59 (C-3', C-5', C-5''), 129.76 (C-6''), 132.09 (C-2', C-6', C-2''), 132.16 (C-1'), 132.98 (C-1'') 158.45 (C-4'), 159.37 (C-4''), 187.24 (C=O).

5.5.7. General deprotection method using boron trifluoride-dimethyl sulphide complex

Boron trifluoride-dimethyl sulphide (0.123M) was added dropwise over 30 min to the methoxy substituted compound (0.0123M) dissolved in dichloromethane (50ml). Stirring was continued for a further 10 hours at room temperature. The solvent was removed using a nitrogen purge and the remaining residue was dissolved in ethyl acetate (100ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and residue was chromatographed on silica gel (eluant: hexane:ethyl acetate 4:1) to yield the hydroxy substituted product.

5.5.8. Synthesis of 4-hydroxy-2'-pivaloyloxybenzophenone (BRI048)

5.5.8.1. Attempted synthesis of 4-hydroxy-2'-pivaloyloxybenzophenone (BRI048)

The following deprotection reaction was carried out according to the general method 5.4. 2-Pivaloyloxy-4'-methoxybenzophenone (BRI020) (0.0017M) was dissolved in N,N-dimethylformamide (30ml) and stirred with sodium ethanethiolate (0.0051M) for 3 hours at 80°C. The N,N-dimethylformamide was distilled off and the product was washed with water and extracted using dichloromethane (2 x 100ml) and then washed with sodium chloride solution (50ml). The product was purified by flash column chromatography

(eluant: hexane:diethyl ether 50:50). This reaction was unsuccessful as it completely deprotected the starting material to afford 2,4'-dihydroxybenzophenone (BRI188).

5.5.8.2. Synthesis of 4-hydroxy-2'-pivaloyloxybenzophenone (BRI048)

The general method 5.4.6 was applied to this reaction. 2-Pivaloyloxy-4'-methoxybenzophenone (BRI020) (0.000467M) was stirred in dichloromethane (8ml) while boron trifluoride-dimethyl sulphide (0.0187M) was added dropwise over 30 min. Stirring was continued for a further 10 hours at room temperature. The solvent was then removed using a nitrogen purge and the remaining residue was dissolved in ethyl acetate (50ml) and washed with saturated sodium bicarbonate solution (50ml), water (50ml) and brine (50ml). The ethyl acetate was dried over Na_2SO_4 and then removed under reduced pressure. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 50:50). The dipivaloylated product was also recovered as a yellow oil (20%), (R_f 0.6 hexane:diethyl ether 1:1), (see below for spectroscopic data). The desired product was obtained as yellow oil (65%), (R_f 0.3 hexane:diethyl ether 1:1). IR ν_{max} (KBr) 2973 (OH), 1751 (C=O of pivaloyl), 1659 (C=O), 1601 (C=C), 1571 (CH_2), cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.37 (9H, s, $(CH_3)_3$), 6.89-6.90 (2H, d, H-3', H-5'), 7.27-7.28 (1H, d, H-3''), 7.45-7.50 (2H, m, H-4'', H-6''), 7.57-7.60 (1H, d, H-5''), 7.7-7.76 (2H, d, H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 26.56 ($(CH_3)_3$, OPiv), 38.70 (C-1, $C(CH_3)_3$), 114.90 (C-3', C-5'), 122.30 (C-3''), 124.70 (C-5''), 126.60 (C-4''), 128.50 (C-6''), 131.28 (C-2', C-6'), 139.17 (C-1''), 150.42 (C-1'), 160.92 (C-4'), 176.93 (C-2''), 194.73 (C=O, OPiv), 208.11 (C=O). HRMS calculated for $C_{18}H_{16}O_4$ (M^++1) 297.1127, observed 297.1117.

4,2'-Dipivaloyloxybenzophenone (BRI048)

^1H NMR $\delta(CDCl_3)$ 1.38 (9H, s, $(CH_3)_3$), 1.39 (9H, s, $(CH_3)_3$), 7.21-7.23 (2H, d, H-3', H-5'), 7.31-7.33 (1H, d, H-3''), 7.50-7.53 (2H, m, H-4'', H-6''), 7.64-7.66 (1H, d, H-5''), 7.87-7.89 (2H, d, H-2', H-6').

5.6. McMurry coupling reaction - General method

Titanium tetrachloride (0.01175M) was added dropwise over 5 minutes to zinc dust (0.0376M) in dry THF (tetrahydrofuran) (10ml) under a nitrogen atmosphere. The mixture was refluxed at 100°C for 2 hours in the dark to form the catalyst. Then an appropriately substituted benzophenone (0.0047M) and 1-phenyl-2-butanone [3] (0.0141M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Flash column chromatography was performed to purify the crude product.

5.6.1. 2-Benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (BRI030)

As in the general method 5.6, titanium tetrachloride (0.01175M) was added dropwise over 5 minutes to zinc dust (0.0376M) in dry THF (tetrahydrofuran) (10ml) under a nitrogen atmosphere. The mixture was refluxed at 100°C for 2 hours in the dark. This formed the catalyst. Then 4-hydroxybenzophenone (0.0047M) and 1-phenyl-2-butanone [3] (0.0141M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 40:60) (80%). The product was obtained a light yellow oil (60%), (R_f 0.4 dichloromethane:hexane 6:4). IR ν_{max} (film) 3360 (OH), 3000-3027, 2965-2854 (CHs), 1608 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.04 (3H, t, J=7.5Hz, H-4, CH_3), 2.11 (2H, q, J=7.5Hz, H-3, CH_2), 3.62-3.66 (2H, s, H-5, CH_2), 5.98 (H, s, OH), 6.76-6.78 (2H, dd, H-3'', H-5''), 7.13-7.15 (2H, dd, H-2'', H-6''), 7.28-7.30 (10H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.80 (C-4, CH_3), 24.31 (C-3, CH_2), 36.86 (C-5, CH_2), 114.5-114.57 (C-3'', C-5''), 125.35 (C-4'), 125.79 (C-4'''), 127.60 (C-2'', C-6''), 127.67 (C-1, C=C), 127.80 (C-2''', C-6'''), 128.10 (C-3''', C-5'''), 128.20 (C-3', C-5'), 128.78 (C-2', C-6'), 138.10 (C-1''), 138.52 (C-2, C=C), 140.25 (C-1'''), 143.02 (C-1'), 153.65 (C-4'').

5.6.2. 2-Benzyl-1-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-but-1-ene (BRI023)

Following the general method 5.6, titanium tetrachloride (0.01175M) and zinc (0.0376M) were refluxed in dry THF (tetrahydrofuran) (10ml) under a nitrogen atmosphere. The mixture was refluxed at 100°C for 2 hours in the dark. This formed the catalyst. Then 4-hydroxy-4'-methoxy-benzophenone (BRI004) (0.0047M) and 1-phenyl-2-butanone [3] (0.0141M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 40:60). The product was also a light yellow oil (88%), (R_f 0.26 dichloromethane:hexane 2:1). IR ν_{max} (film) 3402 (OH), 3000-2963 (CHs), 1606 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.04 (3H, t, J=7.5Hz, H-4, CH_3), 2.16 (2H, q, J=7.5Hz, H-3, CH_2), 3.66 (2H, s, H-5, CH_2), 3.82 (3H, s, OCH_3) 6.18 (H, s, OH), 6.8-6.85 (2H, d, (J=8.56), H-3'', H-5''), 6.84-6.95 (2H, m, H-3''', H-5''') 7.15 –7.18 (2H, m, H-2', H-6'), 7.21-7.25 (2H, m, H-2'', H-6''), 7.27 (1H, s, H-4''), 7.3-7.32 (2H, dd, H-3'', H-5''). ^{13}C NMR δ($CDCl_3$) 11.80 (C-4, CH_3), 24.12 (C-3, CH_2), 25.24 (C-5, CH_2), 53.82 (OCH_3), 113.11-113.16 (C-3'', C-5''), 113.5-113.60 (C-3', C-5'), 127.36 (C-1, C=C), 127.86 (C-2', C-6'), 128.12-128.18 (C-2'', C-6''), 129.8-129.84 (C-3'', C-5'''), 130.23 (C-2''', C-6'''), 135.04 (C-2, C=C), 137.06 (C-4'''), 137.60 (C-1''), 138.26 (C-1''), 140.06-140.10 (C-1'''), 154.96 (C-4'), 162.58 (C-4'').

5.6.3. 2-Benzyl-1-(4-hydroxyphenyl)-1-(4-pivaloyphenyl)-but-1-ene (BRI032)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.01175M) was added to zinc dust (0.0376M) in dry THF (tetrahydrofuran) (10ml) and refluxed at 100°C for 2 hours. Then 4-hydroxy-4'-pivaloyloxy benzophenone (BRI002) (0.0047M) and 1-phenyl-2-butanone [3] (0.0141M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Flash column chromatography was performed to purify the crude product

(eluant: hexane: dichloromethane 40:60). The product was obtained as a light yellow oil (49%), (R_f 0.3 dichloromethane:hexane 6:4). IR ν_{max} (film) 3356 (OH), 3033, 2928 (CHs), 1764 (C=O), 1610 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.97 (3H, t, J=7.5Hz, H-4, CH_3), 1.35 (9H, s, OPiv), 2.07 (2H, q, J=7.5Hz, H-3, CH_2), 3.56-3.59 (2H, m, H-5, CH_2), 5.60 (H, s, OH), 6.72-6.74 (2H, dd, H-3'', H-5''), 7.01 (1H, s, H-4') 7.02-7.04 (2H, dd, H-3''', H-5'''), 7.07-7.10 (2H, dd, H-2'', H-6''), 7.2-7.25 (4H, dd, H-2''', H-6''', H-2', H-2', H-6'), 7.29-7.33 (2H, dd, H-3', H-5'). ^{13}C NMR $\delta(CDCl_3)$ 12.80 (C-4, CH_3), 24.10 (C-3, CH_2), 25.23 ($(\underline{C}H_3)_3$, OPiv), 35.70 (C-1, $\underline{C}(CH_3)_3$), 39.60 (C-5, CH_2), 113.20 (C-2', C-6'), 120-120.68 (C-3', C-5'), 121.70 (C-1, C=C), 124.40 (C-2, C=C), 127.90 (C-3'', C-5''), 128 (C-2'', C-6''), 129.63 (C-3''', C-4''', C-5'''), 130.27 (C-1''', C-2''', C-6'''), 132.61 (C-1''), 137.86 (C-1'), 140.12 (C-4''), 156.12 (C-4'), 173.50 (C=O).

5.6.4. 2-Benzyl-1-(4-hydroxyphenyl)-1-(2-methoxyphenyl)-but-1-ene (BRI033)

The general method 5.6 was used in this reaction. Titanium tetrachloride (0.01175M) and zinc dust (0.0376M) were refluxed in dry THF (tetrahydrofuran) (10ml) for 2 hours. Then 4-hydroxy-2'-methoxy benzophenone (BRI019) (0.0047M) and 1-phenyl-2-butanone [3] (0.0141M) were dissolved in dry THF (5ml) and added to the reaction vessel and refluxed for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Flash column chromatography was performed to purify the crude product (eluant: hexane: dichloromethane 40:60) and the product was obtained as an oil (18%), orange-yellow in colour (R_f 0.15 dichloromethane:hexane 6:4). IR ν_{max} (film) 3490 (OH), 3023-2854 (CHs), 1606 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$0.92 (3H, t, J=7.5Hz, H-4, CH_3), 1.91 (2H, q, J=7.5Hz, H-3, CH_2), 3.62 (2H, s, H-5, CH_2), 3.78 (3H, s, OCH_3), 4.79 (H, s, OH), 6.7-6.73 (2H, dd, H-3'', H-5''), 6.74-6.76 (1H, s, H-4') 6.9-6.92 (1H, dd, H-3'''), 6.96-6.97 (1H, dd, H-5'''), 7.19-7.24 (6H, m, ArH), 7.31-7.32 (2H, dd, H-3', H-5'). ^{13}C NMR $\delta(CDCl_3)$ 11.79 (C-4, CH_3), 25.60 (C-3, CH_2), 28.70 (C-5, CH_2), 56.84 (OCH_3), 113.72-113.80 (C-3', C-5'), 119 (C-3''), 126.8(C-1''), 127.17 (C-5''), 127.50 (C-6''), 127.89 (C-1, C=C), 128 (C-1', C-2', C-6'), 128.29 (C-2''', C-6'''), 128.34 (C-4'''), 129.68 (C-3''', C-5'''), 129.80 (C-2, C=C), 13.01 (C-4''), 135.29 (C-1'''), 155 (C-2''), 156.40 (C-2'').

5.6.5. 2-Benzyl-1-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-but-1-ene (BRI034)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.01175M) was added to zinc dust (0.0376M) in THF (tetrahydrofuran) (10ml) and refluxed at 100°C for 2 hours. Then 4-hydroxy-3'-methoxy benzophenone (BRI015) (0.0047M) and 1-phenyl-2-butanone [3] (0.0141M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Flash column chromatography was performed to purify the crude product (eluant: hexane: dichloromethane 40:60). The product was obtained a light orange oil (80%), (R_f 0.2 dichloromethane:hexane 6:4). IR ν_{max} (film) 3346 (OH), 3027-2904 (CHs), 1607 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$1.07 (3H, t, J=7.5Hz, H-4, CH_3), 2.54 (2H, q, J=7.5Hz, H-3, CH_2), 3.75 (2H, s, H-5, CH_2), 3.84 (3H, s, OCH_3) 5.78 (1H, s, OH) 6.8-6.82 (2H, dd, J=8.04, H-3'', H-5'') 6.86-6.90 (2H, dd, H-2''', H-4'''), 6.92-6.94 (H, d, H-3'''), 7.16-7.19 (2H, dd, H-2'', H-6'') 7.29-7.30 (6H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.79 (C-4, CH_3), 24.30 (C-3, CH_2), 28.36 (C-5, CH_2), 55.70 (OCH_3), 111.68-111.06 (C-2''), 112.65-112.70 (C-4''), 123.28 (C-6''), 124.80 (C-5', C-3'), 126.18 (C-6', C-2', C-1'), 128.80 (C-3''', C-5'''), 129.83 (C-4'''), 129.86-129.94 (C-2''', C-6''', C-5'''), 134.33 (C-1, C=C), 136 (C-2, C=C), 138 (C-1''), 140.07 (C-1'''), 156.96 (C-4'), 158.55 (C-3'').

5.6.6. 2-Benzyl-1-(4-hydroxyphenyl)-1-(3-pivaloyloxyphenyl)-but-1-ene (BRI035)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.01175M) and zinc dust (0.0376M) were refluxed at 100°C for 2 hours in in THF (10ml). Then 4-hydroxy-3'-pivaloyloxy benzophenone (BRI028) (0.0047M) and 1-phenyl-2-butanone [3] (0.0141M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Flash column chromatography was performed to purify the crude product (eluant: hexane: dichloromethane 40:60). The product was obtained as a light yellow oil (90%), (R_f 0.15 dichloromethane:hexane 6:4). IR ν_{max} (film) 3363 (OH), 3027, 2932 (CHs), 1755 (C=O),

1605 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.05 (3H, t, J=7.5Hz, H-4, CH_3), 1.41 (9H, s, OPiv), 2.11 (2H, q, J=7.5Hz, H-3, CH_2), 3.63 (2H, s, H-5, CH_2), 5.78 (1H, s, OH), 6.7-6.72 (2H, dd, H-3'', H-5'') 6.98 (2H, dd, H-2'', H-6''), 7.11-7.13 (2H, dd, H-3''', H-5'''), 7.27-7.28 (2H, dd, H-2''', H-6''') 7.29-7.32 (5H, m, ArH). ^{13}C NMR δ($CDCl_3$) 12.40 (C-4, CH_3), 24.40 (C-3, CH_2), 26.80 (($\underline{C}H_3)_3$, OPiv), 36.70 (C-1, $\underline{C}(CH_3)_3$), 39.60 (C-5, CH_2), 113.70 (C-2''), 121.3(C-4''), 122.80 (C-6''), 123.40 (C-5', C-3'), 128 (C-6', C-2', C-1'), 128.90 (C-3''', C-5'''), 129.63 (C-4'''), 132.27 (C-2''', C-6''', C-5'''), 133.61 (C-1, C=C), 136.86(C-2, C=C), 141.12 (C-1''), 143.07 (C-1'''), 157.60 (C-4'), 158.55 (C-3''), 173.50 (C=O).

5.6.7. 2-Benzyl-1-(4-hydroxyphenyl)-1-(2-pivaloyloxyphenyl)-but-1-ene (BRI043)

The general method 5.6 was used in this reaction. Titanium tetrachloride (0.01175M) and zinc (0.0376M) were refluxed in THF (tetrahydrofuran) (10ml) for 2 hours. To this 4-hydroxy-2'-pivaloyloxy benzophenone (BRI048) (0.0047M) and 1-phenyl-2-butanone [3] (0.0141M), dissolved in dry THF (5ml), was added and refluxed for a further 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Flash column chromatography was performed to purify the crude product (eluant: hexane: dichloromethane 40:60). The product was obtained as also a light yellow oil (85%), (R_f 0.34 dichloromethane:hexane 6:4). IR ν_{max} (film) 3357 (OH), 3034, 2924 (CHs), 1757 (C=O), 1607 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.98 (3H, t, J=7.5Hz, H-4, CH_3), 1.35-1.38 (9H, s, OPiv), 2.08 (2H, q, J=7.5Hz, H-3, CH_2), 3.60 (2H, s, H-5, CH_2), 5.47 (1H, s, OH), 6.71-7.33 (13H, m, ArH). ^{13}C NMR δ($CDCl_3$) 12.75 (C-4, CH_3), 20.45 (C-3, CH_2), 26.65-26.70 (($\underline{C}H_3)_3$, OPiv), 36.75 (C-5, CH_2), 43.08 (C-1, $\underline{C}(CH_3)_3$), 114.31-114.62 (C-3', C-5'), 118.53-118.83 (C-3''), 121.7-121.80 (C-5''), 125.3-125.38 (C-1, C=C), 126-126.10 (C-6''), 127.8-127.86 (C-2''', C-6'''), 128.1-128.42 (C-2', C-6', C-1'), 128.50 (C-2, C=C), 128.96 (C-4''), 130.07-130.60 (C-3''', C-5'''), 144.37 (C-1'''), 150.42 (C-2''), 153.95 (C-4'), 175 (C=O).

5.6.8. Synthesis of 4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenol (BRI159)

Following the general method 5.6, titanium tetrachloride (0.0992M) was added to zinc dust

(0.0198M) in dry THF (tetrahydrofuran) (10ml). The mixture was refluxed for 2 hours in the dark. Then 4,4'-dimethoxy-3-methylbenzophenone (BRI036) (0.0124M) and 4-methoxypropiophenone [20] (0.0248M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (80%) (hexane: dichloromethane 40:60). The product was obtained as a light yellow oil (60%), (R_f 0.32 dichloromethane:hexane 6:4). IR ν_{max} (film) 3395 (OH), 2961 (CHs), 1607 (C=C), 1460 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.92-0.94 (3H, t, J=7.5Hz, H-4, CH_3), 2.25 (3H, s, H-5, CH_3), 2.44-2.54 (2H, m, H-3, CH_2), 3.72 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 6.42-6.44 (1H, d, H-1'''), 6.57-6.59 (1H, m, H-2''') 6.64-6.65 (2H, d, H-2'', H-3''), 6.67-6.72 (2H, m, H-2''', H-3'''), 6.74-6.76 (1H, m, H-3'''), 6.95-7.0 (3H, m H-3'', H-5''', H-3'), 7.1-7.15 (1H, m, H-5'). ^{13}C NMR $\delta(CDCl_3)$ 13.30 (C-4, CH_3), 15.89 (C-3, CH_3), 28.55 (C-5, CH_2), 54.64 (OCH_3), 54.68 (OCH_3), 108.90 (C-2'''), 112.77 (C-3'', C-5''), 114.02 (C-3', C-5'), 127.27 (C-5'''), 128.90 (C-6'''), 131.63 (C-2'', C-6''), 133.02 (C-1, C=C), 134.56 (C-3'''), 135.90 (C-2', C-6'), 136.20 (C-1'), 137.13 (C-2, C=C), 139.76 (C-1''), 139.83 (C-1'''), 153.05 (C-4'), 155.90 (C-4''), 157.13 (C-4''').

5.7. General Method - Addition of basic side chain to hydroxy substituted products

To the hydroxy substituted product (0.00045M) in acetone:water 19:1 (10ml), potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M) were added and refluxed for 5 hours in darkness. The product was washed and extracted with water (10ml) and dichloromethane (3 x 40ml). TLC analysis showed almost complete consumption of the starting material. The reaction mixture was dried using sodium sulphate, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography.

5.7.1. Synthesis of 2-benzyl-1-(4-pyrrolidinyl ethoxy)-1-phenylbut-1-ene (BRI039)

The general method 5.7 was employed for this reaction by dissolving 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (BRI030) (0.00045M) in acetone:water 19:1 (10ml), potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M). These

were refluxed for 5 hours in darkness. The reaction mixture was extracted using dichloromethane (3 x 40ml), dried using sodium sulphate, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (22%), (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 2931, 2789 (CHs), 1606 (C=C), 1507 (NCH_2), 1453 (CH_2), 1364 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.98 (3H, t, J=7.5Hz, H-4, CH_3), 1.90 (4H, s, H-1'''', H-4''''), 2.09 (2H, q, J=7.5Hz, H-3, CH_2), 2.88 (4H, s, H-2'''', H-3''''), 3.03 (2H, m, H-6, CH_2), 3.65 (2H, s, H-5, CH_2), 4.22 (2H, m, H-4, CH_2), 6.8-6.90 (2H, dd, H-3'', H-5''), 7.26-7.36 (10H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.86 (C-4, CH_3), 22.90 (C-2'''', C-3'''', $(CH_2)_2$), 24.27 (C-3, CH_2), 36.67-36.79 (C-5, CH_2), 54.11 (C-1'''', C-4'''', $(CH_2)_2$), 54.30 (C-6, CH_2), 65.44 (C-7, CH_2), 113.63-113.71 (C-3'', C-5''), 125.36 (C-4'), 125.76 (C-4'''), 127.61 (C-1, C=C), 127.68 (C-2'', C-6''), 127.86 (C-2''', C-6'''), 128.79 (C-3''', C-5'''), 129.92 (C-3', C-5'), 129.96 (C-2', C-6'), 138.09 (C-1''), 138.44 (C-2, C=C), 140.10 (C-1'''), 142.90 (C-1'), 156.41 (C-4''). HRMS calculated for $C_{29}H_{34}NO$ 412.2562 (M^++1), observed 412.2640.

5.7.2. Synthesis of 2-benzyl-1-(4-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI040)

The general method 5.7 was used for the addition of aside chain to the hydroxy substitutent of 2-benzyl-1-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-but-1-ene (BRI023). 2-Benzyl-1-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-but-1-ene (BRI023) (0.00045M) was placed in acetone:water 19:1 (10ml) with potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M) and refluxed for 5 hours in darkness. The reaction mixture was extracted with dichloromethane (3 x 40ml) and the organic layers were combined and evaporated. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (36%), (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 2932, 2963 (CHs), 1606 (C=C), 1508 (NCH_2), 1462 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.95 (3H, t, J=7.5Hz, H-4, CH_3), 1.77 (4H, s, H-1'''', H-4''''), 2.03 (2H, q, J=7.5Hz, H-3, CH_2), 2.61-2.63 (4H, s, H-2'''', H-3''''), 3.06-3.10 (2H, m, H-6, CH_2), 3.40 (2H, s, H-5, CH_2), 3.76 (3H, s, OCH_3), 4.26 (2H, m, H-4, CH_2), 6.78-7.28 (13H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.84 (C-4, CH_3), 23.11 (C-2'''', C-3'''', $(CH_2)_2$), 24.23 (C-3, CH_2), 36.72 (C-5, CH_2), 50.03 (C-1'''', C-4'''', $(CH_2)_2$), 53.82 (C-6, CH_2), 54.73 (OCH_3), 63.15 (C-7, CH_2),

113.01-113.06 (C-3'', C-5''), 113.5-113.60 (C-3', C-5'), 125.36 (C-7), 127.86 (C-2', C-6'), 128.12-128.18 (C-2'', C-6''), 129.8-129.84 (C-3''', C-5'''), 130.23 (C-2''', C-6'''), 135.04 (C-8), 137.06 (C-4'''), 137.60 (C-1''), 138.26 (C-1''), 140.06-140.10 (C-1'''), 154.96 (C-4'), 157.58 (C-4''). HRMS calculated for $C_{30}H_{36}NO_2$ 442.2746 (M^++1), observed 442.2782.

5.7.3. Synthesis of 2-benzyl-1-(2-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI041)

5.7.3.1. General method – Mitsunobu basic side chain addition

The hydroxy substituted product (4.626mM) was stirred in dichloromethane (10ml) with triphenylphosphine (9.252mM) and 1-(2-hydroxyethyl)pyrrolidine (13.8mM) at room temperature. Diispropyl azodicarboxylate (11.6mM) was added slowly over 20 minutes. The reaction was left stirring for two days. TLC analysis showed almost complete consumption of the starting material. The reaction mixture was quenched with saturated NH_4Cl solution (50 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic layers were washed with saturated NH_4Cl (50 ml), water (50 ml) and brine (50 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel.

5.7.3.2. Synthesis of 2-benzyl-1-(2-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI041) using new method addition of basic side chain to coupled products

This reaction was attempted using method 5.6.3.1 for basic side chain addition. 2-Benzyl-1-(4-hydroxyphenyl)-1-(2-methoxyphenyl)-but-1-ene (BRI033) (0.00174M) was stirred in dichloromethane (50ml) with triphenylphosphine (0.00348M) and 1-(2-hydroxyethyl)pyrrolidine (0.00522M) at room temperature. Diispropyl azodicarboxylate (0.00435M) was added slowly over 20 minutes. The reaction was left stirring for two days. The reaction mixture was washed with ammonium chloride solution (40ml) and brine (50ml), extracted using dichloromethane (3 x 100ml) and dried over sodium sulphate. The dichloromethane was removed under reduced pressure to afford a brown oil. The product

was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 80:20) and recovered as yellow oil (20%) (R_f 0.2 dichloromethane:methanol 80:20).

5.7.3.3. Synthesis of 2-benzyl-1-(2-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI041)

The reaction was carried out according to the general method 5.7 by placing 2-benzyl-1-(4-hydroxyphenyl)-1-(2-methoxyphenyl)-but-1-ene (BRI033) (0.00045M) in acetone:water 19:1 (10ml) with potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M). The reaction was refluxed for 5 hours and was then extracted using dichloromethane (3 x 40ml). The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (44%), (R_f 0.2 dichloromethane:methanol 80:20). IR ν_{max} (film) 2954, 2786 (CHs), 1604 (C=C), 1505 (NCH_2), 1462 (CH_2), 1279 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.90 (3H, t, J=7.5Hz, H-4, CH_3), 1.85 (2H, q, J=7.5Hz, H-5, CH_2), 1.93 (4H, s, H-1'''', H-4''''), 2.91 (4H, s, H-2'''', H-3''''), 3.09 (2H, m, H-6, CH_2), 3.60 (2H, s, H-5, CH_2), 3.80 (3H, s, OCH_3), 4.19-4.23 (2H, m, H-4, CH_2), 6.77-6.80 (2H, dd, H-3'', H-5''), 6.81-6.83 (1H, d, H-5'''), 6.88-6.90 (1H, d, H-3'''), 6.93-6.95 (1H, d, H-4'''), 7.16-7.17 (2H, dd, H-2'', H-6''), 7.2-7.24 (5H, m, ArH), 7.30 (1H, s, H-2'''). ^{13}C NMR $\delta(CDCl_3)$ 12.25 (C-4, CH_3), 23.90 (C-2'''', C-3'''', $(CH_2)_2$), 24.60 (C-3, CH_2), 29 (C-5, CH_2), 36.60 (C-1'''', C-4'''', $(CH_2)_2$), 53.90 (C-6, CH_2), 54.84 (OCH_3), 65.30 (C-7, CH_2), 113.37-113.50 (C-3', C-5'), 119.96 (C-3''), 125.11(C-1''), 125.17 (C-5''), 127.32 (C-6''), 127.59 (C-1, C=C), 127.70 (C-1', C-2', C-6'), 128.29 (C-2''', C-6'''), 128.34 (C-4'''), 129.68 (C-3''', C-5'''), 129.80 (C-2, C=C), 13.01 (C-4''), 135.29 (C-1'''), 156 (C-2''), 157.55 (C-2''). HRMS calculated for $C_{30}H_{36}NO_2$ (M^++1) 442.2668, observed 442.0740.

5.7.4. Synthesis of 2-benzyl-1-(3-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI042)

5.7.4.1. Synthesis of 2-benzyl-1-(3-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI042)

This reaction was attempted using method 5.6.3.1 for basic side chain addition. Diispropyl azodicarboxylate (0.0023M) was added slowly over 20 minutes at room temperature to 2-benzyl-1-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-but-1-ene (BRI034) (0.00929M) with triphenylphosphine (0.00186M) and 1-(2-hydroxyethyl)pyrrolidine (0.00279M) in dichloromethane (50ml). The reaction was left stirring for two days and then was washed with ammonium chloride solution (50ml) and brine (50ml) and extracted using dichloromethane (3 x 100ml). The organic layer was dried over sodium sulphate and removed under reduced pressure to afford a brown oil. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 80:20) and recovered as yellow oil (15%) (R_f 0.15 dichloromethane:methanol 80:20).

5.7.4.2. Synthesis of 2-benzyl-1-(3-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI042)

Following the general method 5.7, 2-benzyl-1-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-but-1-ene (BRI034) (0.00045M) was dissolved in acetone:water 19:1 (10ml) and reacted with potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M) by refluxing for 5 hours in darkness. This was worked up and the product purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil in a 36% yield (R_f 0.15 dichloromethane:methanol 80:20). IR ν_{max} (film) 2964, 2785 (CHs), 1604 (C=C), 1506 (NCH_2), 1458 (CH_2), 1282 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.90 (3H, t, J=7.5Hz, H-4, CH_3), 2.01-2.03 (4H, s, H-1'''', H-4''''), 2.04-2.06 (2H, q, J=7.5Hz, H-3, CH_2), 3.15 (4H, s, H-2'''', H-3''''), 3.29-3.33 (2H, m, H-4, CH_2), 3.56 (2H, s, H-5, CH_2), 3.77-3.80 (2H, m, H-7, CH_2), 3.86 (3H, s, OCH_3), 6.81-6.83 (H, d, H-3'''), 6.84-6.86 (2H, dd, H-2''', H-4'''), 6.9-6.92 (2H, d, (J=8.04), H-3'', H-5''), 7.11-7.12 (2H, dd, H-2'', H-6''), 7.13-7.14 (4H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.80 (C-4, CH_3), 22.90 (C-2'''', C-3'''', $(CH_2)_2$), 24.27 (C-3, CH_2), 29.26 (C-5, CH_2), 36.82 (C-1'''', C-4'''', $(CH_2)_2$), 53.85 (C-6, CH_2), 54.70 (OCH_3), 64.98 (C-7, CH_2), 112.68-113.06 (C-2''), 113.65-113.70 (C-4''), 125.28 (C-6''), 127.80 (C-5', C-3'), 128.18 (C-6', C-2', C-1'), 129.80 (C-3''', C-5'''), 129.83 (C-4'''), 129.86-129.94 (C-2''', C-6''', C-5'''), 135.33 (C-1, C=C), 136 (C-2, C=C), 137.90 (C-1''), 140.07 (C-1'''), 156.96 (C-4'), 157.55 (C-3''). HRMS calculated for $C_{30}H_{36}NO_2$ (M^++1) 442.2746 observed 442.2772.

5.7.5. Synthesis of 2-benzyl-1-(4-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI037)

The general method 5.7 outlined was applied to 2-benzyl-1-(4-hydroxyphenyl)-1-(4-pivaloyloxyphenyl)-but-1-ene (BRI032) (0.00045M) where it was placed in acetone:water 19:1 (10ml) with potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M) and refluxed for 5 hours in darkness. The reaction mixture was dried using sodium sulphate, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (40%), (R_f 0.35 dichloromethane:methanol 80:20). IR ν_{max} (film) 2919, 2968 (CHs), 1748 (C=O), 1605 (C=C), 1504 (NCH_2), 1462 (CH_2), 1279 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.97 (3H, t, J=7.5Hz, H-4, CH_3), 1.34 (9H, s, OPiv), 1.79 (4H, s, H-1'''', H-4''''), 2.03-2.05 (2H, q, J=7.5Hz, H-3), 2.64 (4H, s, H-2'''', H-3''''), 3.13 (2H, s, H-6, CH_2), 3.73 (2H, s, H-5, CH_2), 3.9-3.94 (2H, m, H-4, CH_2), 6.81-6.84 (2H, dd, H-3'', H-5''), 6.99-7.02 (2H, dd, H-2'',H-6''), 7.17-7.20 (9H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.72 (C-4, CH_3), 21.20 (C-2'''', C-3'''', $(CH_2)_2$), 23.10 (C-3, CH_2), 24.23 ($(\underline{C}H_3)_3$, OPiv), 29.21 (C-5, CH_2), 36.70 (C-1, $\underline{C}(CH_3)_3$), 38.60 (C-6, CH_2), 49.80 (C-1'''', C-4'''', $(CH_2)_2$), 63.24 (C-7, CH_2), 113.70 (C-2', C-6'), 120.63-120.68 (C-3', C-5'), 121.70 (C-1, C=C), 125.40 (C-2, C=C), 127.90 (C-3'', C-5''), 128 (C-2'', C-6''), 129.63 (C-3''', C-4''', C-5'''), 130.27 (C-1''', C-2''', C-6'''), 132.61 (C-1''), 137.86 (C-1'), 139.12 (C-4''), 155.12 (C-4'), 173.50 (C=O). HRMS calculated for $C_{34}H_{42}NO_3$ 512.3164 (M^++1), observed 512.3165.

5.7.6. Synthesis of 2-benzyl-1-(3-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI038)

5.7.6.1. Synthesis of 2-benzyl-1-(3-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI038)

This reaction was attempted using method 5.6.3.1 for basic side chain addition. At room temperature Diispropyl azodicarboxylate (0.0065M) was added slowly over 20 minutes to 2-benzyl-1-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-but-1-ene (BRI034) (0.00259M), triphenylphosphine (0.00517M) and 1-(2-hydroxyethyl)pyrrolidine (0.00777M) in dichloromethane (50ml). The reaction was left stirring for two days and then was washed

with ammonium chloride solution (50ml), brine (50ml) and extracted using dichloromethane (3 x 100ml). The dichloromethane was dried (sodium sulphate) and removed *in vacuo* to afford a brown oil. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 80:20) and obtained as yellow oil (30%) (R_f 0.3 dichloromethane:methanol 80:20).

5.7.6.2. Synthesis of 2-benzyl-1-(3-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI038)

As in the general method 5.7, 2-benzyl-1-(4-hydroxyphenyl)-1-(3-pivaloyloxyphenyl)-but-1-ene (BRI035) (0.00045M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (87%), (R_f 0.3 dichloromethane:methanol 80:20). IR ν_{max} (film) 2931, 2966 (CHs), 1752 (C=O), 1605 (C=C), 1508 (NCH_2), 1470 (CH_2), 1279 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.97 (3H, t, J=7.5Hz, H-4, CH_3), 1.37 (9H, s, OPiv), 1.88 (4H, s, H-1'''', H-4''''), 2.06 (2H, q, J=7.5Hz, H-3, CH_2), 2.76 (4H, s, H-2'''', H-3''''), 2.97 (2H, m, H-6, CH_2), 3.57 (2H, s, H-5, CH_2), 4.1-4.18 (2H, m, H-4, CH_2), 6.82-6.84 (2H, dd, H-3'', H-5''), 6.84-6.85 (1H, d, H-4'), 6.91-6.94 (2H, dd, H-3', H-5'), 7.06-7.08 (1H, d, H-4'''), 7.12-7.16 (2H, dd, H-2'', H-6''), 7.2-7.22 (3H, m, H-2''', H-3''', H-6'''), 7.29-7.31 (2H, dd, H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 11.90 (C-4, CH_3), 22.40 (C-2'''', C-3''''$(CH_2)_2$), 23.54 (C-3, CH_2), 25.68 ($(\underline{C}H_3)_3$, OPiv), 31.29 (C-5, CH_2), 37.70 (C-1, $\underline{C}(CH_3)_3$), 39.60 (C-6, CH_2), 40.32 (C-1'''', C-4'''', $(CH_2)_2$), 61.24 (C-7, CH_2), 113.90 (C-2''), 20.63(C-4''), 122.70 (C-6''), 124.40 (C-5', C-3'), 128 (C-6', C-2', C-1'), 128.90 (C-3''', C-5'''), 129.63 (C-4'''), 131.27 (C-2''', C-6''', C-5'''), 132.61 (C-1, C=C), 137.86(C-2, C=C), 140.12 (C-1''), 143.07 (C-1'''), 156.12 (C-4'), 157.55 (C-3''), 173.50 (C=O). HRMS calculated for $C_{34}H_{42}NO_3$ 511.3165 (M^++1), observed 512.3199.

5.7.7. Synthesis of 2-benzyl-1-(2-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI044)

This reaction was attempted using method 5.6.3.1 for basic side chain addition. At room

temperature Diispropyl azodicarboxylate (0.0035M) was added slowly over 20 minutes to 2-benzyl-1-(4-hydroxyphenyl)-1-(2-pivaloyloxyphenyl)-but-1-ene (BRI043) (0.0013M), triphenylphosphine (0.0026M) and 1-(2-hydroxyethyl)pyrrolidine (0.0039M) in dichloromethane (50ml). The reaction was quenched and washed with saturated NH_4Cl solution (2 x 50ml) and extracted using dichloromethane (3 x 100ml). The dichloromethane was dried over sodium sulphate and removed *in vacuo* to afford a brown oil. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (19%),), (R_f 0.25 dichloromethane:methanol 80:20). IR ν_{max} (film) 2916, 2953 (CHs), 1742 (C=O), 1605 (C=C), 1503 (NCH_2), 1475 (CH_2), 1283 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.95-0.98 (3H, t, J=7.5Hz, H-4, CH_3), 1.36 (9H, s, OPiv), 1.82 (4H, s, H-1'''', H-4''''), 2.07 (2H, q, J=7.5Hz, H-3, CH_2), 2.65-2.66 (4H, s, H-2'''', H-3''''), 2.89-2.94 (2H, m, H-6, CH_2), 3.56 (2H, s, H-5, CH_2), 4.08-4.14 (2H, m, H-4, CH_2), 6.83-7.32 (13H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.74-12.78 (C-4, CH_3), 23.03 (C-2'''', C-3''''), 24.31 (C-3, CH_2), 26.65-26.70 (($\underline{C}H_3)_3$, OPiv), 30.39 (C-5, CH_2), 36.75-36.87 (C-1, $\underline{C}(CH_3)_3$), 38.55-38.60 (C-6, CH_2), 54.17-54.61 (C-1'''', C-4'''', $(CH_2)_2$), 66.41 (C-7, CH_2), 113.49-113.80 (C-2', C-6'), 118.77-118.82 (C-6''), 121.7-121.80 (C-3''), 125.3-125.35 (C-1, C=C), 126-126.07 (C-5''), 127.8-127.84 (C-2''', C-6'''), 128.23-128.43 (C-3', C-5', C-1'', C-4'''), 129.6-130.44 (C-3''', C-5'''), 137.61 (C-4''), 138.78-138.86 (C-2, C=C), 140.07 (C-1'''), 144.27 (C-1'), 150.45 (C-4'), 156.96-156.98 (C-2''), 172.30 (C=O). HRMS calculated for $C_{34}H_{42}NO_3$ 512.3165 (M^++1), observed 512.3148.

5.7.8. Synthesis of 1-(2-{4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI160)

As in the general method 5.7, 4-[1-(4-methoxy-3-methyl-phenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenol (BRI159) (0.0005M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.001M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0006M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (73%), (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 2931 (CHs), 1606 (C=C), 1500 (NCH_2), 1459 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$

0.89-0.95 (3H, t, J=7.28Hz, H-4, CH_3), 1.85 (4H, s, H-2'''', H-3''''), 2.21 (3H, s, CH_3), 2.43-2.47 (2H, q, J=7.28Hz, H-3, CH_2), 2.65-2.75 (4H, s, H-1'''', H-4''''), 2.95-2.98 (2H, t, J=6.02Hz, H-5), 3.77 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.02-4.04 (2H, t, J=6.0Hz, H-6), 6.56-6.59 (1H, d, H-2'''), 6.62-6.66 (1H, m, H-5''') 6.71-6.73 (2H, d, H-3'', H-5''), 6.78-6.81 (2H, m, H-3', H-5'), 6.8-6.90 (1H, m, H-6'''), 7.02-7.17 (4H, m H-2'', H-6'', H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 13.70 (C-4, CH_3), 16 (C-3, CH_3), 22.93 (C-2'''', C-3'''', $(CH_2)_2$), 28.55 (C-5, CH_2), 54.20 (OCH_3), 54.37 (OCH_3), 54.54 (C-6, CH_2), 54.63 (C-1'''', C-4'''', $(CH_2)_2$), 66.55 (C-7, CH_2), 109.82 (C-2'''), 112.74 (C-3'', C-5''), 112.91 (C-3', C-5'), 125.64 (C-5'''), 127.26 (C-6'''), 128.87 (C-1, C=C), 130.30 (C-2'', C-6''), 131.46 (C-2', C-6'), 132.71 (C-3'''), 134.40 (C-1'), 136.31 (C-2, C=C), 139.75 (C-1''), 139.79 (C-1'''), 155.08 (C-4'), 156.80 (C-4''), 157.17 (C-4'''). HRMS calculated for $C_{31}H_{38}NO_3$ 472.2852 (M^++1), observed 472.2838.

5.8. Deprotection of hydroxy groups - General Method

5.8.1. Synthesis of 2-benzyl-1-(3-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI045)

Using the general method described in section 5.4.6 boron trifluoride-dimethyl sulphide (0.00133M) was reacted with 2-benzyl-1-(3-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI042) (0.00667M) in dichloromethane (8ml) The residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield the product 2-benzyl-1-(3-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene as a yellow oil (30%) (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 3400 (OH), 2926, 2854 (CHs), 1681 (C=C), 1596 (NCH_2), 1486 (CH_2), 1264 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.95-0.99 (3H, t, J=7.16Hz, H-4, CH_3), 1.84 (4H, s, H-1'''', H-4''''), 2.0-2.07 (2H, q, J=7.5Hz, H-3, CH_2), 2.70 (4H, s, H-2'''', H-3''''), 2.95-2.96 (2H, s, H-6, CH_2), 3.50 (2H, s, H-5, CH_2), 4.09-4.12 (2H, m, H-4, CH_2), 6.78-7.31 (13H, m, ArH). ^{13}C NMR δ($CDCl_3$) 12.89 (C-4, CH_3), 22.90 (C-3, CH_2), 28.80 (C-2'''', C-3'''', $(CH_2)_2$), 31.30 (C-5, CH_2), 53.32 (C-1'''', C-4'''', $(CH_2)_2$), 54.09 (C-6, CH_2), 65.89 (C-7, CH_2), 113.67 (C-3', C-5'), 118.40 (C-4'''), 120.48 (C-2'''), 125.30 (C-5'''), 126.25 (C-6'''), 127.80 (C-2', C-6'), 128.17 (C-3'', C-5'', C-4''), 129.77 (C-2'', C-6''), 138.86 (C-2, C=C), 140.07 (C-1, C=C), 142.70 (C-1'), 143 (C-1''), 144 (C-1'''), 156.60 (C-4'), 156.98 (C-3'''). HRMS calculated for $C_{29}H_{34}NO_2$ 428.2590 (M^++1), 428.2595 observed.

5.8.2. Synthesis of 2-benzyl-1-(4-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI049)

A deprotection of the methoxy group was performed on 2-benzyl-1-(4-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI040) (0.0067M) using boron trifluoride-dimethyl sulphide (0.00133M) as described in the general method in section 5.4.6. The residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield the product 2-benzyl-1-(4-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI049) as a yellow oil (10%) (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 3300 (OH), 2926, 2855 (CHs), 1606 (C=C), 1508 (NCH_2), 1458 (CH_2), 1241 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.94-0.98 (3H, t, J=7.28Hz, H-4, CH_3), 1.87 (4H, s, H-2'''', H-3''''), 2.05-2.08 (2H, q, J=7.78Hz, H-3, CH_2), 2.80 (4H, s, H-1'''', H-4''''), 3.0 (2H, s, H-6, CH_2), 3.55 (2H, s, H-5, CH_2), 4.09-4.11 (2H, m, H-4, CH_2), 6.67-7.31 (13H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.80 (C-4, CH_3), 22.80 (C-2'''', C-3'''', $(CH_2)_2$), 24.30 (C-3, CH_2), 36.82 (C-5, CH_2), 53.90 (C-1'''', C-4'''', $(CH_2)_2$), 54.39 (C-6, CH_2), 65.22 (C-7, CH_2), 113.46 (C-3', C-5'), 114.73 (C-3''', C-5'''), 125.24 (C-4''), 127.78 (C-2'', C-6''), 128.19 (C-3'',C-5''), 129.85 (C-2', C-6'), 134.53 (C-1, C=C), 135.90 (C-2, C=C), 137.40 (C-1'), 138.20 (C-1''), 140.40 (C-1'''), 154.60 (C-4'''), 156.33 (C-4'). HRMS calculated for $C_{29}H_{34}NO_2$ 428.2590 (M^++1), observed 428.2569.

5.8.3. Synthesis of 2-benzyl-1-(2-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI106)

5.8.3.1. Demethylation of 2-benzyl-1-(2-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI041)

Using the general method 5.4.6, boron trifluoride-dimethyl sulphide (0.123M) was added dropwise over 30 min to 2-benzyl-1-(2-methoxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI041) (0.0123M) in dichloromethane (10ml). Stirring was continued for a further 10 hours at room temperature. The solvent was removed using a nitrogen purge and the remaining residue was dissolved in ethyl acetate (200ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1). The

yield was very low and required more purification so instead a depivaloylation was attempted on 2-benzyl-1-(2-pivaloyloxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI044).

5.8.3.2. General depivaloylation method

The pivaloyl protected compound (0.00664M) was deprotected by stirring with sodium hydroxide (0.0332M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours. The reaction mixture was acidified with 10% HCl (10ml) and the product extracted with dichloromethane (4 x 40ml). The organic layers were combined, dried (sodium sulphate) and evaporated. The product was then purified by column chromatography and identified by spectroscopic data.

5.8.3.3. Depivaloylation of 2-benzyl-1-(2-pivaloyloxyphenyl)-1-[4-pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI044)

2-Benzyl-1-(2-pivaloyloxyphenyl)-1-[4-pyrrolidinyl ethoxy)phenyl]but-1-ene (BRI044) (0.00664M) was depivaloylated using the general method described above by stirring with sodium hydroxide (0.0332M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours. The residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield the product 2-benzyl-1-(4-hydroxyphenyl)-1-[4-(pyrrolidinyl ethoxy)phenyl]but-1-ene as a yellow oil (45%) (R_f 0.32 dichloromethane:methanol 19:1). IR ν_{max} (KBr) 3300 (OH), 2926 (CHs), 1727 (C=C), 1511 (NCH_2), 1458 (CH_2), 1265 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.95-0.97 (3H, t, J=7.78Hz, H-4, CH_3), 1.86 (4H, s, H-2'''', H-3''''), 2.07-2.01 (2H, q, J=7.82Hz, H-3, CH_2), 2.77 (4H, s, H-1'''', H-4''''), 3.09 (2H, s, H-6, CH_2), 3.53 (2H, s, H-5, CH_2), 4.19-4.22 (2H, m, H-4, CH_2), 6.69-7.42 (13H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.81 (C-4, CH_3), 22.81 (C-2'''', C-3'''', $(CH_2)_2$), 24.27 (C-3, CH_2), 36.58 (C-5, CH_2), 53.86 (C-1'''', C-4'''', $(CH_2)_2$), 54.14 (C-6, CH_2), 65.36 (C-7, CH_2), 113.01 (C-3', C-5'), 113.52 (C-3''', C-5'''), 115.91 (C-4''), 125.31 (C-2'', C-6''), 128.19 (C-3'',C-5''), 129.77 (C-2', C-6'), 129.95 (C-1, C=C), 137.92 (C-2, C=C), 138.17 (C-1'), 140.18 (C-1''), 144.45 (C-1'''), 155.63 (C-4'''), 156.34 (C-4'). HRMS calculated for $C_{29}H_{34}NO_2$ (M^++1) 428.2609, observed 428.2590.

5.9. Bromo substituted products

5.9.1. Synthesis of 4-bromo-4'-methoxybenzophenone (BRI102)[145]

Synthesis of 4-bromo-4'-methoxybenzophenone (BRI102) was achieved via Friedel Crafts reaction. Anisole [2] (0.118M) and *para*-bromoanisoyl chloride (0.0256M) were stirred at 0°C under while aluminium trichloride (0.0255M) was added in 5 portions over 10 minutes. The reaction was stirred on ice for 45 minutes. Ice water was then added to the solution and the product extracted with dichloromethane. The sample was dried over sodium sulphate and concentrated under reduced pressure. The product crystallised as clear crystals from hexane:diethylether 1:1 (90.3%), (m.p. 156°C), (ethanol) [lit. m.p. 156-157 °C]. IR ν_{max} (film) 1639 (C=O), 1253 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.04, (3H, s, OCH_3), 6.96-6.98 (2H, d, H-2'', H-3''), 7.61-7.66 (4H, m, H-1', H-4', H-1'', H-4''), 7.79-7.80 (2H, d, H-2', H-3'). ^{13}C NMR δ($CDCl_3$)55.48 (OCH_3), 113.64 (C-3'', C-5''), 131.24 (C-3', C-5'), 131.32 (C-2'', C-6''), 132.42 (C-2', C-6'), 162.90 (C-4'), 194 (C=O).

5.9.2. Synthesis of 4-bromo-4'-hydroxybenzophenone (BRI103)[146]

5.9.2.1. Synthesis of 4-bromo-4'-hydroxybenzophenone (BRI103)[146]

4-Bromo-4'-methoxybenzophenone (BRI102) (0.002M) was heated at 200°C for 90 minutes with pyridine hydrochloride (0.015M). The reaction mixture was then diluted with 10% HCl (100ml) and dichloromethane (2 x 100ml). The organic phase was dried over sodium sulphate and removed under reduced pressure. The residue was purified by flash column chromatography (eluant: diethyl:hexane 1:1). The desired product was recovered as colourless crystals from hexane:diethyl ether 1:1 (30%) (Rf 0.6 diethyl ether:hexane 1:1). (m.p.186-187°C), (ethanol) (lit. m.p. 191°C).

5.9.2.2. Synthesis of 4-bromo-4'-hydroxybenzophenone (BRI103)[146]

The general deprotecting method 5.4 was used in this reaction. 4-Bromo-4'-methoxybenzophenone (BRI102) (0.02M) was dissolved in N,N-dimethylformamide (30ml) and stirring with sodium ethanethiolate (0.071M) for 3 hours at 80°C. N,N-Dimethylformamide was distilled off and the product was washed with water (100ml) and

extracted using DCM (3 x 100ml) and then washed with sodium chloride solution (100ml). The product was purified by flash chromatography (eluant: diethyl:hexane 1:1). The desired product was recovered as colourless crystals from hexane:diethyl ether 1:1 (55%) (Rf 0.6 diethyl ether:hexane 1:1). (m.p.186-187°C), (ethanol) (lit. m.p. 191°C). IR ν_{max} (film) 3400 (OH), 1638 (C=O), 1286 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 6.92-6.94 (2H, d, H-2'', H-3''), 7.64 (4H, m, H-1', H-4', H-1'', H-4''), 7.76-7.78 (2H, d, H-2', H-3'). ^{13}C NMR δ($CDCl_3$) 114.85 (C-3', C-5'), 126.50 (C-4''), 130.73 (C-3'', C-5''), 130.85 (C-2', C-6'), 132.37 (C-2'', C-6''), 159.72 (C-4'), 194 (C=O).

5.9.3. Synthesis of 1-(2-benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104)

The general method 5.6 was applied to this reaction. Zinc (0.012M) powder was suspended in dry tetrahydrofuran (100ml) and titanium tetrachloride (0.006M) was added to the mixture, which was then refluxed for 2 hours. Then 4-bromo-4'-hydroxybenzophenone (BRI103) (0.0014M) and 1-phenyl-2-butanone [3] (0.0141M) were dissolved in dry tetrahydrofuran (20ml) and added in one portion and the mixture refluxed for a further 4 hours before being poured into sodium carbonate solution (100ml) and extracted with ethyl acetate (4 x 200ml) and dichloromethane (200ml). The organic phases were dried over anhydrous sodium sulphate, filtered and evaporated to dryness *in vacuo*. The product was purified by flash column chromatography on silica gel (eluant: hexane:diethyl ether 1:1). The desired product was recovered as a brown oil (100%) (R_f 0.3 hexane). IR ν_{max} (film) 3368(OH), 2966 (CHs), 1594 (C=C), 1484 (CH_2), 1263 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.98-1.0 (3H, t, J=7.52Hz, H-4, CH_3), 2.03-2.08 (2H, q, J=7.52Hz, H-3, CH_2), 3.60 (2H, s, H-5, CH_2), 6.77-6.79 (2H, d, H-3', H-5'), 7.08-7.10 (4H, m, H-2'', H-6'', H-2', H-6'), 7.22 (3H, m, H-3'', H-5'', H-4''), 7.3-7.33 (2H, d, H-2''', H-6'''), 7.44-7.47 (2H, d, H-3''', H-5'''). ^{13}C NMR δ($CDCl_3$) 13.70 (C-4, CH_3), 24.28 (C-3, CH_2), 36.70 (C-5, CH_2), 114.70 (C-3', C-5'), 125.47 (C-4''), 127.90 (C-2', C-6'), 128.22 (C-3'', C-5''), 129.01 (C-1, C=C), 130.22 (C-2''', C-6'''), 130.60 (C-2'', C-6''), 130.77 (C-3''', C-5'''), 134.48 (C-2, C=C), 137.38 (C-4'''), 138.80 (C-1'), 139.88 (C-1''), 141.92 (C-1'''), 154.05 (C-4').

5.9.4. Synthesis of 1-(3-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenyl}-propyl)-pyrrolidine (BRI110)

As in the general method 5.7, 1-(2-benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104) (0.0004M) was refluxed for 5 hours in acetone:water 19:1 (10ml) with potassium carbonate (0.00046M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0019M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: hexane:diethyl ether:methanol 1:1:1). The product was obtained as a light brown oil (100%), (R_f 0.1 hexane:diethyl ether:methanol 1:1:1). IR ν_{max} (KBr) 2966 (CHs), 1605 (C=C), 1500 (NCH_2), 1265, 1242 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.97-0.99 (3H, t, J=7.52Hz, H-4, CH_3), 1.84 (4H, s, H-2'''', H-3''''), 2.05-2.40 (2H, q, J=7.48Hz, H-3, CH_2), 2.66-2.68 (4H, s, H-1'''', H-4''''), 2.9-2.93 (2H, t, J=5.8Hz, H-6, CH_2), 3.58 (3H, s, OCH_3), 4.08-4.11 (2H, t, J=5.8Hz, H-4, CH_2), 6.81-6.82 (2H, d, H-3', H-5'), 7.08-7.11 (4H, m, H-2', H-6', H-3'', H-6''), 7.18-7.23 (4H, t, J=8.88Hz, H-2''', H-6''', H-2'', H-4''), 7.28-7.32 (5H, H-3''', H-5'''), 7.43-7.44 (1H, d, H-5''). ^{13}C NMR $\delta(CDCl_3)$ 12.79 (C-4, CH_3), 22.98 (C-2'''', C-3'''', $(CH_2)_2$), 24.24 (C-3, CH_2), 36.70 (C-5, CH_2), 54.17 (C-1'''', C-4'''', $(CH_2)_2$), 54.52 (C-6, CH_2), 66.27 (C-7, CH_2), 113.79 (C-3', C-5'), 119.72 (C-4'''), 125.42 (C-2, C=C), 127.57 (C-4''), 127.88 (C-2', C-6'), 128.18 (C-3'', C-5''), 129.86 (C-2''', C-6'''), 130.53 (C-2'', C-6''), 130.57 (C-3''', C-5'''), 130.75 (C-1, C=C), 134.544 (C-1'), 138.80 (C-1''), 139.95 (C-1'''), 141.87 (C-4'). HRMS calculated for $C_{29}H_{33}NOBr$ 490.1746 (M^++1), observed 490.1740.

5.9.5. Synthesis of 4-(2-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenoxy}-ethyl)-morpholine (BRI111)

The general method 5.7 was used for basic side chain addition at the hydroxy substitutent. 1-(2-Benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104) (0.00025M) was placed in acetone:water 19:1 (10ml) with potassium carbonate (0.0003M), and 1-(2-chloroethyl)morpholine.HCl (0.00127M) and refluxed for 5 hours. The reaction mixture was washed and extracted according to the general method and purified by flash column chromatography (eluant: dichloromethane:methanol 19:1). The product was obtained as a light brown oil (100%), (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (KBr) 2944, 2856 (CHs), 1605 (C=C), 1500 (NCH_2), 1453 (CH_2), 1279, 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$

0.93-0.97 (3H, t, J=7.54Hz, H-4, CH_3), 2.01-2.03 (2H, q, J=7.56Hz, H-3, CH_2), 2.56-2.58 (4H, s, H-2'''', H-3''''), 2.78-2.80 (2H, t, J=5.52Hz, H-4, CH_2), 3.52-3.56 (2H, s, H-5, CH_2), 3.73-3.74 (4H, s, H-1'''', H-4''''), 4.06-4.10 (2H, t, J=5.78Hz, H-7, CH_2), 6.8-6.81 (2H, d, H-3''', H-5'''), 7.07-7.13 (4H, m, H-2', H-6', H-3'', H-5''), 7.15-7.22 (4H, t, H-2''', H-6''', H-2'', H-6''), 7.26-7.31 (4H, H-3', H-5'), 7.41-7.44 (1H, d, H-4''). ^{13}C NMR δ($CDCl_3$) 12.79 (C-4, CH_3), 24.23 (C-3, CH_2), 28.90 (C-5, CH_2), 53.60 (C-1'''', C-4'''', $(CH_2)_2$), 57.05 (C-6, CH_2), 65.19 (C-7, CH_2), 66.50 (C-2'''', C-3'''', $(CH_2)_2$), 113.80 (C-3', C-5'), 119.74 (C-4'''), 125.44 (C-2, C=C), 127.58 (C-2'', C-6''), 127.80 (C-2''', C-6'''), 127.89 (C-4''), 128.16 (C-3'', C-5''), 130.17 (C-2', C-6'), 130.55 (C-3''', C-5'''), 130.79 (C-1, C=C), 134.65 (C-1'''), 137.25 (C-1''), 139.79 (C-1'), 157.90 (C-4'). HRMS calculated for $C_{29}H_{33}NOBr$ 506.1695 (M^++1), observed 506.1695.

5.9.6. Synthesis of 1-(2-4-{-[1-(4-bromo-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-methoxy (BRI112)

As in the general method 5.6, titanium tetrachloride (0.004M) was added to zinc (0.008M) in dry THF (tetrahydrofuran). The mixture was refluxed at 100°C for 2 hours. Then 4-bromo-4'-methoxybenzophenone (BRI102) (0.001M) and 1-phenyl-2-butanone [3] (0.002M) were dissolved in dry THF and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (2 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated under reduced pressure to afford impure oil. The oil was purified by flash column chromatography (eluant:chloroform) to afford clear oil (R_f 0.8 chloroform) (100%). IR ν_{max} (KBr) 2963, 2929 (CHs), 1605 (C=C), 1265, 1243 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.0-1.056 (3H, t, J=6.82Hz, H-4, CH_3), 2.06-2.15 (2H, q, J=7.5Hz, H-3, CH_2), 3.59-3.65 (2H, s, H-5, CH_2), 3.81 (3H, s, OCH_3), 6.86-6.88 (2H, d, H-3', H-5'), 7.14-7.16 (4H, m, H-2', H-6', H-3'', H-5''), 7.22-7.29 (4H, m, H-2''', H-6''', H-2'', H-6''), 7.3-7.37 (2H, d, H-3''', H-5'''), 7.4-7.50 (1H, d, H-4''). ^{13}C NMR δ($CDCl_3$) 12.92 (C-4, CH_3), 24.37 (C-3, CH_2), 37.02 (C-5, CH_2), 54.76 (OCH_3), 113.23 (C-3''', C-5'''), 119.86 (C-2, C=C), 125.40 (C-4'), 125.50 (C-4''), 125.80 (C-2''', C-6'''), 127.60 (C-2', C-6'), 127.70 (C-3'', C-5''), 130 (C-2'', C-6''), 130.85 (C-3', C-5'), 134.50 (C-1'), 135.10 (C-1''), 137.37 (C-1, C=C), 137.42 (C-1'''), 157.90 (C-4''').

5.9.7. Synthesis of (2-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenoxy}-ethyl)-diethyl-amine (BRI115)

The reaction was carried out according to the general method 5.7 by placing 1-(2-benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104) (0.0004M) in acetone:water 19:1 (10ml) with potassium carbonate (0.00048M), and 2-diethylaminoethylchloride.HCl (0.00076M). The reaction was refluxed for 5 hours and then was and extracted using dichloromethane (2 x 50ml) and potassium carbonate solution (50ml). The product was purified by flash column chromatography (eluant: dichloromethane:methanol 19:1). The product was obtained as a light brown oil (80%), (R_f 0.2 dichloromethane). IR ν_{max} (film) 2968 (CHs), 1605 (C=C), 1500 (NCH_2), 1452 (CH_2), 1243 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.07-1.10 (7H, m, H-4, CH_3, H-2'''', H-3'''', $(CH_2)_2$), 2.0-2.06 (2H, q, J=7.52Hz, H-3, CH_2), 2.64-2.70 (4H, s, H-1'''', H-4'''', $(CH_3)_2$), 2.88-2.90 (2H, t, J=6.28Hz, H-5), 3.57 (2H, s, H-5, CH_2), 4.03-4.06 (2H, t, J=6.02Hz, H-4), 6.8-6.81 (2H, d, H-3', H-5'), 7.08-7.12 (4H, m, H-2', H-6', H-3'', H-5''), 7.19-7.23 (4H, t, H-2''', H-6''', H-2'', H-6''), 7.28-7.30 (2H, d, H-3''', H-5'''), 7.42-7.44 (1H, d, H-4''). ^{13}C NMR $\delta(CDCl_3)$ 11.15 (C-4, CH_3), 12.82 (C-2'''', C-3'''', $(CH_2)_2$), 24.25 (C-3, CH_2), 36.70 (C-5, CH_2), 47.24 (C-1'''', 4'''', $(CH_2)_2$), 51.15 (C-6, CH_2), 65.67 (C-7, CH_2), 113.70 (C-3', C-5'), 119.73 (C-2, C=C), 125.40 (C-4'), 127.80 (C-2', C-6'), 127.89 (C-3'', C-5''), 128.12 (C-2''', C-6'''), 129.88 (C-2'', C-6''), 130.70 (C-3''', C-5'''), 134.50 (C-1'), 137.30 (C-1''), 138.80 (C-1'''), 139.90 (C-1, C=C), 141.87 (C-4''), 156.90 (C-4'''). HRMS calculated for $C_{29}H_{35}NOBr$ 492.1902 (M^++1), observed 490.1894.

5.9.8. Synthesis of 1-(2-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenoxy}-ethyl)-piperidine (BRI116)

As in the general method 5.7, 1-(2-benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104) (0.00025M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.0003M), and 1-(2-chloroethyl)piperidinemonohydrochloride (0.0005M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 19:1). The product was obtained as a light brown oil (80%), (R_f 0.25 dichloromethane). IR ν_{max} (film) 2933 (CHs), 1605 (C=C), 1506 (NCH_2), 1453 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.94-0.99 (3H, t, J=7.48Hz, H-4, CH_3), 1.46-1.46

(2H, s, H-3'''', CH_2), 1.58-1.99 (4H, m, H-2'''', H-4'''', $(CH_2)_2$), 2.01-2.08 (2H, q, J=7.48Hz, H-3, CH_2), 2.19 (4H, s, H-1'''', H-5''''), 2.65-2.80 (2H, t, J=6.16Hz, H-5), 3.5-3.58 (2H, s, H-5, CH_2), 4.06-4.12 (2H, t, J=6.14Hz, H-4), 6.8-6.82 (2H, d, H-3', H-5'), 7.07-7.09 (4H, m, H-2', H-6', H-3'', H-5''), 7.17-7.23 (4H, t, H-2''', H-6''', H-2'', H-6''), 7.25-7.32 (2H, d, H-3''', H-5'''), 7.42-7.44 (1H, d, H-4''). ^{13}C NMR $\delta(CDCl_3)$ 12.83 (C-4, CH_3), 23.60 (C-3, CH_2), 25.28 (C-2'''', C-4'''', $(CH_2)_2$), 28.90 (C-3'''', CH_2), 36.70 (C-5, CH_2), 54.47 (C-1'''', 5'''', $(CH_2)_2$), 57.41 (C-6, CH_2), 65 (C-7, CH_2), 113.79 (C-3', C-5'), 119.72 (C-4'''), 125.71 (C-4''), 127.88 (C-2', C-6'), 128.21 (C-2''', C-6'''), 129.87 (C-3'', C-5''), 130.56 (C-2'', C-6''), 130.78 (C-3''', C-5'''), 134.50 (C-1'), 137.30 (C-1, C=C), 138 (C-2, C=C), 138.80 (C-1'''), 141.87(C-1''), 156.90 (C-4'). HRMS calculated for $C_{30}H_{35}NOBr$ 504.1902 (M^++1), observed 504.1899.

5.9.9. Synthesis of (2-{4-[2-benzyl-1-(4-bromo-phenyl)-but-1-enyl]-phenoxy}-ethyl)-dimethyl-amine (BRI122)

As in the general method 5.7, 1-(2-benzyl-1-phenylbut-1-enyl)-4-bromobenzene (BRI104) (0.0005M) was refluxed for 5 hours in darkness in acetone:water 19:1 (30ml) with potassium carbonate (0.0006M), and 2-dimethylaminoethyl.HCl (0.001M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 19:1). The product was obtained as a light brown oil (43%), (R_f 0.25 dichloromethane:methanol 19:1). IR ν_{max} (KBr) 2933 (CHs), 1605 (C=C), 1506 (NCH_2), 1453 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.95-0.99 (3H, t, J=7.52Hz, H-4, CH_3), 2.0-2.09 (2H, q, J=7.52Hz, H-3, CH_2), 2.63-2.68 (6H, s, H-1'''', H-2''''), 2.87-2.90 (2H, t, J=6.52Hz, H-5), 3.53-3.57 (2H, s, H-5, CH_2), 4.02-4.07 (2H, t, J=6.52Hz, H-4), 6.81-6.83 (2H, d, H-3', H-5'), 7.08-7.13 (4H, m, H-2', H-6', H-3'', H-5''), 7.2-7.21 (4H, m, H-2''', H-6''', H-2'', H-6''), 7.28-7.31 (2H, m, H-3''', H-5'''), 7.43-7.45 (1H, d, H-4''). ^{13}C NMR $\delta(CDCl_3)$ 12.80 (C-4, CH_3), 24.25 (C-3, CH_2), 36.70 (C-5, CH_2), 47.30 (C-1'''', C-2'''', $(CH_2)_2$), 51.20 (C-6, CH_2), 65.80 (C-7, CH_2), 113.73 (C-3', C-5'), 125.43 (C-4''), 127.80 (C-2''', C-6'''), 127.89 (C-2', C-6'), 129.87 (C-3'', C-5''), 130.19 (C-2'', C-6''), 130.70 (C-3''', C-5'''), 134.50 (C-4'''), 137.30 (C-2, C=C), 138.80 (C-1, C=C), 139.90 (C-1'), 141.88''' (C-1''), 144.02 (C-1''), 156.90 (C-4'). HRMS calculated for $C_{27}H_{31}NOBr$ 464.1589 (M^++1), observed 464.1586.

5.10. Synthesis of fluoro substituted products

5.10.1. Synthesis of 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-methoxy (BRI077)[154]

As in the general method 5.6, titanium tetrachloride (0.01175M) was added to zinc (0.0376M) in THF (tetrahydrofuran). The mixture was refluxed for 2 hours. Then 4-fluoro-4'-methoxybenzophenone (BRI003) (0.0047M) and propiophenone [26] (0.0094M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The reaction afforded an impure oil which was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1) to give the product as a pure white powder (R_f 0.8 hexane:diethyl ether 1:1) (80%) (m.p. 126°C), (hexane) [lit. m.p. 125 °C]. IR ν_{max} (film) 2926 (CHs), 1510 (C=C), 1250 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.91-0.99 (3H, t, J=7.76Hz, H-4, CH_3), 2.48-2.50 (2H, q, J=7.52Hz, H-3, CH_2), 3.72 (3H, s, OCH_3), 6.6-6.62 (2H, d, H-3', H-5'), 6.8-6.83 (2H, d, H-3''', H-5'''), 6.93-6.96 (1H, s, H-4''), 7.06-7.10 (2H, t, J=8.78Hz, H-3'', H-5''), 7.16-7.18 (4H, d, H-2', H-6', H-2''', H-6'''), 7.2-7.23 (2H, m, H-2'', H-5''). ^{13}C NMR δ($CDCl_3$) 13.16 (C-4, CH_3), 28.62 (C-3, CH_2), 54.56 (OCH_3), 112.38 (C-3''', C-5'''), 113.12 (C-3', C-5'), 114.70 (C-2'', C-6''), 125.70 (C-2, C=C), 127.50 (C-2', C-6'), 129.22 (C-2''', C-6'''), 130.10 (C-1, C=C), 130.64 (C-4''), 131.45 (C-3'', C-5''), 131.84 (C-1'''), 134.84 (C-1''), 136.75 (C-1'), 141.78 (C-4'), 157.13 (C-4'''). ^{19}F NMR ppm ($CDCl_3$) -116.47 (Ar<u>F</u>).

5.10.2. Synthesis of 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078)[155]

This coupling was performed according to the general McMurry 5.10 coupling method. Titanium tetrachloride (0.01175M) was added to zinc (0.0376M) in THF (tetrahydrofuran). The mixture was refluxed for 2 hours. Then 4-hydroxy-4'-fluorobenzophenone [4] (0.0047M) and propiophenone [26] (0.0094M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (50ml), brine (50ml) and extracted with ethyl acetate (2 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated to afford an impure oil (55%). Flash column chromatography was performed to purify the crude product (eluant: 1. hexane:diethyl ether 7:3, 2. dichloromethane). The

product was present with an impurity of the same polarity, which was difficult to remove. An acetylation was performed on the product to aid purification (see section 5.18.3 below).

5.10.3. General method - Acetylation of hydroxy substituted product

The hydroxy substituted product (0.0016M) was stirred with acetic anhydride (0.035M) and pyridine (0.045M) overnight in the dark. The reaction mixture was poured into an ice:water mixture 1:1 (100ml) and extracted with diethyl ether (2 x 100ml). This was then washed with hydrochloride solution (0.1M) (100ml). The product was dried over sodium sulphate, reduced under reduced pressure and finally purified by flash column chromatography.

5.10.3.1. Acetylation of BRI078 to produce BRI081 {4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol}-acetic acid

The general acetylation method 5.11.3 was applied to this reaction. The 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (0.0016M) was stirred with acetic anhydride (0.035M) and pyridine (0.045M) overnight in the dark. Then the reaction mixture was poured into an ice water (100ml) and extracted with diethyl ether (2 x 100ml). This was then washed with hydrochloride solution (0.1M) (100ml). The product was dried over sodium sulphate, reduced under reduced pressure and finally purified by flash column chromatography to afford the product as an oil (eluant: hexane:diethyl ether 4:1), (64%), (R_f 0.64 hexane:diethyl ether 4:1). IR ν_{max} (film) 2926 (CHs), 1605 (C=C), 1501 (NCH_2), 1454 (CH_2), 1250 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.93-0.97 (3H, t, J=8.28Hz, H-4, CH_3), 2.23 (3H, s, H-3), 2.46-2.53 (2H, q, J=7.26Hz, H-3, CH_2), 6.77-6.78 (2H, d, H-3', H-5'), 6.85-6.88 (2H, d, H-3''', H-5'''), 7.04-7.07 (2H, d, H-3'', H-5''), 7.09-7.12 (3H, m, H-2', H-6', H-4''), 7.18-7.22 (2H, d, H-2''', H-6'''), 7.26-7.27 (2H, d, H-2'', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 13.06 (C-4, CH_3), 20.70 ($CO\underline{C}H_3$), 28.50 (C-3, CH_2), 113.77 (C-3', C-5'), 119 (C-3''', C-5'''), 125.87 (C-4''), 127.50 (C-2', C-6'), 129.13 (C-3'', C-5''), 129.90 (C-2''', C-6'''), 130.60 (C-1, C=C), 131.21 (C-2'', C-6''), 131.90 (C-2, C=C), 136.34 (C-1'), 139.82 (C-1''), 141.30 (C-1'''), 148.13 (C-4'''), 148 (C-4'), 159.55 (C=O). ^{19}F NMR ppm $(CDCl_3)$ -117.49 (Ar$\underline{F}$).

5.10.4. General method - Deacetylation of acetylated product

The acetylated product (1.38M) was refluxed at 80°C for 24 hours in methanol (20ml) with water (20ml) and potassium carbonate (9.66M). The product was washed with water (100ml) and extracted using ethyl acetate (2 x 100ml). The product was dried (sodium sulphate) and solvent removed under reduced pressure. No further purification of the product should be necessary.

5.10.4.1. Deacetylation of BRI081 to produce 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078)

Using the general method 5.11.4, {4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol}-acetic acid (BRI081) (1.38M) was refluxed at 80°C for 24 hours in methanol (20ml) with water (20ml) and potassium carbonate (9.66M). The product was washed with water (100ml), extracted using ethyl acetate (2 x 100ml), dried (sodium sulphate) and the solvent removed under reduced pressure to afford a pure yellow oil (70%) (R_f 0.7 dichloromethane). IR ν_{max} (film) 3386 (OH), 2963 (CHs), 1507 (C=C), 1225 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.00-1.02 (3H, t, J=7.6Hz, H-4, CH_3), 2.52-2.57 (2H, q, J=7.6Hz, H-3, CH_2), 6.55-6.57 (2H, d, H-3', H-5'), 6.74-6.79 (2H, t, J=8.76Hz, H-3''', H-5'''), 6.8-6.82 (2H, d, H-2', H-6'), 6.89-6.91 (2H, d, H-3'', H-5''), 7.09-7.14 (2H, t, J=8.76Hz, H-2''', H-6'''), 7.18-7.19 (2H, d, H-2'', H-6''), 7.2-7.23 (1H, d, H-4''). ^{13}C NMR $\delta(CDCl_3)$ 13.21 (C-4, CH_3), 28.64 (C-3, , CH_2), 114.54 (C-3''', C-5'''), 114.79 (C-3', C-5'), 127.58 (C-3'', C-5'', C-4''), 129.30 (C-2', C-6'), 130.40 (C-2''', C-6'''), 130.70 (C-1, C=C), 131.72 (C-2'', C-6''), 132 (C-2, C=C), 135 (C-1'), 141.87 (C-1'''), 154.00 (C-1''), 160.04 (C-4''), 162.49 (C-4'''). ^{19}F NMR ppm $(CDCl_3)$ -116.99 (Ar<u>F</u>).

5.10.5. Addition of basic side

5.10.5.1. Attempted synthesis of (4-fluoro-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone (BRI079) using basic side chain addition method

As in the general method 5.7, 4-hydroxy-4'-fluorobenzophenone [4] (0.00045M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M). The resulting mixture was

dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 80:20). The product (4-fluoro-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone was a yellow oil (36%).

5.10.5.2. Attempted synthesis of (4-fluoro-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone (BRI079) using Mitsunobu method for basic side chain addition method

Using the general method for sidechain addition 5.6.3.1 4-hydroxy-4'-fluorobenzophenone [4] (4.626mM) was stirred in dichloromethane (50ml) with triphenylphosphine (9.252mM) and 1-(2-hydroxyethyl)pyrrolidine (13.8mM) at room temperature. Diispropyl azodicarboxylate (11.6mM) was added slowly over 20 minutes. The reaction was left stirring for two days. TLC analysis showed almost complete consumption of the starting material. The reaction mixture was extracted using dichloromethane (3 x 100ml) and dried over sodium sulphate. The dichloromethane was removed under reduced pressure to afford a brown oil. The product was purified by flash column chromatography on silica gel (eluant: acetone) and recovered as yellow oil (70%) (R_f 0.36 acetone). IR ν_{max} (KBr) 2929 (CHs), 1647 (C=O), 1504 (NCH_2) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.77 (4H, s, H-2'''', H-3''''), 2.59 (4H, s, H-1'''', H-4''''), 2.88-2.90 (2H, t, J=5.52Hz, H-3, CH_2), 4.13-4.16 (2H, t, J=5.52Hz, H-4, CH_2), 6.9-6.95 (2H, d, H-2', H-3'), 7.07-7.11 (2H, t, J=8.02Hz, H-1'', H-4''), 7.28-7.41 (2H, d, H-2'', H-3''), 7.43-7.47 (2H, m, H-1', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 22.90 (C-2'''', C-3'''', $(CH_2)_2$), 54.23 (C-1'''', C-4'''', $(CH_2)_2$), 54.35 (C-2, CH_2), 66.88 (C-1, CH_2), 113.69 (C-2'', C-6''), 114.92 (C-2'', C-6''), 127.94 (C-1''), 118.08 (C-3', C-5'), 131.52 (C-1''), 131.87 (C-3'', C-5''), 162.08 (C-4'), 165.77 (C-4''), 193.55 (C=O). ^{19}F NMR ppm ($CDCl_3$) -107.39 (Ar<u>F</u>). HRMS calculated for $C_{19}H_{21}FNO_2$ 314.1556 (M^++1), observed 314.1570.

5.10.6. Synthesis of 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-pyrrolidine (BRI080)

5.10.6.1. Method 1 - synthesis of 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-pyrrolidine (BRI080) using the Mitsunobu side chain addition method

As in the basic side chain addition method 5.6.3.1, 2 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078) (4.626mM) was stirred in dichloromethane (50ml) with triphenylphosphine (9.252mM) and 1-(2-hydroxyethyl)pyrrolidine (13.8mM) at room temperature. Diispropyl azodicarboxylate (11.6mM) was added slowly over 20 minutes. The reaction was left stirring for one day. TLC analysis showed almost complete consumption of the starting material. The reaction mixture was washed with ammonium chloride solution (50ml) and brine (50ml), extracted using dichloromethane (3 x 100ml) and dried over sodium sulphate. The dichloromethane was removed under reduced pressure leaving a brown oil. The product was purified by flash column chromatography on silica gel (eluant: hexane:ethyl acetate 1:1) and recovered as yellow oil (78%) (R_f 0.83 hexane:diethyl ether 1:1).

5.10.6.2. Method 2 - synthesis of 1-(2-4-{-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-pyrrolidine (BRI080) using the new McMurry coupling method

Following the general method 5.6, titanium tetrachloride (0.01175M) was added to zinc dust (0.0376M) in dry THF (tetrahydrofuran) (80ml) and the mixture was refluxed at 100°C for 2 hours. Then 4-fluoro-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone (BRI079) (0.0047M) and propiophenone [26] (0.0141M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. Flash column chromatography on silica gel was performed to purify the crude product (78% yellow oil) (eluant: dichloromethane:methanol 19:1) (R_f 0.5 (dichloromethane:methanol 19:1). IR ν_{max} (KBr) 2956, 2871 (CHs), 1604 (C=C), 1506 (NCH_2), 1438 (CH_2), 1240 (CH_3) cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.93-0.96 (3H, t, J=7.28Hz, H-4, CH_3), 1.81 (4H, s, H-2'''', H-3''''), 2.43-2.47 (2H, q, J=7.26Hz, H-3, CH_2), 2.62 (4H, s, 1'''', 4''''), 2.84 (2H, t, J=6.02Hz, H-4, CH_2), 3.99-4.02 (2H, t, J=5.76Hz, H-5, CH_2), 6.57-6.59 (2H, d, H-3', H-5'), 6.76-6.78 (2H, d, H-3''', H-5'''), 7.03-7.08 (2H, t, J=8.78Hz, H-3'', H-5''), 7.12-7.14 (3H, m, H-2', H-6', H-4''), 7.17-7.22 (4H, d, H-2''', H-6''', H-2'', H-6''). ^{13}C NMR δ($CDCl_3$) 13.14 (C-4, CH_3), 23 (C-2'''', C-3'''', $(CH_2)_2$), 28.56 (C-3, CH_2), 54.25 (C-1'''', C-4'''', $(CH_2)_2$), 54.60 (C-5, CH_2), 66.20 (C-6, CH_2), 112.99 (C-3', C-5'), 114.60 (C-3''', C-5'''), 125.70 (C-4''), 127.47 (C-2'', C-6''), 129.18 (C-1, C=C), 130.08 (C-2''', C-6'''), 130.50 (C-2', C-6'), 131.38 (C-3'', C-5''), 131.60 (C-2, C=C), 134.87 (C-1'), 136.85 (C-1''), 139.27 (C-1'''), 141.21 (C-4'), 157 (C-4'''). ^{19}F NMR ppm ($CDCl_3$) -116.53 (ArF). HRMS calculated for $C_{28}H_{31}FNO$ 415.5423 (M^++1), observed 416.2390.

5.10.7. Synthesis of 4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082)

The general method 5.6 was applied to this reaction. Zinc (0.0376M) powder was suspended in dry tetrahydrofuran (100ml) and titanium tetrachloride (0.01175M) was added to the mixture, which was then refluxed for 2 hours. Then 4-hydroxy-4'-fluorobenzophenone [4] (0.0047M) and propiophenone [26] (0.0141M) were dissolved in dry tetrahydrofuran (50ml) and added in one portion and the mixture refluxed for a further 4 hours. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:hexane 1:1). The desired product was recovered as a brown oil (100%) (R_f 0.85 dichloromethane). IR ν_{max} (film) 2967 (CHs), 1601 (C=C), 1506 (NCH_2), 1453 (CH_2), 1221 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.0-1.04 (3H, t, J=6.78Hz, H-4, CH_3), 2.43-2.47 (2H, q, J=7.52Hz, H-3, CH_2), 3.65 (2H, s, H-5, CH_2), 6.85-6.87 (2H, d, H-3', H-5'), 7-7.06 (2H, t, J=8.87Hz, H-3''', H-5'''), 7.11-7.15 (2H, d, H-2'', H-6''), 7.22-7.28 (5H, m, H-3'', H-5'', H-4'', H-2', H-6'), 7.32-7.34 (3H, m, H-2''', H-6'''). ^{13}C NMR $\delta(CDCl_3)$ 12.85 (C-4, CH_3), 24.30 (C-3, CH_2), 36.78 (C-5, CH_2), 114.36 (C-3''', C-5'''), 114.80 (C-3', C-5'), 125.47 (C-4''), 127.78 (C-2''', C-6'''), 128.08 (C-2''', C-6'''), 130.01 (C-2', C-6'), 130.55 (C-3'', C-5''), 134.31 (C-1, C=C), 137.60 (C-2, C=C), 139.07 (C-1''), 140.18 (C-1'), 154.60 (C-1'''), 159.73 (C-4'''), 162.17 (C-4'). ^{19}F NMR ppm ($CDCl_3$) -117.0 (ArF). HRMS calculated for $C_{23}H_{22}FO$ (M^++1) 314.1663, observed 314.1671.

5.10.8. Synthesis of (2-{4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-dimethyl-amine (BRI083)

The Mitsunobu basic side chain addition method 5.6.3.1 was used here. 4-[1-(4-Fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078) (4.626mM) was stirred in dichloromethane (50ml) with triphenylphosphine (9.252mM) and N,N-dimethylethanolamine (13.8mM) at room temperature. Diispropyl azodicarboxylate (11.6mM) was added slowly over 20 minutes. Stirring was continued at room temperature for 4 hours. The reaction mixture was quenched with saturated NH_4Cl solution (50ml) and extracted with dichloromethane (3 x 100ml). The combined organic layers were washed with saturated NH_4Cl (50ml), water (50ml) and brine (50ml). The product was purified by flash column chromatography on silica gel (eluant: acetone) and recovered as yellow oil (27%) (R_f 0.36 (dichloromethane:methanol 19:1). IR ν_{max} (film) 2981, 2934 (CHs), 1615 (C=C), 1507

(NCH_2), 1467 (CH_2), 1241 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.93-0.97 (3H, t, J=7.28Hz, H-4, CH_3), 2.44 (6H, s, 1'''', 2''''), 2.46-2.53 (2H, q, J=7.52Hz, H-3, CH_2), 2.76-2.87 (2H, t, J=5.26Hz, H-5, CH_2), 4.14-4.17 (2H, t, J=5.26Hz, H-4, CH_2), 6.68-6.72 (2H, t, J=8.54Hz, H-3', H-5'), 6.8-6.85 (2H, m, H-3''', H-5'''), 6.9-6.92 (2H, d, H-2', H-6'), 7.02-7.05 (2H, d, H-3'', H-5''), 7.08-7.20 (5H, m, H-2'', H-6'', H-2''', H-6''', H-4''). ^{13}C NMR δ($CDCl_3$) 13.27 (C-4, CH_3), 21.60 (C-3, CH_2), 45.32 (C-1'''', C-2'''', $(CH_3)_2$), 54.80 (C-5, CH_2), 60.96 (C-6, CH_2), 109.80 (C-3''', C-5'''), 113.11 (C-3', C-5'), 125.86 (C-1, C=C), 127.60 (C-3'', C-4'', C-5''), 129.36 (C-2', C-6'), 129.90 (C-2'', C-6''), 129.99 (C-2, C=C), 130.26 (C-2''', C-6'''), 131.50 (C-1'), 132 (C-1''), 136.27 (C-1'''), 138.21 (C-4'), 141.55 (C-4'''). ^{19}F NMR ppm ($CDCl_3$) -117.32 (ArF). HRMS calculated for $C_{26}H_{29}FNO$ 389.2155 (M^++1), observed 390.2233.

5.10.9. Synthesis of 4-(2-{4-[1-4-fluoro-phenyl]-2-phenyl-but-1-enyl}-[phenoxy]-ethyl)-morpholine (BRI084)

Using the basic side chain addition method 5.6.3.1 4-[1-(4-Fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078) (4.626mM) was stirred in dichloromethane (50ml) with triphenylphosphine (9.252mM) and N-(-2-hydroxyethyl)-morpholine (13.8mM) at room temperature. Diispropyl azodicarboxylate (11.6mM) was added left stirring overnight. The product was extracted using dichloromethane (3 x 100ml), washed with ammonium chloride solution (50ml), brine (50ml) and dried over sodium sulphate. The dichloromethane was removed under reduced pressure to afford a brown oil. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:ethyl acetate 3:1) and recovered as yellow oil (86%) (R_f 0.58 dichloromethane:methanol 19:1). IR ν_{max} (KBr) 2980, 2935 (CHs), 1631 (C=C), 1497 (NCH_2), 1469 (CH_2), 1239 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.92-0.95 (3H, t, J=7.28Hz, H-4, CH_3), 2.44-2.47 (2H, q, J=7.26Hz, H-3, CH_2), 2.57-2.58 (4H, s, 2'''', 3''''), 2.74-2.77 (2H, t, J=5.52Hz, H-4, CH_2), 3.72-3.75 (4H, s, 1'''', 4''''), 3.99-4.02 (2H, t, J=5.52Hz, H-5, CH_2), 6.55-6.57 (2H, d, H-3', H-5'), 6.76-6.78 (2H, d, H-3''', H-5'''), 7.03-7.07 (2H, t, J=8.78Hz, H-2', H-6'), 7.11-7.14 (3H, m, H-2'', H-3'', H-4''), 7.19-7.28 (4H, m, H-2'', H-6'', H-2''', H-6'''). ^{13}C NMR δ($CDCl_3$) 13.10 (C-4, CH_3), 28.57 (C-3, CH_2), 53.57 (C-2'''', C-3'''', $(CH_2)_2$), 57.17 (C-5, CH_2), 64.90 (C-6, CH_2), 66.35 (C-1'''', C-4'''', $(CH_2)_2$), 112.99 (C-3', C-5'), 114.45 (C-3''', C-5'''), 125.66 (C-4''), 127.46 (C-2''', C-6'''), 129.17 (C-3'', C-5''), 130.11 (C-2', C-6'), 131.58 (C-2'', C-6''), 135.06 (C-2, C=C), 136.64 (C-1, C=C), 139.17 (C-1''),

141.33 (C-1'), 141.75 (C-1'''), 156.19 (C-4'''), 162.39 (C-4'). ^{19}F NMR ppm ($CDCl_3$) -116.52 (ArF). HRMS calculated for $C_{28}H_{31}FNO$ 431.5417 (M^++1), observed 432.2339.

5.10.10. Synthesis of 1-(2-{4-[4-fluoro-phenyl)-2-phenyl-but-1-enyl]-[phenoxy]-ethyl)-piperidine (BRI085)

Side group addition was performed using the method 5.6.3.1. Diispropyl azodicarboxylate (11.6mM) was added dropwise to a solution of 4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenol (BRI078) (4.626mM), triphenylphosphine (9.252mM) and N-(-2-hydroxyethyl)piperidine (13.8mM) in dichloromethane (50ml) and left stirring overnight. The product was extracted using dichloromethane (3 x 100ml), washed and dried over sodium sulphate. The dichloromethane was removed under reduced pressure to afford a brown oil. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 18:3) and recovered as yellow oil (27%) (R_f 0.27 dichloromethane:methanol 19:1). IR ν_{max} (KBr) 2934 (CHs), 1634 (C=C), 1507 (NCH_2), 1466 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.93-0.97 (3H, t, J=7.26Hz, H-4, CH_3), 1.45-1.46 (2H, d, H-3''''), 1.59-1.62 (4H, t, J=5.52Hz, H-2'''', H-4''''), 1.66-1.69 (4H, q, J=5.52Hz, H-1'''', H-5''''), 2.44-2.50 (2H, q, J=7.52Hz, H-3, CH_2), 2.65-2.67 (2H, t, J=6.04Hz, H-4, CH_2), 4.25-4.28 (2H, t, J=6.04Hz, H-5, CH_2), 6.67-6.70 (2H, t, J=8.78Hz, H-3', H-5'), 6.8-6.83 (2H, d, H-3''', H-5'''), 6.89-6.91 (2H, d, H-2', H-6'), 7.02-7.08 (2H, t, J=8.78Hz, H-3'', H-5''), 7.08-7.10 (2H, d, H-2'', H-6''), 7.13-7.16 (3H, m, H-2''', H-6''', H-4''). ^{13}C NMR δ($CDCl_3$) 13.10 (C-4, CH_3), 21.33 (C-3, CH_2), 25.31 (C-2'''', C-4'''', $(CH_2)_2$), 28.56 (C-3'''', CH_2), 54.30 (C-1'''', C-5'''', $(CH_2)_2$), 56.77 (C-5, CH_2), 64.50 (C-6, CH_2), 112.90 (C-3''', C-5'''), 113.69 (C-3', C-5'), 127.44 (C-3'', C-4'', C-5''), 129.18 (C-2', C-6'), 129.50 (C-1''), 130.10 (C-2'', C-6''), 130.50 (C-1'''), 131.78 (C-2''', C-6'''), 132.06 (C-1, C=C), 132.50 (C-1'), 141.72 (C-2, C=C), 144.50 (C-4'''), 154.39 (C-4'). ^{19}F NMR ppm ($CDCl_3$) -117.34 (ArF). HRMS calculated for $C_{29}H_{33}FNO$ 429.5789 (M^++1), observed 430.2546.

5.10.11. Synthesis of diethyl-(2-{4-[1-(4-fluoro-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-ethyl)-amine (BRI086)

The basic side chain addition method 5.6.3.1 was used here. Diispropyl azodicarboxylate (11.6mM) was added slowly over 20 minutes to a solution of 4-[1-(4-fluoro-phenyl)-2-

phenyl-but-1-enyl]-phenol (BRI078) (4.626mM), triphenylphosphine (9.252mM) and 2-diethylaminoethanol (13.8mM) in dichloromethane (50ml) at room temperature and left stirring for 12 hours. The product was extracted with dichloromethane (3 x 100ml), dried over sodium sulphate and the dichloromethane was removed *in vacuo* to afford a brown oil. Flash column chromatography was used to purify the product with silica gel as the stationary phase (eluant: dichloromethane:methanol 18:2) and recovered as yellow oil (100%) (R_f 0.3 dichloromethane:methanol 19:1). IR ν_{max} (film) 2981, 2934 (CHs), 1628 (C=C), 1506 (NCH_2), 1469 (CH_2), 1240 (CH_3) $cm^{-1}cm^{-1}$. ^1H NMR $\delta(CDCl_3)$ 1.13-1.16 (3H, t, J=7.02Hz, H-4, CH_3), 1.27-1.29 (6H, s, H-2'''', H-3''''), 2.46-2.50 (2H, q, J=7.52Hz, H-3, CH_2), 2.75-2.78 (4H, t, J=6.52Hz, 1'''', 4''''), 2.98-3.0 (2H, t, J=5.76Hz, H-5, CH_2), 4.13-4.16 (2H, t, J=6.02Hz, H-4, CH_2), 6.68-6.70 (2H, t, J=8.78Hz, H-3', H-5'), 6.8-6.84 (2H, m, H-3''', H-5'''), 6.88-6.90 (2H, d, H-2', H-6'), 7.02-7.07 (2H, t, J=8.78Hz, H-3'', H-5''), 7.08-7.10 (2H, m, H-2'', H-6''), 7.13-7.16 (3H, d, H-2''', H-6''', H-4''). ^{13}C NMR $\delta(CDCl_3)$ 13.10 (C-4, CH_3), 21.50 (C-3, CH_2), 30.50 (C-2'''', C-3'''', $(CH_3)_2$), 47.12 (C-1'''', C-4'''', $(CH_2)_2$), 50.99 (C-5, CH_2), 65.29 (C-6, CH_2), 112.90 (C-3''', C-5'''), 113.64 (C-3'', C-5''), 113.84 (C-4''), 114.63 (C-3', C-5'), 125.69 (C-1, C=C), 127.44 (C-2''', C-6'''), 129.18 (C-2', C-6'), 130.10 (C-2'', C-6''), 130.50 (C-2, C=C), 130.59 (C-4'), 131.40 (C-4'''), 131.80 (C-1'), 131.86 (C-1''), 141.73 (C-1'''). ^{19}F NMR ppm $(CDCl_3)$ -117.34 (Ar<u>F</u>). HRMS calculated for $C_{28}H_{33}FNO$ 417.5582 (M^++1), observed 418.2546.

5.10.12. Synthesis of 1-(2-{4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI087)

Following the basic side chain addition method 5.6.3.1, to a solution of 4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082) (4.626mM), triphenylphosphine (9.252mM) and 1-(2-hydroxyethyl)pyrrolidine (13.8mM) in dichloromethane (50ml), Diispropyl azodicarboxylate (11.6mM) was added and left stirring for 12 hours. The reaction mixture was washed with NH_4CL solution (50ml) and brine (50ml), using dichloromethane (3 x 100ml) to extract the product. The dichloromethane was dried over sodium sulphate and removed *in vacuo* to afford a brown oil. Flash column chromatography was used to purify the product with silica gel as the stationary phase (eluant: chloroform:methanol 4:1) and recovered as yellow oil (80%) (R_f 0.6 chloroform:methanol 4:1). IR ν_{max} (film) 2853, 2930 (CHs), 1604 (C=C), 1505 (NCH_2), 1458 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.97-0.99 (3H, t, J=7.56, H-4, CH_3), 1.85 (4H, s, H-2'''', H-3''''), 2-2.06 (2H, q, J=7.3, H-3,

CH_2), 2.65 (4H, s, 1'''', 4''''), 2.92-2.95 (2H, t, J=6.00Hz, H-6, CH_2), 3.58 (2H, s, H-5, CH_2), 4.1-4.13 (2H, t, J=5.98, H-4, CH_2), 6.83-6.85 (2H, d, H-2', H-6'), 6.98-7.02 (2H, t, J=8.76, H-3''', H-5'''), 7.11-7.14 (2H, d, H-3', H-5'), 7.14-7.22 (5H, m, H-2'', H-3'', H-4'', H-5'', H-6''), 7.29-7.31 (2H, d, H-2''', H-6'''). ^{13}C NMR δ($CDCl_3$) 12.80 (C-4, CH_3), 23 (C-2'''', C-3'''', $(CH_2)_2$), 24.23 (C-3, CH_2), 36.70 (C-5, CH_2), 54.18 (C-1'''', C-4'''', $(CH_2)_2$), 54.60 (C-6, CH_2), 66.20 (C-7, CH_2), 113.75 (C-3', C-5'), 114.57 (C-3''', C-5'''), 114.60 (C-4''), 125.40 (C-1, C=C), 127.88 (C-2''', C-6'''), 128.14 (C-3'', C-5''), 129.80 (C-2'', C-6''), 130.28 (C-2', C-6'), 135 (C-1'), 137.42 (C-1'''), 138.53 (C-2, C=C), 140.07 (C-1''), 156.80 (C-4'''), 159.70 (C-4'). ^{19}F NMR ppm ($CDCl_3$) -117.12 (Ar**F**). HRMS calculated for $C_{29}H_{33}FNO$ 430.2546 (M^++1), observed 430.2545.

5.10.13. Synthesis of (2-{4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenoxy}-ethyl)-dimethyl-amine (BRI088)

The basic side chain addition method 5.6.3.1 was applied to this reaction. 4-[2-Benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082) (4.626mM), triphenylphosphine (9.252mM) and N,N-dimethylethanolamine (13.8mM) were dissolved in dichloromethane (50ml) and Diispropyl azodicarboxylate (11.6mM) was added and left stirring overnight. Dichloromethane (3 x 100ml) was used to extract the product from the reaction mixture, which was then washed with NH_4CL (50ml) solution and brine (50ml). The organic layers were combined and dried over sodium sulphate and the dichloromethane removed *in vacuo* leaving a brown oil. The product was purified by flash column chromatography (eluant: chloroform:methanol 4:1) and recovered as yellow oil (75%) (R_f 0.5 chloroform:methanol 4:1). IR ν_{max} (film) 2972 (CHs), 1604 (C=C), 1507 (NCH_2), 1453 (CH_2), 1242 (CH_3) cm^{-1}. ^{1}H NMR δ($CDCl_3$) 0.99-1.01 (3H, t, J=7.52Hz, H-4, CH_3), 2.02-2.08 (2H, q, J=7.54Hz, H-3, CH_2), 2.6-2.70 (6H, m, 1'''', 2''''), 2.87-2.90 (2H, t, J=6.02Hz, H-6, CH_2), 3.59 (2H, s, H-5, CH_2), 4.02-4.05 (2H, t, J=6.02Hz, H-4, CH_2), 6.83-6.85 (2H, d, H-2', H-6'), 6.98-7.03 (2H, t, J=8.54Hz, H-3''', H-5'''), 7.12-7.15 (2H, d, H-2'', H-6''), 7.17-7.23 (5H, m, H-3', H-5', H-4'', H-3'', H-5''), 7.29-7.31 (2H, d, H-2''', H-6''',). ^{13}C NMR δ($CDCl_3$) 12.80 (C-4, CH_3), 23 (C-2'''', C-3'''', $(CH_2)_2$), 24.23 (C-3, CH_2), 36.70 (C-5, $(CH_2)_2$), 54.18 (C-1'''', C-4'''', $(CH_2)_2$), 54.60 (C-6, CH_2), 66.20 (C-7, CH_2), 113.75 (C-3', C-5'), 114.57 (C-3''', C-5'''), 114.60 (C-4''), 125.40 (C-1, C=C), 127.88 (C-2''', C-6'''), 128.14 (C-3'', C-5''), 129.80 (C-2'', C-6''), 130.28 (C-2', C-6'), 135 (C-1'), 137.42 (C-1'''), 138.53 (C-2,

C=C), 140.07 (C-1''), 156.80 (C-4'''), 159.70 (C-4'). ^{19}F NMR ppm ($CDCl_3$) -117.07 (ArF). HRMS calculated for $C_{27}H_{31}FNO$ 403.2316 (M^++1), observed 404.2390.

5.10.14. Synthesis of (2-{4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenoxy}-ethyl)-dimethyl-amine (BRI089)

Using the basic side chain addition method 5.6.3.1, 4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082) (4.626mM), triphenylphosphine (9.252mM) and N-(-2-hydroxyethyl)-morpholine (13.8mM) were stirred in dichloromethane (50ml) until dissolved and Diispropyl azodicarboxylate (11.6mM) was added. The reaction was left stirring overnight. Dichloromethane (3 x 100ml) was used to extract the product from the reaction mixture. The organic layers were combined, dried (sodium sulphate) and removed under reduced pressure to afford a brown oil. The product was purified by flash column chromatography (eluant: chloroform) and recovered as yellow oil (84%) (R_f 0.5 chloroform). IR ν_{max} (KBr) 2817 (CHs), 1654 (C=C), 1505 (NCH_2), 1456 (CH_2), 1275 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.95-0.97 (3H, t, J=7.00Hz, H-4, CH_3), 1.69 (4H, s, H-1'''', H-2''''), 2.02-2.08 (2H, q, J=7.00Hz, H-3, CH_2), 2.85 (2H, s, H-6, CH_2), 3.55 (2H, s, H-5, CH_2), 3.68 (2H, s, H-6, CH_2), 4.05 (2H, s, H-4, CH_2), 6.76-6.79 (2H, d, H-3', H-5'), 6.98-7.02 (2H, m, H-3''', H-5'''), 7.11-7.13 (2H, d, H-2'', H-6''), 7.18-7.20 (5H, m, H-2', H-6', H-3'', H-5'', H-4''), 7.30 (2H, s, H-2''', H-6'''). ^{13}C NMR δ($CDCl_3$) 13.49 (C-4, CH_3), 18.55 (C-3, CH_2), 46.25 (C-1'''', C-4''''), 51.04 (C-6, CH_2), 56.03 (C-2'''', C-3'''', $(CH_2)_2$), 61.42 (C-7, CH_2), 106.26 (C-3', C-5'), 107.96 (C-3''', C-5'''), 107.67 (C-3'', C-5''), 108.45 (C-2''', C-6'''), 119.32 (C-1, C=C), 119.54 (C-2'', C-6''), 120.84 (C-2, C=C), 121.32 (C-2', C-6'), 132.39 (C-1'), 134.74 (C-1'''), 135.74 (C-1''), 145.74 (C-4'), 146.60 (C-4''), 147.38 (C-4'''). ^{19}F NMR ppm ($CDCl_3$) -117.10 (ArF). HRMS calculated for $C_{29}H_{33}FNO_2$ 445.5683 (M^++1), observed 445.5683.

5.10.15. Synthesis of 1-(2-{4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenoxy}-ethyl)-piperidine (BRI090)

This reaction was carried out according to the method 5.6.3.1. 4-[2-Benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082) (4.626mM), triphenylphosphine (9.252mM) and N-(-2-hydroxyethyl)piperidine (13.8mM) were dissolved in dichloromethane (50ml) and Diispropyl azodicarboxylate (11.6mM) was added and left stirring for 12 hours.

Dichloromethane (3 x 100ml) was used to extract the product from the reaction mixture. The dichloromethane removed *in vacuo* leaving a brown oil. The product was purified by flash column chromatography (eluant: chloroform:methanol 4:1) and recovered as yellow oil (73%) (R_f 0.4 chloroform). IR ν_{max} (KBr) 2934 (CHs), 1604 (C=C), 1505 (NCH_2), 1463 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.95-0.99 (3H, t, J=7.52Hz, H-4, CH_3), 1.28-1.33 (2H, s, H-3''''), 1.62-1.66 (4H, m, H-2'''', H-4''''), 2.01-2.08 (2H, q, J=7.52Hz, H-3, CH_2), 2.55 (4H, s, H-1'''', H-5''''), 2.78-2.80 (2H, t, J=6.02Hz, H-6, CH_2), 3.59 (2H, s, H-5, CH_2), 4.09-4.11 (2H, t, J=6.02Hz, H-4, CH_2), 6.82-6.84 (2H, d, H-3', H-5'), 6.98-7.02 (2H, t, J=8.04Hz, H-3''', H-5'''), 7.11-7.15 (2H, d, H-2'', H-6''), 7.17-7.20 (5H, m, H-2', H-6', H-3'', H-5'', H-4''), 7.28-7.32 (2H, d, H-2''', H-6'''). ^{13}C NMR δ($CDCl_3$) 12.80 (C-4, CH_3), 23.60 (C-3, CH_2), 24.23 (C-3'''', CH_2), 25.29 (C-2'''', C-4'''', $(CH_2)_2$), 36.70 (C-5, CH_2), 54.33 (C-1'''', C-5'''', $(CH_2)_2$), 57.40 (C-6, CH_2), 65.16 (C-7, CH_2), 113.67 (C-3', C-5'), 114.35 (C-3''', C-5'''), 125.07 (C-4''), 128.12 (C-2''', C-6'''), 129.80 (C-2'', C-6''), 130.27 (C-3'', C-5''), 134.90 (C-2', C-6'), 137.44 (C-1, C=C), 138.50 (C-2, C=C), 138.85 (C-1'), 138.88 (C-1'''), 156.55 (C-1''), 159 (C-4'), 164 (C-4'''). ^{19}F NMR ppm ($CDCl_3$) -117.04 (ArF). HRMS calculated for $C_{30}H_{35}FNO$ 444.2703 (M^++1), observed 444.2691.

5.10.16. Synthesis of (2-{4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenoxy}-ethyl)-diethyl-amine (BRI091)

The basic side chain addition method 5.6.3.1 was used in this reaction. Diispropyl azodicarboxylate (11.6mM) was added to a solution of 4-[2-benzyl-1-(4-fluoro-phenyl)-but-1-enyl]-phenol (BRI082) (4.626mM), triphenylphosphine (9.252mM) and 2-diethylaminoethanol (13.8mM) in dichloromethane (50ml) and left stirring overnight. Dichloromethane (3 x 100ml) was used to extract the product. The dichloromethane was removed *in vacuo* leaving a brown oil. The product was purified by flash column chromatography (eluant: dichloromethane 4:1) and recovered as yellow oil (43%) (R_f 0.66 chloroform). IR ν_{max} (KBr) 2963, 2928 (CHs), 1604 (C=C), 1505 (NCH_2), 1458 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.94-0.98 (3H, t, J=7.02Hz, H-4, CH_3), 1.17 (4H, s, H-2'''', H-3''''), 2.00-2.06 (2H, q, J=7.52Hz, H-3, CH_2), 2.77 (4H, s, H-1'''', H-4''''), 3.03 (4H, s, H-5, CH_2), 3.57 (2H, s, H-5, CH_2), 4.07 (2H, s, H-4, CH_2), 6.82-6.84 (2H, d, H-3', H-5'), 6.97-7.01 (2H, t, J=8.02Hz, H-3''', H-5'''), 7.12-7.14 (2H, d, H-2'', H-6''), 7.15-7.21 (5H, m, H-2', H-6', H-3'', H-5'', H-4''), 7.28-7.32 (2H, d, H-2''', H-6'''). ^{13}C

5.11.2. Synthesis of 1-(4-hydroxyphenyl)-3-phenylpropenone (BRI060)[164]

This reaction was performed according to the general method 5.12. A solution 4-hydroxyacetophenone [7] (0.0167M) in 50ml ethanol three pellets of sodium hydroxide and benzaldehyde [5] (0.015M) were added. The solution was left stirring overnight. Normally the product precipitated out of solution but in this case flash column chromatography was required (eluant: hexane:diethyl ether 50:50) and the product was recovered as yellow crystals and was shown to of high purity by melting point (60%) (R_f 0.4 hexane:diethyl ether 1:1), (m.p. 106°C), [lit. m.p. 104 °C]. IR ν_{max} (film) 3124 (OH), 1654 (C=O), 1597 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 6.95-6.97 (2H, d, H-2'', H-4''), 7.4-7.45 (3H, m, H-1', H-4', H-3''), 7.55-7.58 (1H, d, H-2), 7.66-7.68 (2H, m, H-2', H-3'), 7.8-7.85 (1H, d, H-1), 8.02-8.04 (2H, d, H-1'', H-5''). ^{13}C NMR δ($CDCl_3$) 115.40 (C-2'', C-6''), 121.75 (C-1'), 128.34 (C-4', C-6'), 128.89 (C-3', C-5'), 130.35 (C-1''), 130 (C-1, CH), 131.11 (C-3'', C-5'', C-4''), 144.14 (C-2, CH), 208.90 (C=O).

5.11.3. Synthesis of 1-(3-hydroxyphenyl)-3-phenylpropenone (BRI061)[162]

According to the general method 5.12, 3-hydroxyacetophenone [8] (0.0167M) and benzaldehyde [5] (0.015M) were reacted with sodium hydroxide (3 pellets) in ethanol (50ml) overnight. The product required purification and so flash column chromatography was performed (eluant: hexane:diethyl ether 50:50). The product was recovered as yellow crystals and was shown to of high purity by melting point (45%) (R_f 0.35 hexane:diethyl ether 1:1), (m.p. 106°C), [lit. m.p. 105-106 °C]. IR ν_{max} (film) 3026 (OH), 1654 (C=O), 1597 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 7.09-7.14 (1H, d, H-1), 7.74-7.44 (5H, m, H-1'', H-2'', H-3'', H-4'', H-5''), 7.63-7.65 (4H, m, H-1', H-2', H-3', H-4'), 7.75-7.79 (1H, d, H-2). ^{13}C NMR δ($CDCl_3$) 124.90 (C-4''), 127.98 (C-1'', C-2'', C-3'', C-5'', C-6''), 128.54 (C-1', C-2', C-4', C-5', C-6'), 130.10 (C-1, CH), 134.34 (C-3'), 142.89 (C-2, CH), 188.48 (C=O).

5.11.4. Synthesis of 1,3-diphenylpropenone (BRI050)[171]

The general method 5.12 was used here. Acetophenone [9] (0.0216M), sodium hydroxide (3 pellets) and benzaldehyde [5] (0.0216M) were stirred overnight in ethanol (50ml). The

product did not precipitate as expected so the ethanol was removed by vacuum. The solution was then washed with water, extracted using dichloromethane and finally dried over sodium sulphate. The product crystallised from dichloromethane as light yellow crystals (73%). (R_f 0.6 hexane:diethyl ether 1:1), (m.p.59°C), [lit. m.p. 58°C]. IR ν_{max} (film) 1664 (C=O), 1608 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 7.44-7.46 (3H, m, H-2'', H-3'', H-4''), 7.52-7.56 (2H, m, J=7.52Hz, H-3', H-1), 7.59-7.62 (2H, m, H-1', H-5'), 7.67-7.69 (2H, m, H-2', H-4'), 7.83-7.86 (1H, d, J=7.76Hz, H-2), 8.04-8.06 (2H, d, H-1', H-5') ^{13}C NMR $\delta(CDCl_3)$ 121.60 (C-4''), 128 (C-2'', C-6''), 128.07 (C-3'', C-5''), 128.19 (C-3', C-5'), 128.50 (C-2', C-6'), 130.12 (C-4'), 132.36 (C-1''), 134.42 (C-2, CH), 137.75 (C-1, CH), 144.43 (C-1'), 190.14 (C=O).

5.11.5. Synthesis of 1-(3-methoxyphenyl)-3-phenylpropenone (BRI046)[169]

This reaction was performed according to the general method 5.12. To a solution 3-methoxyacetophenone [10] (0.0166M) in ethanol (50ml) three pellets of sodium hydroxide were added at room temperature while stirring vigorously. To this solution benzaldehyde [5] (0.0166M) was added. The solution was left stirring overnight. The product did not precipitate so the ethanol was removed by vacuum and the mixture dissolved in dichloromethane (100ml). The solution was then washed with water (100ml), dried over sodium sulphate and left to crystallise. The product did not crystallise and so flash chromatography was performed on silica gel (eluant: hexane). The desired product was recovered as yellow crystals (75%). (R_f 0.5) (hexane:ethyl acetate 4:1), (m.p. 42°C), [lit. m.p. 41-43 °C]. IR ν_{max} (film) 2938 (CHs), 1681 (C=O), 1596 (C=C), 1264 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 3.91 (3H, s, OCH_3), 7.15-7.17 (1H, dd, H-2'), 7.26-7.28 (1H, m, H-2), 7.42-7.46 (4H, m, H-1'', H-2'', H-4'', H-5''), 7.52-7.57 (2H, m, H-1', H-3'), 7.6-7.64 (1H, d, H-3''), 7.66-7.68 (1H, m, H-4'). ^{13}C NMR $\delta(CDCl_3)$ 55.05 (OCH_3), 112.20 (C-3', C-5'), 118.89 (C-3, CH), 121.64 (C-2'', C-6''), 128.95 (C-3'', C-5''), 129.14 (C-4'') 130.13 (C-2', C-6'), 134.41 (C-1'), 137.99 (C-1''), 144.44 (C-2, CH), 159.45 (C-4'), 189.84 (C=O).

5.11.6. Synthesis of 1-(4-methoxyphenyl)-3-phenylpropenone (BRI055)[173]

This reaction was performed according to the general method 5.12. 4-Methoxyacetophenone [11] (0.017M) benzaldehyde [5] (0.017M) and sodium hydroxide (3 pellets) were stirred overnight in ethanol (50ml). The product crystallised of solution and

was filtered and washed with cold ethanol (50ml), water (50ml) and dried (under vacuum and heat) and was shown to be of high purity by melting point. A yield of yellow crystals were recrystallised from dichloromethane (98%), (R_f 0.5 hexane:diethyl ether 1:1), (m.p. 106°C), [lit. m.p. 106-107°C]. IR ν_{max} (film) 2936 (CHs), 1656 (C=O), 1605 (C=C), 1260 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.89 (3H, s, OCH_3), 6.99-7.01 (2H, d, H-3', H-5'), 7.42-7.44 (3H, m, H-3'', H-4'', H-5''), 7.55-7.60 (1H, d, H-1), 7.65-7.67 (2H, d, H-2'', H-6''), 7.8-7.84 (1H, d, H-2) 8.05-8.08 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 55.06 (OCH_3), 113.40 (C-3', C-5'), 121.39 (C-3, CH), 127.90 (C-2'', C-6''), 128.48 (C-3'', C-5''), 129.90 (C-4'') 130.38 (C-2', C-6'), 130.62 (C-1'), 134.62 (C-1''), 143.52 (C-2, CH), 162.99 (C-4'), 188.26 (C=O).

5.11.7. Synthesis of 3-(4-methoxyphenyl)-1-(3-methoxyphenyl)-propenone (BRI047)[170]

This reaction was carried out according to the general method 5.12. A solution of 3-methoxyacetophenone [10] (0.017M), three pellets of sodium hydroxide and *para*-anisaldehyde [12] (0.018M) was stirred in ethanol (50ml) at room temperature overnight. The product did not precipitate as expected so the ethanol was removed by vacuum. The solution was then washed with water (100ml), extracted using dichloromethane (3 x 100ml) and finally dried over sodium sulphate. The product did not crystallise and so flash chromatography was performed on silica gel (eluant: hexane). The desired product was recovered as yellow crystals (71%). (R_f 0.7 hexane:diethyl ether 1:1) , (m.p. 53°C), [lit. m.p. 52 °C]. IR ν_{max} (KBr) 2936, 2036 (CHs), 1681.90 (C=O), 1596.90 (C=C), 1251 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.77-3.78 (3H, s, OCH_3), 3.81-3.82 (3H, s, OCH_3), 6.86-6.89 (2H, dd, H-3'', H-5''), 7.06-7.09 (1H, d, H-1), 7.35-7.39 (2H, dd, H-2'', H-6''), 7.52-7.55 (4H, m, H-2', H-3', H-4', H-6'), 7.73-7.77 (1H, d, H-2). ^{13}C NMR δ($CDCl_3$) 54.82 (OCH_3), 54.85 (OCH_3), 112.37 (C-1'), 113.90 (C-2'', C-6''), 118.44 (C-6'), 119.09 (C-4'), 120.43 (C-3'), 127 (C-3, CH), 129.04 (C-2'), 129.80 (C-3'', C-5''), 139.34 (C-2, CH), 144.13 (C-1''), 159.35 (C-4''), 161.20 (C-4'), 189.48 (C=O).

5.11.8. Synthesis of 3-(4-methoxyphenyl)-1-(2-methoxyphenyl)-propenone (BRI056)[174]

The general method 5.12 was used in this reaction. Acetophenone [9] (0.0166M), benzaldehyde [5] (0.0166M) were reacted with sodium hydroxide (3 pellets) in ethanol (50ml). The solution was then washed with water (50ml), extracted using dichloromethane

(3 x 100ml) and finally dried over sodium sulphate. The product did not crystallise and so flash chromatography was performed on silica gel (eluant: hexane). Yellow crystals were recovered (60%) (R_f 0.55 hexane:diethyl ether 1:1), (m.p. 57°C), [lit. m.p. 58 °C]. IR ν_{max} (film) 1629 (C=O), 1601 (C=C), 1251 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 3.78 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 6.86-6.89 (2H, d, H-2'', H-3''), 7.06-7.08 (1H, d, H-1), 7.35-7.40 (2H, d, H-1'', H-4''), 7.5-7.55 (4H, m, H-1', H-2', H-3', H-4'), 7.7-7.77 (1H, d, H-2). ^{13}C NMR $\delta(CDCl_3)$ 55.22 (OCH_3), 55.26 (OCH_3), 112 (C-3'), 113.90 (C-3'', C-5''), 118.44 (C-5'), 119.10 (C-6'), 120.40 (C-4'), 127 (C-1''), 129 (C-1'), 129.80 (C-2'', C-6''), 139.34 (C-2, CH), 144 (C-1, CH), 159.35 (C-4''), 161.20 (C-2'), 189.49 (C=O).

5.11.9. Synthesis of 1,3-bis-(4-methoxyphenyl)-propenone (BRI054)[172]

The reaction was carried out following the general method 5.12. 4-Methoxyacetophenone [11] (0.0166M) was dissolved in 50ml ethanol and three pellets of sodium hydroxide were added at room temperature while stirring vigorously. To this *para*-anisaldehyde [12] (0.0166M) was added and stirring was continued stirring overnight. The product precipitated out of solution and the crystalline product was filtered and washed with cold ethanol (50ml), water (50ml) and dried (under vacuum and heat) and the product was shown to be of high purity by the melting point. Yellow crystals recrystallised from dichloromethane (40%), (R_f 0.55 hexane:diethyl ether 1:1), (m.p. 100°C), [lit. m.p. 102°C]. IR ν_{max} (film) 1647 (C=O), 1599 (C=C), 1266 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.95-6.98 (2H, d, H-2'', H-6''), 6.99-7.01 (2H, d, H-3'', H-5''), 7.44-7.47 (1H, d, H-2), 7.61-7.63 (2H,d, H-3', H-5'), 7.78-7.82 (1H, d, H-1), 8.05-8.07 (2H, dd, H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 54.95 (OCH_3), 55.03 (OCH_3), 113.33 (C-3'', C-5''), 113.90 (C-3', C-5'), 119.08 (C-3, CH), 127.35 (C-1''), 129.65 (C-2'', C-6''), 130.25 (C-2', C-6'), 130.90 (C-1'), 143.36 (C-2, CH), 161.04 (C-4''), 162.80 (C-4'), 188.31 (C=O).

5.11.10. Synthesis of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062)[179]

5.11.10.1. Synthesis of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062)

The general method 5.12 was used here. To a solution 4-hydroxyacetophenone [7] (0.0166M) in ethanol (50ml), *para*-anisaldehyde [12] (0.0166M) and sodium hydroxide (3 pellets) were added. The solution was left stirring overnight. Normally the product precipitated out of solution and the crystalline product was filtered and washed with cold ethanol (50ml), water (50ml) and dried (under vacuum and heat) and was shown to of high purity by melting point. The product obtained as yellow crystals (23.7%), (R_f 0.5 hexane:ethyl acetate 70:30).

5.11.10.2. Synthesis of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062)

This reaction was attempted again but using a different method. 4-hydroxyacetophenone [7] (0.0118M), pyridinium *para*-toluene sulphonate (0.00047M) and dihydropyran (0.0188M) were stirred in dichloromethane (20ml) for 4 hours at room temperature. The solution was then washed with sodium carbonate solution (1M) (2 x 20ml) and the organic layer was dried (sodium sulphate) and removed under reduced pressure. The residue was dissolved in 10ml of methanol added to *para*-anisaldehyde [12] dissolved in 5ml of methanol while stirring at room temperature. Sodium hydroxide solution (3% w/v in methanol) (10ml) was added dropwise to the mixture. Finally the reaction mixture was diluted with water (50ml), neutralised and extracted with ethyl acetate (2 x 100ml). The ethyl acetate was dried (sodium sulphate) and removed *in vacuo* and flash column chromatography performed to purify the product (eluant: hexane:diethyl ether 1:1). Yellow crystals were produced (46%), (R_f 0.3 hexane: diethyl ether 1:1), (m.p. 188°C), [lit. m.p. 187-189 °C]. IR ν_{max} (film) 3322 (OH), 1654 (C=O), 1610 (C=C), 1257 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.88 (3H, s, OCH_3), 6.94-6.95 (2H, d, H-3'', H-5''), 6.95-6.97 (2H, d, H-3', H-5'), 7.42-7.46 (1H, d, H-2), 7.61-7.64 (2H, d, H-2'', H-6''), 7.79-7.80 (1H, d, H-1), 8-8.03 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 54.96 (OCH_3), 113.14 (C-2', C-6'), 114.92 (C-2'', C-6''), 119.02 (C-1), 129.60 (C-3'', C-5''), 130.58 (C-3', C-5'), 143.59 (C-2), 159.33 (C-4''), 161.23 (C-4''), 188.42 (C=O).

5.11.11. Synthesis of 1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)-propenone (BRI066)[180]

The method described in section 5.12.10.2 was applied to this reaction. 4-Hydroxyacetophenone [7] (0.0118M), pyridinium *para*-toluene sulphonate (0.0047M) and dihydropyran (0.0188M) were stirred in dichloromethane (20ml) for 4 hours at room temperature. The reaction mixture was then washed with sodium carbonate solution (1M) (2 x 20ml) and the organic layer was dried (sodium sulphate) and removed under reduced pressure. 3-Methoxybenzaldehyde [13] was dissolved in 5ml of methanol while stirring at room temperature. The tetrahydropyran ether was resuspended in 10ml of methanol and was added to the solution of 3-methoxybenzaldehyde [13]. Sodium hydroxide solution (3% w/v in methanol) (10ml) was added dropwise to the mixture. Finally the reaction mixture was diluted with water (50ml), neutralised with dilute hydrochloric acid and extracted with ethyl acetate (2 x 100ml). The ethyl acetate was dried (sodium sulphate) and removed *in vacuo* and flash column chromatography performed to purify the product (eluant: hexane:diethyl ether 1:1). Yellow crystals were produced (53%), (R_f 0.3 hexane: diethyl ether 1:1), (m.p. 199°C), [lit. m.p. 200 °C]. IR ν_{max} (film) 3301 (OH), 1654 (C=O), 1577 (C=C), 1257 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.87 (3H, s, OCH_3), 6.9-6.93 (1H, d, H-2', H-3'), 7-7.02 (1H, d, H-1), 7.31-7.37 (3H, m, H-3'', H-4'', H-2), 7.71-7.77 (2H, m, H-1'', H-2''), 8.05-8.07 (2H, d, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 53.96 (OCH_3), 112.45 (C-6''), 114.59 (C-3', C-5'), 115.60 (C-2''), 120.36 (C-4''), 121.30 (C-3''), 129.14 (C-5''), 130.58 (C-2', C-6'), 143.25 (C-4'), 178.12 (C=O).

5.11.12. Synthesis of 1-(4-hydroxyphenyl)-3-(2-methoxyphenyl)-propenone (BRI070)[181]

The general method 5.12 was followed in this reaction by reacting 4-hydroxyacetophenone [7] (0.0166M) in 50ml ethanol with three pellets of sodium hydroxide and 2-methoxybenzaldehyde [14] (0.0166M) was added. The solution was left stirring overnight. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1) and the product was crystalline yellow (75%), (R_f 0.3 hexane: diethyl ether 1:1), (m.p. 196°C), [lit. m.p. 194-198 °C]. IR ν_{max} (KBr) 3187 (OH), 1647 (C=O), 1578 (C=C), 1259 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.14 (3H, s, OCH_3), 6.09-6.11 (2H, d, H-2', H-3'), 6.2-6.23 (1H, t, J=7.52Hz, H-3''), 6.26-6.28 (1H, d, H-5''), 6.59-6.63 (1H, t, J=7.28Hz, H-2''), 6.95-6.96 (2H, d, H-4'', H-1), 7.2-7.22 (2H, d, H-1', H-4'), 7.28-7.32 (1H, d, H-2). ^{13}C NMR δ($CDCl_3$) 57.06 (OCH_3), 113.38 (C-3''), 117.36 (C-3', C-5'), 122.80 (C-5''), 123.90

(C-4''), 130.70 (C-3''), 133.25 (C-2', C-6'), 133.98 (C-2, CH), 141.32 (C-1, CH), 164.50 (C-4'), 191.75 (C=O).

5.11.13. Synthesis of 3-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-propenone (BRI074)[182]

According to the general method 5.12, to a solution of 3-methoxyacetophenone [10] (0.017M) and sodium hydroxide (3 pellets) in ethanol (50ml), 4-hydroxybenzaldehyde [15] (0.017M) was added. The solution was left stirring overnight. The product crystallised from solution as yellow crystals and was filtered and washed with cold ethanol (50ml), water (50ml) and dried (under vacuum and heat), and were recrystallised from dichloromethane (55%), (R_f 0.35 hexane: diethyl ether 1:1), (m.p. 114°C), [lit. m.p. 112-116 °C]. IR v_{max} (film) 3294 (OH), 1648 (C=O), 1568 (C=C), 1256 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.90 (3H, s, OCH_3), 6.9-6.92 (2H, d, H-2'', H-3''), 6.99-7.01 (2H, d, H-1', H-2'), 7.4-7.46 (1H, d, H-2), 7.56-7.58 (2H, d, H-3', H-4'), 7.77-7.80 (1H, d, H-1), 8.04-8.07 (2H, d, H-1'', H-4''). ^{13}C NMR δ($CDCl_3$) 55.05 (OCH_3), 112.44 (C-2'), 115.60 (C-3'', C-5''), 118.82 (C-4'), 119.15 (C-1''), 120.63 (C-6'), 126.90 (C-1, CH), 129.14 (C-5'), 130.17 (C-2'', C-6''), 139.27 (C-2, CH), 144.96 (C-1'), 158 (C-3'), 159.40 (C-4''), 190.62 (C=O).

5.11.14. Synthesis of 3-(4-hydroxyphenyl)-1-(2-methoxyphenyl)-propenone (BRI075)[183]

Following the general method 5.12, a solution 2-methoxyacetophenone [6] (0.0147M), sodium hydroxide and 4-hydroxybenzaldehyde [15] (0.0143M) in ethanol (50ml) was stirred overnight. The product precipitated out of solution and the yellow crystals were filtered and washed with cold ethanol (50ml), water (50ml) and dried (under vacuum and heat). The product was a yellow oil (63%), (R_f 0.26 hexane: diethyl ether 1:1). IR v_{max} (film) 3317 (OH), 1643 (C=O), 1598 (C=C), 1247 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.90 (3H, s, OCH_3), 6.88-6.90 (2H, d, H-2'', H-3''), 7.0-7.07 (2H, m, H-1', H-3'), 7.2-7.28 (1H, d, H-1), 7.46-7.50 (3H, m, H-1'', H-4'', H-2'), 7.56-7.60 (2H, m, H-4', H-2). ^{13}C NMR δ($CDCl_3$) 55.05 (OCH_3), 112.44 (C-2'), 115.60 (C-3'', C-5''), 118.82 (C-4'), 119.15 (C-1''), 120.63 (C-6'), 126.90 (C-1, CH), 129.14 (C-5'), 130.17 (C-2'', C-6''), 139.27 (C-2, CH), 144.96 (C-1'), 158 (C-3'), 159.40 (C-4''), 190.62 (C=O).

5.11.15. Synthesis of 3-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-propenone (BRI073)[184]

The general method 5.12 was applied to this reaction. 4-Methoxyacetophenone [11] (0.0133M) was dissolved in 50ml ethanol with six pellets of sodium hydroxide and reacted with 4-hydroxybenzaldehyde [15] (0.0133M) overnight. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1) as was yellow crystals (72%), (R_f 0.21 hexane: diethyl ether 1:1), (m.p. 178°C), [lit. m.p. 177-179 °C]. IR ν_{max} (film) 3269 (OH), 1643 (C=C), 1598 (C=O), 1222 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 3.92 (3H, s, OCH_3), 6.89-6.92 (2H, d, H-3'', H-5''), 6.99-7.02 (2H, d, H-3', H-5'), 7.4-7.47 (1H, d, H-1), 7.57-7.59 (2H, d, H-2'', H-6''), 7.77-7.80 (1H, d, H-2), 8.04-8.07 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 55.06 (OCH_3), 113.37 (C-3', C-5'), 115.50 (C-3'', C-5''), 119 (C-1''), 127.39 (C-1'), 129.90 (C-2'', C-6''), 130.32 (C-2', C-6'), 130.80 (C-2,CH), 143.55 (C-1, CH), 157.46 (C-4''), 162.90 (C-4'), 188.60 (C=O).

5.12. Catalytic hydrogenation of Chalcones – general method

The chalcone (0.0125M) was dissolved in 50ml of ethanol and 260mg of 10% palladium on charcoal added to the solution while stirring. The flask was flushed with nitrogen to remove air, and then the reaction mixture was stirred under hydrogen. The reaction observed by TLC for product development. The reaction was stopped when a third spot started to appear on TLC as this indicated completion of the hydrogenation of the chalcone and a slight overhydrogenation. The solution was the filtered to remove the catalyst and the product allowed to crystallise out of the ethanol.

5.12.1. Synthesis of 1-(3-hydroxyphenyl)-3-phenyl-propan-1-one (BRI171)[165]

This reaction was carried out according to the general method 5.13. 1-(3-Hydroxyphenyl)-3-phenyl-propan-1-one (BRI061) (0.009M) was dissolved in 50ml of ethanol and 227mg of 10% palladium on charcoal added to the solution while stirring under hydrogen. The progression of the reaction was observed by thin layer chromatography. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1) and product crystallised as clear crystals (72%) from the mobile phase, (R_f 0.5 hexane:diethyl ether

1:1), (m.p. 108°C), [lit. m.p. 105-109 °C]. IR ν_{max} (film) 3072 (OH), 2926 (CHs), 1713 (C=O), 1495, 1453 (CH_2), 1289 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 2.72-2.76 (3H, t, J=7.52Hz, H-2, CH_3), 2.90-2.94 (3H, t, J=7.78Hz, H-1, CH_3), 7.18-7.32 (9H, m, H-1', H-2', H-3', H-4', H-1'', H-2'', H-3'', H-4'', H-5''). ^{13}C NMR δ($CDCl_3$) 29.28 (C-1, CH_2), 44.07 (C-3, CH_2), 125.67 (C-1', C-1''), 127.87 (C-2'', C-4'', C-5'', C-6''), 128.02 (C-2'', C-4'', C-5'', C-6''), 140.57 (C-3'), 208.70 (C=O).

5.12.2. Synthesis of 1-(2-hydroxyphenyl)-3-phenyl-propan-1-one (BRI143)[166]

In this reaction the general method 5.13 was used. 1-(2-Hydroxyphenyl)-3-phenylpropenone (BRI057) (0.00736M) and 187mg of 10% palladium on charcoal were stirred in 50ml of ethanol and hydrogen was flushed through the system. The progression of the reaction was observed by thin layer chromatography. The product crystallised as clear crystals from hexane:diethyl ether 1:1 (79%), (R_f 0.7 hexane:diethyl ether 1:1), (m.p. 33°C), [lit. m.p. 32-34 °C]. IR ν_{max} (film) 3028 (OH), 2929 (CHs), 1683 (C=O), 1458 (CH_2), 1232 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 2.77-2.82 (4H, m, H-2, H-1, $(CH_2)_2$), 6.85-6.87 (1H, d, H-1'), 6.96-7.00 (1H, t, J=7.52Hz, H-3'), 7.16-7.20 (1H, t, J=7.86, H-3''), 7.23-7.25 (1H, d, H-2'), 7.3-7.33 (3H, m, H-1'', H-5'', H-4'), 7.38-7.42 (2H, d, H-2'', H-4'').^{13}C NMR δ($CDCl_3$) 34.11 (C-1, CH_2), 34.97 (C-2, CH_2), 114.78 (C-3'', C-5''), 125.34 (C-4''), 127.92 (C-4', C-6'), 127.99 (C-3', C-5'), 129.13 (C-2'', C-6''), 135.51 (C-1''), 141.98 (C-1'), 153.03 (C-2'), 208.70 (C=O).

5.12.3. Synthesis of 1-(4-hydroxyphenyl)-3-phenyl-propan-1-one (BRI170)[164]

Following the general method 5.13, 1-(4-hydroxyphenyl)-3-phenyl-propan-1-one (BRI060) (0.009M) was dissolved in 50ml of ethanol and 227mg of 10% palladium on charcoal added to the solution while stirring under hydrogen. The progression of the reaction was observed by thin layer chromatography. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1) and crystallised as clear crystals (57%) from the mobile phase. (R_f 0.5 hexane:diethyl ether 1:1), (m.p.102°C), [lit. m.p.104 °C]. IR ν_{max} (film) 3369 (OH), 2933 (CHs), 1607 (C=O), 1452 (CH_2), 1236 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 2.53-2.56 (3H, t, J=7.76Hz, H-2, CH_3), 2.58-2.62 (3H, t, J=7.76Hz, H-1, CH_3), 6.71-6.73 (2H, d, H-2', H-3'), 7.00-7.02 (2H, d, H-1'', H-5''), 7.14-7.17 (3H, m, H-2'', H-3'', H-4''), 7.23-7.24 (2H, d, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 34.11 (C-1, CH_2), 34.97 (C-

2, CH_2), 125.35 (C-4''), 125.67 (C-3', C-5'), 127.93 (C-3'', C-5''), 128.03 (C-2'', C-6''), 129.13 (C-2', C-6'), 134.14 (C-1''), 141.98 (C-1'), 153.03 (C-4'), 208.70 (C=O).

5.12.4. Synthesis of 1-(3-methoxyphenyl)-3-phenyl-propan-1-one (BRI052)[162]

The general method 5.13 was applied to this reaction. 1-(3-Methoxyphenyl)-3-phenylpropenone (BRI046) (0.0124M) was dissolved in 50ml of ethanol with 300mg of 10% palladium on charcoal and stirred under hydrogen. The reaction observed by TLC for product development and stopped after 3 hours. The solution was filtered to remove the palladium on charcoal. Flash column chromatography was performed (eluant: hexane:diethyl ether 1:1). The product was recovered as a clear crystals (38%) (R_f 0.7 hexane:diethyl ether 1:1). IR ν_{max} (film) 2929 (CHs), 1689 (C=O), 1255 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 3.07-3.11 (2H, J=7.78Hz, H-2, CH_2), 3.3-3.34 (2H, J=7.78Hz, H-1, CH_2), 3.87 (3H, s, OCH_3), 7.12-7.14 (1H, dd, H-2'), 7.22-7.26 (1H, m, H-3'), 7.27-7.28 (2H, m, H-2'', H-4''), 7.32-7.33 (2H, m, H-1'', H-5''), 7.38-7.40 (1H, d, H-3'), 7.5-7.53 (1H, m, H-1'), 7.56-7.57 (1H, d, H-4'). ^{13}C NMR $\delta(CDCl_3)$ 54.98 (OCH_3), 31.50 (C-1, CH_3), 40.12 (C-2, CH_3), 111.76 (C-2'), 119.15 (C-4'), 120.22 (C-6'), 125.69 (C-5'), 127.98 (C-2'', C-6''), 128.10 (C-3'', C-5''), 129.13 (C-4''), 159.38 (C-3'), 198.58 (C=O).

5.12.5. Synthesis of 3-(4-methoxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI053)

This reaction was carried out according to the general method 5.13. 3-(4-Methoxyphenyl)-1-(3-methoxyphenyl)-propenone (BRI047) (0.01M) was dissolved in 50ml of ethanol and 260mg of 10% palladium on charcoal added to the solution while stirring under hydrogen. The progression of the reaction was observed by thin layer chromatography. The resulting solution was filtered and the ethanol removed under reduced pressure. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1) and crystallised as clear crystals (68%), (R_f 0.75 hexane:diethyl ether 1:1). IR ν_{max} (film) 2938 (CHs), 1686 (C=O), 1249 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 3.02-3.06 (2H, t, J=7.76Hz, H-1, H-2, CH_2) 3.26-3.30 (2H, t, J=7.78Hz, H-3, H-4, CH_2), 3.80 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 6.85-6.90 (2H, dd, H-2'', H-6''), 7.1-7.13 (1, dd, H-6'), 7.19-7.20 (2H, d, H-3'', H-5''), 7.35-7.39 (1H, t, J=8.04Hz, H-3'), 7.5-7.57 (2H, m, H-2', H-4'). ^{13}C NMR

δ($CDCl_3$) 28.88 (C-3, CH_2), 40.35 (C-2, CH_2), 54.77 (OCH_3), 54.93 (OCH_3), 111.83 (C-6'), 113.48 (C-3'', C-5''), 119.03 (C-4'), 120.22 (C-2'), 128.90 (C-2'', C-6''), 129.15 (C-3'), 132.84 (C-1''), 137.80 (C-1'), 157.56 (C-4''), 159.39 (C-5'), 198.67 (C=O). HRMS calculated for $C_{17}H_{18}O_3$ (M^++1) 271.1324, observed 271.1334.

5.12.6. Synthesis of 1,3-diphenyl-propan-1-one (BRI051)[176]

This reaction was carried out according to the general method 5.13. 1,3-Diphenylpropenone (BRI050) (0.0125M) was dissolved in 50ml of ethanol and 260mg of 10% palladium on charcoal added to the solution while stirring under hydrogen. The reaction observed by TLC for product development. The reaction was stopped after 1 hour and 45minutes when a third spot started to appear on. The solution was then filtered to remove the catalyst and the product allowed to crystallise out of the ethanol. The product crystallised as clear crystals (68%). (R_f 0.6 hexane:diethyl ether 1:1), (m.p. 71°C), [lit. m.p. 72 °C]. IR v_{max} (film) ^{1}H NMR δ($CDCl_3$) 3.08-3.12 (2H, t, J=7.76Hz, H-1, H-2, CH_2) 3.32-3.35 (2H, t, J=7.5Hz, H-3, H-4, CH_2), 7.22-7.35 (5H, m, H-2'', H-3'', H-4'', H-5'', H-6''), 7.46-7.50 (2H, t, H-3', H-5'), 7.56-7.60 (1H, t, H-4'), 7.98-8 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$)29.67 (C-3, CH_2), 40.02 (C-2, CH_2), 125.69 (C4''). 127.6-128.16 (C-2', C-3', C-5', C-6', C-2'', C-3'', C-5'', C-6''), 132.64 (C-4'), 136.37 (C-1'), 140.84 (C-5'), 198.80 (C=O).

5.12.7. Synthesis of 1,3-bis-(4-methoxyphenyl)-propan-1-one (BRI058)[177]

The general method 5.13 was applied to this reaction. 1,3-Bis-(4-methoxyphenyl)-propenone (BRI054) (0.00668M) was dissolved in 50ml of ethanol and reacted with 250mg of 10% palladium on charcoal while under a hydrogen atmosphere. The progression of the reaction was observed by thin layer chromatography. After filtration the product was allowed to crystallise. The product crystallised as clear crystals from the ethanol (40%). (R_f 0.5 hexane:diethyl ether 1:1), (m.p. 46°C), [lit. m.p. 44-45°C]. IR v_{max} (KBr) 2915 (CHs), 1670 (C=O), 1254 (CH_3) cm^{-1}. ^{1}H NMR δ($CDCl_3$) 3.0-3.04 (2H, t, J=7.52Hz, H-1, H-2, CH_2) 3.2-3.24 (2H, t, J=7.78Hz, H-3, H-4, CH_2), 3.78 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 6.85-6.88 (2H, d, H-2'', H-6''), 6.9-6.94 (2H, d, H-3'', H-5''), 7.18-7.20 (2H, d, H-3', H-5'), 7.94-7.97 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 29 (C-3, CH_2), 39.80 (C-2, CH_2), 54.77 (OCH_3), 54.98 (OCH_3), 113.28 (C-3'', C-5''), 113.33 (C-3'', C-5''), 128.90 (C-2'',

C-6''), 128.90 (C-1''), 129.86 (C-2', C-6'), 133 (C-1'), 157.52 (C-4''), 162.99 (C-4'), 197.49 (C=O).

5.12.8. Synthesis of 3-(4-methoxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI137)

The general method 5.13 was applied to this reaction. 3-(4-Methoxyphenyl)-1-(2-methoxyphenyl)-propenone (BRI056) (0.009M) and 228mg of 10% palladium on charcoal were stirred in 50ml of ethanol under a hydrogen atmosphere. The progression of the reaction was observed by thin layer chromatography. The product crystallised as clear crystals from ethanol (88%), (R_f 0.6 hexane:diethyl ether 1:1). (m.p.103°C). IR ν_{max} (film) 2938, 2832 (CHs), 1656 (C=O), 1464 (CH_2), 1299, 1243 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 2.97-3.01 (2H, t, J=7.78Hz, H-2, CH_2), 3.28-3.32 (2H, t, J=7.78Hz, H-1, CH_2), 3.79 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 6.84-6.87 (2H, d, H-2'', H-3''), 6.9-6.98 (1H, d, H-1'), 6.99-7.03 (1H, t, J=7.04Hz, H-3'), 7.16-7.19 (2H, d, H-1'', H-4''), 7.44-7.48 (1H, t, J=8.52, H-2'), 7.69-7.72 (1H, m, H-4'). ^{13}C NMR δ($CDCl_3$) 29.17 (C-2, CH_2), 45.30 (C-1, CH_2), 54.78 (OCH_3), 55.03 (OCH_3), 111.10 (C-3'), 113.35 (C-3'', C-5''), 120.19 (C-5''), 127.84 (C-6'), 128.92 (C-2', C-6'), 129.87 (C-4'), 132.99 (C-1'), 133.28 (C-1''), 157.38 (C-4''), 158.08 (C-2''), 201.38 (C=O).

5.12.9. Synthesis of 1-(4-methoxyphenyl)-3-phenyl-propan-1-one (BRI059)[178]

The general method 5.13 was used in this reaction. 1-(4-Methoxyphenyl)-3-phenylpropenone (BRI055) (0.00168M) was reacted with 250mg of 10% palladium on charcoal while dissolved in 50ml of ethanol and under a hydrogen atmosphere. The progression of the reaction was observed by thin layer chromatography. The reaction solution was filtered, evaporated and the residue was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1). The product was recovered as clear crystals (50%). (R_f 0.6 hexane:diethyl ether 1:1), (m.p. 97°C), [lit. m.p. 97 °C]. IR ν_{max} (KBr) 1670 (C=O), 1261 (CH_3) cm^{-1}. ^1H NMR δ ($CDCl_3$) 3.07-3.11 (2H, t, J=7.76Hz, H-1, H-2, CH_2) 3.25-3.30 (2H, t, J=7.78Hz, H-3, H-4, CH_2), 3.87 (3H, s, OCH_3), 6.94-6.96 (2H, dd, H-3', H-5'), 7.24-7.26 (1H, d, H-4''), 7.28-7.34 (4H, m, H-2'', H-3'', H-5'', H-6''), 7.96-7.98 (2H, dd, H-2', H-6'). ^{13}C NMR δ($CDCl_3$)29.80 (C-3, CH_2), 39.66 (C-2, CH_2),

55.01 (OCH_3), 113.29 (C-3', C-5'), 125.65 (C-4''), 128.01 (C-3'', C-5''), 128.08 (C-2', C-6'), 129.49 (C-1'), 129.80 (C-2'', C-6''), 141.04 (C-1''), 163.01 (C-4'), 197.34 (C=O).

5.12.10. Synthesis of 1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)-propan-1-one (BRI071)[180]

The general method 5.13 was applied to this reaction. In 50ml of ethanol 180mg of 10% palladium on charcoal and 1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)-propenone (BRI066) (0.0041M) were stirred under a hydrogen atmosphere. The progression of the reaction was observed by thin layer chromatography. The product crystallised as clear crystals from hexane:diethyl ether 1:1 (73%). (R_f 0.45 hexane:diethyl ether 1:1). IR ν_{max} (film) 3362 (OH), 1651 (C=O), 1259 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 3.03-3.07 (2H, t, J=7.86Hz, H-1, H-2, CH_2) 3.25-3.29 (2H, t, J=7.86Hz, H-3, H-4, CH_2), 3.81 (3H, s, OCH_3), 6.76-6.78 (2H, m, H-1'', H-2'', H-4''), 6.8-6.88 (2H, d, H-2', H-3'), 7.21-7.25 (1H, t, J=7.86, H-3''), 7.91-7.93 (2H, d, H-1', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 29.90 (C-1, CH_3), 39.58 (C-2, CH_2), 54.74 (OCH_3), 110.95 (C-4''), 113.76 (C-2''), 114.96 (C-3', C-5'), 120.34 (C-6''), 129.08 (C-5''), 129.29 (C-1'), 130.27 (C-2', C-6'), 142.50 (C-1''), 159.20 (C-4'), 160.04 (C-3''), 197.97 (C=O).

5.12.11. Synthesis of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072)[164]

Using the general method 5.13 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062) (0.0071M) was reacted with in 50ml of ethanol with 180mg of 10% palladium on charcoal while stirring under a hydrogen atmosphere. The progression of the reaction was observed by thin layer chromatography. The product was obtained clear crystals from ethanol (80%), (R_f 0.3 hexane:diethyl ether 1:1), (m.p. 124°C), [lit. m.p. 122 °C]. IR ν_{max} (film) 3179 (OH), 1648 (C=O), 1238 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 2.99-3.03 (2H, t, J=7.5Hz, H-1, H-2, CH_2) 3.22-3.26 (2H, t, J=7.5Hz, H-3, H-4, CH_2), 3.80 (3H, s, OCH_3), 6.85-6.87 (2H, d, H-3'', H-5''), 6.88-6.90 (2H, d, H-2'', H-6''), 7.17-7.19 (2H, d, H-3', H-5'), 7.9-7.93 (2H, d, H-2', H-6').^{13}C NMR $\delta(CDCl_3)$ 29 (C-2, CH_3), 39.90 (C-1, CH_2), 54.84 (OCH_3), 113.47 (C-3'', C-5''), 114.90 (C-3', C-5'), 128.90 (C-2'', C-6''), 130.24 (C-2', C-6'), 132.90 (C-1'), 134.60 (C-1''), 159.78 (C-4'), 156.20 (C-4'') 198 (C=O).

5.12.12. Synthesis of 1-(4-hydroxyphenyl)-3-(2-methoxyphenyl)-propan-1-one (BRI142)

The general method 5.13 was used in this reaction. 1-(4-Hydroxyphenyl)-3-(2-methoxyphenyl)-propenone (BRI070) (0.002M) was stirred in 50ml of ethanol with 50mg of 10% palladium on charcoal under hydrogen. The progression of the reaction was observed by thin layer chromatography. The product crystallised as clear crystals from hexane:diethyl ether 1:1 (86%), (R_f 0.4 hexane:diethyl ether 1:1), (m.p. 124°C). IR ν_{max} (KBr) 2939 (OH), 1684 (C=O), 1493 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR δ ($CDCl_3$) 3.02-3.06 (2H, t, J=7.16Hz, H-2, CH_2), 3.21-3.24 (2H, t, J=7.16Hz, H-1, CH_2), 3.84 (3H, s, OCH_3), 6.86-6.92 (4H, m, H-1'', H-2'', H-3'', H-4''), 7.2-7.28 (2H, d, H-2', H-3'), 7.92-7.95 (2H, d, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 30.50 (C-2, CH_2), 38.17 (C-1, CH_2), 54.73 (OCH_3), 109.70 (C-3''), 114.82 (C-3', C-5'), 120.04 (C-5''), 123.04 (C-6''), 127.01 (C-4''), 130.28 (C-2', C-6'), 137.85 (C-4'), 140.47 (C-2''), 154.77 (C-1''), 159.76 (C-1'), 197.49 (C=O).

5.12.13. Synthesis of 3-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI067)[187]

Following the general method 5.13, 3-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-propenone (BRI074) (0.0071M) was dissolved in 50ml of ethanol and 180mg of 10% palladium on charcoal added to the solution while stirring under a hydrogen atmosphere. The progression of the reaction was observed by thin layer chromatography. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1) and crystallised as clear crystals from the mobile phase (80%). (R_f 0.4 hexane:diethyl ether 1:1), (m.p. 115°C), [lit. m.p. 113-114 °C]. IR ν_{max} (film) 3433 (OH), 1677 (C=O), 1251 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 2.99-3.03 (2H, t, J=7.5Hz, H-1, H-2, CH_2) 3.2-3.24 (2H, t, J=7.5Hz, H-3, H-4, CH_2), 3.86 (3H, s, OCH_3), 6.78-6.80 (2H, d, H-2'', H-3''), 7.11-7.14 (3H, m, H-1'', H-4'', H-2'), 7.36-7.40 (1H, t, H-3'), 7.5-7.51 (1H, s, H-1'), 7.54-7.57 (1H, d, H-4'). ^{13}C NMR δ($CDCl_3$) 28.90 (C-2, CH_2), 40.41 (C-1, CH_2), 55 (OCH_3), 111.82 (C-2'), 114.94 (C-3'', C-5''), 119.24 (C-4'), 120.33 (C-6'), 129.07 (C-5'), 129.18 (C-2'', C-6''), 132.63 (C-1''), 137.69 (C-1'), 153.70 (C-4''), 159.34 (C-3'), 199.37 (C=O).

5.12.14. Synthesis of 3-(4-hydroxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI017)

The reaction was performed according to the general method 5.13. 3-(4-Hydroxyphenyl)-1-(2-methoxyphenyl)-propenone (BRI075) (0.0048M) was dissolved in 50ml of ethanol and to this 122mg of 10% palladium on charcoal was added and stirring was continued under an atmosphere of hydrogen. The progression of the reaction was observed by thin layer chromatography. The product was obtained as a clear oil (67%) was produced. (R_f 0.4 hexane:diethyl ether 1:1). IR ν_{max} (film) 3381 (OH), 1668 (C=O), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 2.86-2.90 (2H, t, J=7.54Hz, H-1, H-2), 3.18-3.20 (2H, t, J=7.54Hz, H-3, H-4), 3.81 (3H, s, OCH_3), 6.68-6.70 (2H, d, H-2'', H-3''), 6.88-6.93 (2H, t, J=9.78, H-1', H-3'), 7.02-7.19 (2H, d, H-1'', H-4''), 7.36-7.41 (2H, t, J=17.08, H-2'), 7.6-7.62 (1H, d, H-4'). ^{13}C NMR $\delta(CDCl_3)$ 29.18 (C-1, CH_2), 45.25 (C-2, CH_2), 55 (OCH_3), 111 (C-5'), 114.80 (C-3'', C-5'', C-3'), 120.23 (C-4'), 129.07 (C-2'', C-6'', C-6'), 129.90 (C-1'), 133.07 (C-1''), 153.40 (C-4'), 158.10 (C-2'), 201.96 (C=O).

5.12.15. Synthesis of 3-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-propan-1-one (BRI097)[164]

Applying the general method 5.13 to this reaction, the chalcone 3-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-propenone (BRI073) (0.004M) was dissolved in 50ml of ethanol with 180mg of 10% palladium on charcoal and stirred under hydrogen. The progression of the reaction was observed by thin layer chromatography. The product was recovered as a yellow oil (65%), (R_f 0.4 hexane:diethyl ether 1:1). IR ν_{max} (film) 3394 (OH), 2932, 2855 (CHs), 1613 (C=O), 1443 (CH_2), 1246 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 2.99-3.03 (2H, t, J=7.86Hz, H-1, H-2, CH_2), 3.22-3.26 (2H, t, J=7.86Hz, H-3, H-4, CH_2), 3.80 (3H, s, OCH_3), 6.84-6.87 (2H, d, H-2'', H-3''), 6.9-6.92 (2H, d, H-2', H-3'), 7.16-7.19 (2H, d, H-1'', H-4''), 7.9-7.92 (2H, d, H-1', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 33.01 (C-1, CH2), 33.99 (C-2, CH2), 54.88 (OCH3), 113.32 (C-2'', C-3''), 114.72 (C-2', C-3'), 128.92 (C-1', C-4'), 129.07 (C-1'', C-4''), 134.04 (C-1''), 134.13 (C-1'), 153.14 (C-4''), 157.13 (C-4').

5.13. Addition of basic side chain to hydrogenated chalcones

5.13.1. Synthesis of 3-Phenyl-1-[3-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI173)

As in the general method 5.7, 1-(3-hydroxyphenyl)-3-phenyl-propan-1-one (BRI171) (0.0045M) was refluxed for 5 hours in acetone:water 19:1 (10ml) with potassium carbonate (0.0054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.009M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (73%), (R_f 0.3 acetone). IR ν_{max} (KBr) 2926, 2954 (CHs), 1611 (C=O), 1513 (NCH_2), 1455 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.82 (4H, s, H-2'''', H-3''''), 1.92-1.96 (2H, t, J=7.52Hz, H-1, CH_2), 2.59-2.63 (2H, t, J=7.52Hz, H-4, CH_2), 2.64-2.67 (4H, m, H-2, CH_2, H-1'''', H-4''''), 2.9-2.93 (2H, t, J=6.02Hz, H-4), 4.09-4.12 (2H, t, J=6.02Hz, H-3), 6.85-6.87 (2H, d, H-2', H-3'), 7.09-7.11 (2H, d, H-1'', H-5''), 7.19-7.20 (3H, m, H-2'', H-3'', H-4''), 7.28-7.31 (H-1', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 23.02 (C-2'''', C-3'''', $(CH_2)_2$), 32.73 (C-2, CH_3), 34.06 (C-1, CH_2), 54.25 (C-1'''', C-4'''', $(CH_2)_2$), 54.68 (C-4, CH_2), 66.53 (C-3, CH_2), 113.95 (C-2', C-5'), 125.24 (C-4''), 127.82 (C-2'', C-6''), 127.99 (C-3'', C-5''), 128.81 (C-4', C-6') 133.97 (C-1'), 141.91 (C-1''), 156.51 (C-3'), 184.61 (C=O).

5.13.2. Synthesis of 3-Phenyl-1-[2-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI165)

As in the general method 5.7, 1-(2-hydroxyphenyl)-3-phenyl-propan-1-one (BRI143) (0.0045M) was refluxed in acetone:water 19:1 (10ml) with potassium carbonate (0.0054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.009M) for 5 hours. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (40%), (R_f 0.2 acetone). IR ν_{max} (film) 2925 (CHs), 1612 (C=O), 1513 (NCH_2), 1455 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.89 (4H, s, H-2'''', H-3''''), 2.61-2.65 (2H, t, J=7.26, H-2, CH_2), 2.73-2.76 (2H, t, J=7.26, H-1, CH_2), 2.95 (4H, s, H-1'''', H-4''''), 3.11-3.12 (2H, t, J=5.52Hz, H-4, CH_2), 4.19-4.21 (2H, t, J=4.26, H-3, CH_2), 6.72-6.76 (3H, m, H-2', H-1'', H-5''), 6.82-6.84 (1H, d, H-4''), 6.99-7.01 (2H, d, H-2'', H-3''), 7.08-7.19 (1H, d, H-3'), 7.84-7.86 (2H, d, H-1', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 32.70 (C-

2'''', C-3'''', $(CH_2)_2$), 34.06 (C-2, CH_3), 34.92 (C-1, CH_3), 54.25 (C-4, CH_2), 54.68 (C-1'''', C-4'''', $(CH_2)_2$), 66.54 (C-3, CH_2), 113.96 (C-3', C-5'), 125.24 (C-4''), 127.82 (C-2'', C-6''), 127.99 (C-3'', C-5''), 128.81 (C-4', C-6') 133.97 (C-1''), 141.92 (C-1'), 156.52 (C-2'), 184.61 (C=O).

5.13.3. Synthesis of 3-Phenyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI172)[168]

As in the general method 5.7, 1-(4-hydroxyphenyl)-3-phenyl-propan-1-one (BRI170) (0.0045M) was heated under reflux for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.0054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.009M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (62%), (R_f 0.25 acetone), (m.p. 65°C), [lit. m.p. 61-63 °C]. IR v_{max} (film) 2925 (CHs), 1612 (C=O), 1513 (NCH_2), 1462 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.82 (4H, s, H-2'''', H-3''''), 1.92-1.96 (2H, t, J=7.78Hz, H-1, CH_2), 2.59-2.67 (6H, m, H-2, CH_2, H-1'''', H-4''''), 2.90-2.93 (2H, t, J=6.02Hz, H-4, CH_2), 4.09-4.12 (2H, t, J=6.02Hz, H-3), 6.85-6.87 (2H, d, H-2', H-3'), 7.09-7.11 (2H, d, H-1'', H-5''), 7.19-7.21 (3H, m, H-2'', H-3'', H-4''), 7.28-7.31 (H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 23.01 (C-2'''', C-3'''', $(CH_2)_2$), 29.21 (C-2, CH_3), 32.72 (C-1, CH_3), 34.92 (C-4, CH_2), 54.68 (C-1'''', C-4'''', $(CH_2)_2$), 54.24 (C-4, CH_2), 66.53 (C-3, CH_2), 113.95 (C-3', C-5'), 125.23 (C-4'), 127.81 (C-2'', C-6''), 127.98 (C-3'', C-5''), 128.81 (C-2', C-6') 133.96 (C-1'), 141.91 (C-1''), 156.51 (C-4'), 184.61 (C=O).

5.13.4. Synthesis of 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145)

The reaction was carried out according to the general method 5.7 by placing 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (0.047M) in acetone:water with potassium carbonate (0.094M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.094M) and refluxing for 5 hours. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (70%), (R_f 0.2 acetone). IR v_{max} (film) 2955, 2786 (CHs), 1675 (C=O), 1513 (NCH_2), 1462, 1420 (CH_2), 1252 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.68 (4H, s, H-2'''', H-3''''), 2.51 (4H, s, H-1'''', H-4''''), 2.77-2.80

(2H, t, J=7.52Hz, H-2, CH_2), 2.83-2.86 (2H, t, J=7.52Hz, H-1, CH_2), 3.07-3.11 (2H, t, J=5.8Hz, H-4, CH_2), 3.65 (3H, s, OCH_3), 4.02-4.06 (2H, t, J=5.8Hz, H-3, CH_2), 6.69 (2H, d, H-2'', H-3''), 6.81-6.83 (2H, d, H-2'', H-3''), 7.03-7.05 (2H, d, H-1'', H-4''), 7.78-7.80 (2H, d, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 22.80 (C-2'''', C-3'''', $(CH_2)_2$), 28.80 (C-2, CH_2), 39.62 (C-1, CH_2), 54.04 (C-1'''', C-4'''', $(CH_2)_2$), 54.21 (OCH_3), 54.64 (C-4, CH_2), 66.70 (C-3, CH_2), 113.28 (C-3'', C-5''), 113.98 (C-3', C-5'), 128.57 (C-2'', C-6''), 129.32 (C-1'), 129.70 (C-2', C-6'), 132.84 (C-1''), 157.33 (C-4'), 162.17 (C-4''), 197.49 (C=O). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2057 (M^++1), observed 354.2069.

5.13.5. Synthesis of 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI166)

As in the general method 5.7, 1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)-propan-1-one (BRI071) (0.006M) acetone:water 19:1 with potassium carbonate (0.0072M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.011M) were refluxed for 5 hours. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (80%), (R_f 0.2 acetone). IR ν_{max} (film) 2929, 2785 (CHs), 1640 (C=O), 1513 (NCH_2), 1448 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.94-1.95 (4H, s, H-2'''', H-3''''), 2.95 (4H, s, H-1'''', H-4''''), 3.00-3.04 (2H, t, J=7.76Hz, H-1, CH_2), 3.15-3.17 (2H, t, J=5.26, H-3, CH_2), 3.22-3.27 (2H, t, J=7.52Hz, H-2, CH_2), 3.80 (OCH_3), 4.33-4.35 (2H, t, J=5.52Hz, H-4, CH_2), 6.7-6.62 (1H, d, H-2''), 6.76-6.77 (1H, s, H-1'), 6.8-6.85 (1H, d, H-4'), 6.9-6.95 (2H, d, H-2', H-3'), 7.2-7.28 (1H, t, J=7.78Hz, H-3''), 7.92-7.94 (2H, d, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 22.91 (C-2'''', C-3'''', $(CH_2)_2$), 29.85 (C-2, CH_2), 39.58 (C-1, CH_2), 53.94 (C-1'''', C-4'''', $(CH_2)_2$), 53.94 (C-4, CH_2), 54.70 (OCH_3), 65.34 (C-3, CH_2), 110.90 (C-4''), 113.72 (C-6''), 113.80 (C-3', C-5'), 120.29 (C-2''), 129.03 (C-3''), 129.87 (C-2', C-6'), 129.90 (C-1'), 142.57 (C-1''), 159 (C-5''), 161.54 (C-4''), 197.31 (C=O). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2082 (M^++1), observed 354.2069.

5.13.6. Synthesis of 3-(2-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI068)

As in the general method 5.7, 1-(4-hydroxyphenyl)-3-(2-methoxyphenyl)-propan-1-one (BRI142) (0.00045M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.00054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M).

The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a brown oil (55%), (R_f 0.3 acetone). IR ν_{max} (KBr) 2936 (CHs), 1675 (C=O), 1600 (C=O, OPiv), 1494 (NCH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.72 (4H, s, H-2'''', H-3''''), 2.56 (4H, s, H-1'''', H-4''''), 2.85 (2H, s, H-2, CH_2), 2.97 (2H, s, H-1, CH_2), 3.01 (2H, s, H-4, CH_2), 3.84 (3H, s, OCH_3), 4.17 (2H, m, H-3, CH_2), 6.86-6.95 (2H, m, H-1'', H-3''), 7.00-7.02 (2H, d, H-2'', H-4''), 7.17-7.23 (2H, m, H-2', H-3'), 7.98-8.00 (2H, d, H-1', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 22.90 (C-2'''', C-3'''', $(CH_2)_2$), 24.99 (C-2, CH_2), 37.67 (C-1, CH_2), 53.84 (C-1'''', C-4'''', $(CH_2)_2$), 54.04 (OCH_3), 54.32 (C-4, CH_2), 65.22 (C-3, CH_2), 109.84 (C-3''), 113.71 (C-2', C-6'), 119.89 (C-5''), 126.90 (C-6''), 129.06 (C-4''), 129.40 (C-1'), 129.58 (C-1''), 129.74 (C-3', C-5'), 157.12 (C-2''), 162.27 (C-4'), 196.83 (C=O). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2069 (M^++1), observed 354.2069.

5.13.7. Synthesis of 1-(3-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI136)

As in the general method 5.7, 3-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI067) (0.006M) was refluxed in acetone:water for 5 hours with potassium carbonate (0.0072M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.011M). The product was purified by flash column chromatography (eluant:acetone). The product was obtained as a light brown oil (92%), (R_f 0.2 acetone). IR ν_{max} (film) 2932, 2833 (CHs), 1681 (C=O), 1508 (NCH_2), 1454 (CH_2), 1243 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.73-1.74 (4H, s, H-2'''', H-3''''), 2.57 (4H, s, H-1'''', H-4''''), 2.81-2.83 (2H, t, J=5.78Hz, H-4, CH_2), 2.85-2.83 (2H, t, J=7.52Hz, H-1, CH_2), 2.94-2.98 (2H, t, J=7.52Hz, H-2, CH_2), 3.99 (3H, s, OCH_3), 4.01-4.03 (2H, t, J=5.02, H-3, CH_2), 6.86-6.95 (2H, m, H-1'', H-3''), 7.00-7.02 (2H, d, H-2'', H-4''), 7.17-7.23 (2H, m, H-2', H-3'), 7.98-8.00 (2H, d, H-1', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 22.90 (C-2'''', C-3'''', $(CH_2)_2$), 30.70 (C-2, CH_2), 40.67 (C-1, CH_2), 54.08 (OCH_3), 54.45 (C-1'''', C-4'''', $(CH_2)_2$), 54.62 (C-4, CH_2), 66.13 (C-3, CH_2), 110.83 (C-2'), 112.22 (C-4'), 113.71 (C-3'', C-5''), 117.84 (C-6'), 128.71 (C-2'', C-4''), 132.80 (C-5'), 133.75 (C-1''), 137.72 (C-1'), 146.88 (C-4''), 159.30 (C-3'), 198.71 (C=O). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2081 (M^++1), observed 354.2069.

5.13.8. Synthesis of 1-(2-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI167)

The general method 5.7 was used for basic side chain addition to 3-(4-hydroxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI017) (0.004M) by dissolving the starting material in acetone:water and refluxing for 5 hours with potassium carbonate (0.0047M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0078M). The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (36%), (R_f 0.3 dichloromethane:methanol 19:1). IR ν_{max} (film) 2942 (CHs), 1606 (C=O), 1509 (NCH_2), 1462 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 2.03-2.04 (4H, s, H-2'''', H-3''''), 2.9-2.98 (2H, t, J=7.78Hz, H-2, CH_2), 3.16 (4H, s, H-1'''', H-4''''), 3.25-3.27 (2H, t, J=7.78Hz, H-1, CH_2), 3.28-3.30 (2H, t, J=5.26, H-4, CH_2), 3.90 (3H, s, OCH_3), 4.34-4.36 (2H, t, J=5.26, H-3, CH_2), 6.82-6.85 (2H, d, H-2'', H-3''), 6.96-6.98 (2H, t, J=9.28, H-1'', H-4''), 7.14-7.16 (2H, d, H-2', H-4'), 7.44-7.48 (1H, t, J=8.87Hz, H-3'), 7.67-7.69 (1H, d, H-1'). ^{13}C NMR $\delta(CDCl_3)$ 22.80 (C-2'''', C-3'''', $(CH_2)_2$), 29.10 (C-1, CH_2), 45.17 (C-2, CH_2), 53.82 (C-1'''', C-4'''', $(CH_2)_2$), 53.93 (OCH_3), 55.06 (C-4, CH_2), 64.20 (C-3, CH_2), 111.04 (C-3'), 113.95 (C-3', C-5'), 120.19 (C-5'), 127.77 (C-1''), 129.07 (C-2', C-6'), 129.90 (C-4'), 133.0 (C-6'), 134.29 (C-1'), 155.56 (C-2'), 158.07 (C-4''), 198.49 (C=O). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2054 (M^++1), observed 354.2069.

5.13.9. Synthesis of 1-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI168)

The reaction was carried out according to the general method 5.7 by placing 3-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-propan-1-one (BRI097) (0.004M) in acetone:water with potassium carbonate (0.0047M), 1-(2-chloroethyl)pyrrolidine.HCl (0.0078M) and refluxing the solution for 5 hours. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (70%), (R_f 0.2 acetone). IR ν_{max} (film) 2955, 2786 (CHs), 1676 (C=O), 1510 (NCH_2), 1461 (CH_2), 1246 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.79-1.81 (4H, t, J=7.04Hz, H-2'''', H-3''''), 2.56-2.60 (4H, t, J=7.78Hz, H-1'''', H-4''''), 2.63-2.64 (4H, s, H-2, H-1, $(CH_2)_2$), 2.88-2.91 (2H, t, J=6.26, H-3, CH_2), 3.80 (3H, s, OCH_3), 4.08-4.11 (2H, t, J=6.02Hz, H-4, CH_2), 6.82-6.86 (4H, m, H-2', H-3', H-2'', H-3''), 7.07-7.11 (4H, m, H-1', H-4', H-1'', H-4'').

^{13}C NMR δ($CDCl_3$) 31.30 (C-2'''', C-3'''', $(CH_2)_2$), 32.96 (C-2, CH_2), 33.97 (C-1, CH_2), 53.31 (C-1'''', C-4'''', $(CH_2)_2$), 54.23 (OCH_3), 54.68 (C-4, CH_2), 69.06 (C-3, CH_2), 113.21 (C-3', C-4', C-5'), 113.93 (C-3'', C-5''), 128.80 (C-1'', C-2'', C-6''), 128.83 (C-1', C-2', C-6'), 134.03 (C-4''), 210.46 (C=O). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2062 (M^++1), observed 354.2069.

5.14. Deprotection of hydrogenated chalcones with basic side chains

5.14.1. Synthesis of 3-(4-hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI177)

Using the general method 5.4.6, boron trifluoride-dimethyl sulphide (0.123M) was added dropwise over 30 min to 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) (0.0123M) in dichloromethane (30ml). Stirring was continued for a further 10 hours at room temperature. The solvent was removed using a nitrogen purge and the remaining residue was dissolved in ethyl acetate (200ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield a yellow oil (76%) (R_f 0.2 acetone). IR ν_{max} (film) 3400 (OH), 2928, 2815 (CHs), 1673 (C=O), 1514 (NCH_2), 1461 (CH_2), 1255 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.72 (4H, s, H-2'''', H-3''''), 2.59 (4H, s, H-1'''', H-4''''), 2.80-2.84 (2H, t, J=7.78Hz, H-2, CH_2), 2.85-2.88 (2H, t, J=5.76Hz, H-4, CH_2), 2.83-2.86 (2H, t, J=7.54Hz, H-1, CH_2), 4.05 (2H, s, H-3, CH_2), 6.6-6.62 (2H, d, H-2'', H-3''), 6.72-6.74 (2H, d, H-2'', H-3''), 6.92-6.93 (2H, d, H-1'', H-4''), 7.76-7.78 (2H, d, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 22.84 (C-2'''', C-3'''', $(CH_2)_2$), 29.06 (C-2, CH_2), 40.02 (C-1, CH_2), 54.09 (C-1'''', C-4'''', $(CH_2)_2$), 54.27 (C-4, CH_2), 66.19 (C-3, CH_2), 113.73 (C-3'', C-5''), 115.12 (C-3', C-5'), 128.90 (C-2'', C-6''), 129.48 (C-1'), 129.80 (C-2', C-6'), 131.75 (C-1''), 154.68 (C-4''), 162.02 (C-4''), 197.95 (C=O). HRMS calculated for $C_{21}H_{25}NO_3$ (M^++1) 340.1907, observed 340.1913.

5.14.2. Synthesis of 3-(3-hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI178)

Deprotection of the methoxy group was performed on 3-(3-methoxyphenyl)-1-[4-(2

-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI166) using the general method 5.4.6, where boron trifluoride-dimethyl sulphide (0.123M) was added dropwise over 30 min to 3-(3-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI166) (0.0123M) in dichloromethane (8ml). Stirring was continued for a further 10 hours at room temperature. The solvent and reagent were removed using a nitrogen purge and the remaining residue washed, extracted with ethyl acetate (2 x 50ml), and was dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (eluant:acetone) to yield the product as a yellow oil (55%) (R_f 0.2 acetone). IR ν_{max} (film) 3436 (OH), 2929, 2864 (CHs), 1674 (C=O), 1509 (NCH_2), 1456 (CH_2), 1255 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.89 (4H, s, H-2'''', H-3''''), 2.81 (4H, s, H-1'''', H-4''''), 2.90-2.94 (2H, t, J=7.78Hz, H-1, CH_2), 3.04-3.06 (2H, t, J=5.52Hz, H-4, CH_2), 3.1-3.14 (2H, t, J=7.78Hz, H-2, CH_2), 4.18-4.21 (2H, t, J=5.52Hz, H-3, CH_2), 6.66-6.74 (3H, m, H-1'', H-3'', H-4''), 6.81-6.83 (2H, d, H-2', H-3'), 7.01-7.14 (1H, t, J=7.78Hz, H-2''), 7.8-7.84 (2H, d, H-1', H-4'). ^{13}C NMR $\delta(CDCl_3)$ 22.85 (C-2'''', C-3'''', $(CH_2)_2$), 29.71 (C-2, CH_2), 39.46 (C-1, CH_2), 54.17 (C-1'''', C-4'''', $(CH_2)_2$), 54.23 (C-4, CH_2), 65.91 (C-3, CH_2), 112.91 (C-2''), 113.72 (C-3', C-5'), 115.15 (C-4''), 119.37 (C-5''), 129.16 (C-6''), 129.52 (C-1''), 129.80 (C-2', C-6'), 142.59 (C-1'), 156.88 (C-3''), 161.86 (C-4'), 191.29 (C=O). HRMS calculated for $C_{21}H_{25}NO_3$ (M^++1) 340.1913, observed 340.1926.

5.14.3. Synthesis of 1-(2-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI183)

The general method 5.4.6 was applied to this reaction. Boron trifluoride-dimethyl sulphide (0.123M) was added dropwise over 30 min to 1-(2-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI167) (0.0123M) in dichloromethane (8ml). The reaction was left stirring overnight. The solvent was removed using a nitrogen purge and the remaining residue was resuspended in ethyl acetate (200ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml). The ethyl acetate was dried over Na_2SO_4 and removed under reduced pressure. The oil was chromatographed on silica gel (eluant:acetone) to yield the product as a yellow oil (71%) (R_f 0.24 acetone). IR ν_{max} (film) 3300 (OH), 2925, 2854 (CHs), 1674 (C=O), 1600 (NCH_2), 1456 (CH_2), 1256 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.81 (4H, s, H-2'''', H-3''''), 2.62 (4H, s, H-1'''', H-4''''), 2.88-2.91 (2H, t, J=4.52, H-4, CH_2), 2.99-3.03 (2H, t, J=7.52Hz, H-2, CH_2), 3.28-3.32 (2H,

t, J=5.28Hz, H-1, CH_2), 4.07-4.11 (2H, t, J=5.28Hz, H-3, CH_2), 6.8-6.89 (2H, d, H-2'', H-3''), 6.89 (1H, s, H-1'), 6.98-7.00 (1H, d, H-3'), 7.15-7.17 (2H, d, H-1'', H-4''), 7.45-7.49 (1H, t, J=8.54Hz, H-2'), 7.7-7.77 (1H, d, H-4'). ^{13}C NMR $\delta(CDCl_3)$ 23.0 (C-2'''', C-3'''', $(CH_2)_2$), 28.78 (C-2, CH_2), 39.86 (C-1, CH_2), 54.23 (C-1'''', C-4'''', $(CH_2)_2$), 54.32 (C-4, CH_2), 65.22 (C-3, CH_2), 113.95 (C-2'', C-6''), 118.08 (C-3'), 118.45 (C-6'), 118.82 (C-1'), 128.72 (C-3'', C-5''), 129.41 (C-5'), 132.28 (C-4'), 135.86 (C-1''), 156.92 (C-4''), 161.98 (C-2'), 196.83 (C=O). HRMS calculated for $C_{21}H_{25}NO_3$ (M^++1) 340.1899, observed 340.1913.

5.15. McMurry chalcone couplings

5.15.1. Synthesis of 3,4,6-triphenyl-hex-3-ene (BRI029)

5.15.1.1. Attempted synthesis of 3,4,6-triphenyl-hex-3-ene (BRI029)

This reaction was first attempted following the general method 5.6 using a Reaction Carousel. Zinc dust (0.00456M) was dissolved in dry THF (tetrahydrofuran) (10ml) and placed in reaction vessel under nitrogen. Titanium tetrachloride (0.00228M) was added via syringe and the reaction was left reacting in the dark for 2 hours. Then 1,3-diphenylpropan-1-one (BRI051) (0.00057M) and propiophenone [26] (0.00114M) were dissolved in dry THF and added via syringe to the vessel. The reaction continued for another 5 hours. The reaction was then poured into potassium carbonate solution (100ml) and extracted with ethyl acetate (2 x 100ml). The ethyl acetate was removed *in vacuo* and TLC showed the reaction mixture contained many impurities. So flash column chromatography was performed on silica gel (eluant: hexane) but it was found that many of the different fractions were inseparable. The main product retrieved was self-coupled propiophenone (BRI031).

5.15.1.2. Attempted synthesis of 3,4,6-triphenyl-hex-3-ene (BRI029)

This reaction was then attempted in a round bottom flask. 1,3-Diphenyl-propan-1-one (BRI051) (0.00143M) and propiophenone [26] (0.00143M) were stirred in dry dioxane (25ml) for 10 minutes in ice at 0°C to 5°C under nitrogen. Titanium tetrachloride (0.00568M) was added dropwise over 15 minutes and the reaction stirred for a further 30

minutes. Zinc dust (0.011M) was added and the reaction allowed warm to room temperature. The reaction was then refluxed for 4 hours. The product was extracted with ethyl acetate (2 x 100ml) and washed with potassium carbonate solution (2 x 50ml). Flash column chromatography was performed on silica gel to separate the fractions of the product mixture. This reaction was unsuccessful. The starting material was recovered and the self-coupled propiophenone (BRI031).

5.15.1.3. Synthesis of 3,4,6-triphenyl-hex-3-ene (BRI029)

The reaction was then attempted in a round bottom two-neck flask using a larger volume of THF. Titanium tetrachloride (0.00228M) was added dropwise over 5 minutes to zinc dust (0.00456M) in dry THF (tetrahydrofuran) (80ml) under a nitrogen atmosphere. The mixture was refluxed at 100°C for 2 hours in the dark forming the catalyst. Then 1,3-diphenylpropan-1-one (BRI051) (0.00057M) and propiophenone [26] (0.00114M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (50ml), brine (50ml) and extracted with ethyl acetate (2 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: hexane). The product was obtained as a yellow oil (80%), (R_f 0.8 hexane). IR ν_{max} (film) 2968, 2930 (CHs), 1600 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.92-0.95 (3H, t, J=7.5Hz, H-6, CH_3), 2.44-2.50 (2H, q, J=7.5Hz, H-5, CH_2), 2.66-2.68 (2H, t, J=8.16Hz, H-1, CH_2), 2.87-2.89 (2H, t, J=8.2Hz, H-2, CH_2), 6.94-6.96 (2H, d, J=8.2, H-4'', H-4'''), 7.03-7.07 (5H, m, H-2'', H-6'', H-2''', H-6''', H-4'), 7.08-7.10 (2H, d, J=7.52Hz, H-3'', H-5''), 7.1-7.12 (2H, d, J=7.52Hz, H-3''', H-5'''), 7.21-7.28 (2H, d, J=8.2, H-3', H-5'), 7.31-7.34 (2H, d, H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 12.64 (C-6, CH_3), 26.94 (C-5, CH_2), 34.28 (C-1, CH_2), 35.86 (C-2, CH_2), 125.11-125.17 (C-2'', C-6'', C-4', C-4''), 126.94-127.04 (C-3'', C-5'', C-4'), 127.8-128.05 (C-3''', C-5''', C-2''', C-6'''), 129.3-129.46 (C-3', C-5', C-2', C-6'), 135.99 (C-3, C=C), 140.20 (C-4, C=C), 141.75 (C-1'''), 142.48 (C-1''), 142.56 (C-1''').

5.15.2. Synthesis of (*E*)-3,4-diphenyl-3-hexene (BRI031)[190]

5.15.2.1. Attempted synthesis of 1-[2-phenyl-vinyl]-1,2-diphenyl-but-1-enyl (BRI031)

As in the general method 5.6, this reaction was first attempted using a Reaction Carousel. In a reaction vessel Zinc dust (0.00456M) was suspended in dry THF (tetrahydrofuran) (10ml) under nitrogen and titanium tetrachloride (0.00228M) was added via syringe. The reaction was left reacting in the dark for 2 hours. Then 1,3-diphenylpropenone (BRI050) (0.00057M) and propiophenone [26] (0.00114M) were dissolved in dry THF (5ml) and added via syringe to the vessel. The reaction was refluxed for another 5 hours. The reaction was extracted with ethyl acetate (2 x 100ml) and washed with potassium carbonate solution (50ml). The solution was concentrated and a TLC performed which showed many impurities. Flash column chromatography was performed on silica gel (eluant: hexane) to separate the different fractions. Self-coupled propiophenone was found to be the main product.

5.15.3. Attempted synthesis of 1-[2-phenyl-vinyl]-1,2-diphenyl-but-1-enyl (BRI031)

Titanium tetrachloride (0.05M) was added dropwise over 5 minutes to zinc dust (0.01M) in dry THF (tetrahydrofuran) (100ml) under a nitrogen atmosphere in a two-neck round bottom flask. This mixture formed the catalyst when refluxed at 100°C for 2 hours in the dark. 1,3-Diphenylpropenone (BRI050) (0.0125M) and propiophenone [26] (0.025M) were added via syringe in dry THF to the reaction and left to reflux for 5 hours. The reaction was washed with 10% K_2CO_3 solution (50ml), brine (50ml) and extracted with ethyl acetate (2 x 100ml). The ethyl acetate was dried (sodium sulphate) and reduced under reduced pressure. Flash column chromatography was used to separate the products of the reaction (eluant: hexane) this reaction only produced an oil, which was identified as the self-coupled propiophenone (BRI031) (*E*)-3,4-diphenyl-3-hexene (BRI031) both E and Z isomers in a 1:1 ratio. Some starting material was also recovered. IR ν_{max} (film) 2963, 2929 (CHs), 1600 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.99-1.03 (6H, t, J=7.54Hz, H-4, $(CH_3)_2$), 2.57-2.60 (4H, q, J=7.52Hz, H-3, $(CH_2)_2$), 6.97-6.99 (8H, m, H-3'', H-5'', H-3', H-5'), 7.03-7.04 (4H, d, H-4', H-4''), 7.07-7.09 (8H, d, J=7.52Hz, H-2', H-6', H-2'', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 12.87 (C-1, $(CH_3)_2$), 26.86 (C-3, $(CH_2)_2$), 125 ((C-2'', C-6'', C-2', C-6'))$_2$, 126.90 ((C-3'', C-5'', C-4''))$_2$, 129.34 ((C-3', C-5', C-4'))$_2$, 138.74 ((C-1'))$_2$, 142.76 ((C-1''))$_2$.

5.15.4. Synthesis of 3-phenyl-4,6-bis-(4-methoxyphenyl)-hex-3-ene (BRI154)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.0034M) was added dropwise over 5 minutes to zinc dust (0.0068M) in dry THF (tetrahydrofuran) under a nitrogen atmosphere. The mixture was refluxed for 2 hours in the dark. Then 1,3-bis-(4-methoxyphenyl)-propan-1-one (BRI058) (0.0085M) and propiophenone [26] (0.0017M) were added to the reaction vessel and refluxed for 5 hours. The residue recovered from the reaction was chromatographed on silica gel (eluant: hexane:dichloromethane 40:60), (100%), (R_f 0.7 hexane:dichloromethane 40:60). IR ν_{max} (film) 2942 (CHs), 1608 (C=O), 1510 (C=C), 1287, 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.97-1.00 (3H, t, J=7.16Hz, H-6, CH_3), 2.49-2.55 (2H, q, J=7.5Hz, H-5, CH_2), 2.64-2.68 (2H, t, J=7.84Hz, H-1, CH_2), 2.87-2.90 (2H, t, J=7.86, H-2, CH_2), 3.77 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 6.71-6.73 (2H, d, H-3''', H-5'''), 6.91-6.93 (2H, d, H-3', H-5'), 6.99-7.01 (4H, m, H-2''', H-6''', H-3'', H-5''), 7.2-7.25 (5H, m, H-2', H-6', H-2'', H-6'', H-4''). ^{13}C NMR $\delta(CDCl_3)$ 12.73 (C-6, CH_3), 27.07 (C-5, CH_2), 33.46 (C-1, CH_2), 36.25 (C-2, CH_2), 54.58 (OCH_3), 54.85 (OCH_3), 112.53 (C-3', C-5'), 113.26 (C-3'', C-5''), 125.04 (C-4'''), 127.08 (C-3''', C-5'''), 128.99 (C-2'', C-6''), 129.40 (C-2''', C-6'''), 130.50 (C-2', C-6'), 133.98 (C-3, C=C), 134.81 (C-3, C=C), 135.50 (C-1'), 139.70 (C-1''), 142.90 (C-1'''), 156.99 (C-4'''), 157.39 (C-4').

5.15.5. Synthesis of 3-phenyl-4-(4-methoxyphenyl)-6-(3-methoxyphenyl)-hex-3-ene (BRI076)

Following the general method 5.6, zinc dust (0.01136M) was stirred in dry THF (tetrahydrofuran) (80ml) while titanium tetrachloride (0.0071M) was added. The mixture was refluxed at 100°C for 2 hours. Then 3-(4-methoxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI053) (0.00142M) and propiophenone [26] (0.00142M) were dissolved in dry THF (20ml) and added to the round bottom flask in one portion and left to reflux for 5 hours. Column chromatography was performed to purify the crude product (eluant: hexane:diethyl ether 1:1). The product was obtained as a light yellow oil (50%), (R_f 0.8 hexane:diethyl ether 1:1). IR ν_{max} (film) 2959, 2931 (CHs), 1609 (C=C), 1465 (CH_2), 1245, 1216 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.92 (3H, t, J=7.02Hz, H-6, CH_3), 2.45-2.50 (2H, q, J=7.02Hz, H-5, CH_2), 2.6-2.64 (2H, t, J=7.78Hz, H-1, CH_2), 2.8-2.86 (2H, t, J=7.78Hz, H-

2, CH_2), 3.62 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 6.52-6.53 (1H, m, H-2'''), 6.59-6.65 (2H, m, H-4''', H-6'''), 6.86-6.87 (2H, d, H-3', H-5'), 6.95-6.97 (2H, d, H-2', H-6'), 7.02-7.07 (2H, m, H-4'', H-5'''), 7.09-7.14 (4H, m, H-2'', H-3'', H-5'', H-6''). ^{13}C NMR δ($CDCl_3$) 12.56 (C-6, CH_3), 26.90 (C-5, CH_2), 29.22 (C-1, CH_2), 35.90 (C-2, CH_2), 54.56 (OCH_3), 54.83 (OCH_3), 111.01 (C-4'''), 113.26 (C-3', C-5'), 115.21 (C-2'''), 121.87 (C-6'''), 125 (C-5'''), 126.97 (C-2'', C-6''), 128.73 (C-2', C-6'), 128.90 (C-3'', C-5''), 133.88 (C-4, C=C), 135.97 (C-3, C=C), 140.16 (C-1'), 142.69 (C-1''), 143.94 (C-1'''), 157.40 (C-3'''), 158.38 (C-4'). HRMS calculated for $C_{26}H_{28}O_2$ 372.4993 (M^++1), observed 372.5001.

5.16. McMurry coupling of 1,3-diphenylpropan-1-ones with propiophenones and basic side chain alkylation

5.16.1. Synthesis of 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI095)

The reaction was repeated using a two-neck round bottom flask and a larger volume of dry THF (80ml). Titanium tetrachloride (0.0066M) was to zinc dust (0.0013M) in dry THF (tetrahydrofuran) (80ml) and refluxed for 2 hours. To this 3-(4-hydroxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI017) (0.0017M) and propiophenone [26] (0.0033M) were added in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 40:60). A mixture of product and self-coupled propiophenone (BRI031) was produced as an oil (43%). Separation was difficult (R_f 0.4 hexane:dichloromethane 40:60). IR ν_{max} (film) 2930 (CHs), 1610 (C=C), 1489, 1461 (CH_2), 1243 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.99-1.03 (3H, t, J=7.5Hz, H-6, CH_3), 2.45-2.48 (2H, q, J=7.28Hz, H-5, CH_2), 2.54-2.59 (2H, t, J=7.8Hz, H-1, CH_2), 2.83-2.89 (2H, t, J=7.8Hz, H-2, CH_2), 3.64 (3H, s, OCH_3), 6.82-6.87 (2H, d, H-3', H-5'), 7.05-7.09 (3H, d, H-2', H-6'), 7.11-7.16 (5H, m, H-2''', H-3''', H-4''', H-5''', H-4''), 7.3-7.34 (2H, d, H-2'', H-3'', H-5'', H-6''). ^{13}C NMR δ($CDCl_3$) 12.69 (C-6, CH_3), 30.06 (C-5, CH_2), 33.49 (C-1, CH_2), 36.06 (C-2, CH_2), 55.6 (OCH_3), 110.15 (C-6'''), 113.21 (C-3', C-5'), 114.30 (C-4'''), 121.14 (C-2'''), 127.24 (C-4''), 128.50 (C-3'', C-

5''), 128.74 (C-5'''), 129.9 (C-2'', C-6''), 130.11 (C-2', C-6'), 130.34 (C-3''') 131.05 (C-4, C=C), 131.14 (C-3, C=C), 132.34 (C-1'), 133.56 (C-1''), 140.32 (C-1'''), 155.77 (C-4'), 160.20 (C-5''').

5.16.2. Synthesis of 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI094)

The general method 5.6 was applied to this reaction. In a round bottom two-neck flask zinc dust (0.218M) was refluxed in dry THF (tetrahydrofuran) (80ml) with titanium tetrachloride (0.109M) for 2 hours. 3-(4-Hydroxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI067) (0.0137M) and propiophenone [26] (0.0273M) were dissolved in dry THF (20ml) and added to the reaction in one portion. The reaction was refluxed for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and removed under reduced pressure. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 4:6). The product was obtained as a yellow oil (49%), (R_f 0.3 hexane:dichloromethane 4:6). IR ν_{max} (film) 3408 (OH), 2949 (CHs), 1604 (C=C), 1446 (CH_2), 1259 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.97-1.00 (3H, t, J=7.52Hz, H-6, CH_3), 2.49-2.54 (2H, q, J=7.28Hz, H-5, CH_2), 2.63-2.68 (2H, t, J=7.76Hz, H-1, CH_2), 2.88-2.90 (2H, t, J=7.78Hz, H-2, CH_2), 3.64 (3H, s, OCH_3), 6.84-6.86 (2H, d, H-3', H-5'), 7.03-7.02 (3H, d, H-2', H-6'), 7.08-7.16 (5H, m, H-2''', H-3''', H-4''', H-5''', H-4''), 7.3-7.34 (2H, d, H-2'', H-3'', H-5'', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 12.69 (C-6, CH_3), 30.06 (C-5, CH_2), 33.49 (C-1, CH_2), 36.06 (C-2, CH_2), 54.63 (OCH_3), 111.15 (C-6'''), 114.82 (C-3', C-5'), 115.30 (C-4'''), 122.04 (C-2'''), 125.24 (C-4''), 126.50 (C-3'', C-5''), 126.74 (C-5'''), 126.99 (C-2'', C-6''), 127.30 (C-2', C-6'), 128.04 (C-3''') 129.05 (C-4, C=C), 129.14 (C-3, C=C), 129.23 (C-1'), 133.44 (C-1''), 139.83 (C-1'''), 153.78 (C-4'), 158.20 (C-5''').

5.16.3. Synthesis of 4-[3-(4-methoxyphenyl)-4-phenyl-hex-3-enyl]-phenol (BRI108)

The general method 5.6 was applied to this reaction. In a round bottom two-neck flask zinc dust (0.014M) was stirred in dry THF (tetrahydrofuran) (80ml) under a nitrogen atmosphere. Titanium tetrachloride (0.007M) was added. The mixture was refluxed for 2 hours. 3-(4-Hydroxyphenyl)-1-(4-methoxyphenyl)-propan-1-one (BRI097) (0.00175M)

and propiophenone [26] (0.0035M) were dissolved in dry THF (20ml) and added to the reaction in one portion. The reaction was refluxed for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and removed under reduced pressure. Column chromatography was performed to purify the crude product (eluant: dichloromethane) and a yellow oil was obtained (43%), (R_f 0.4 dichloromethane). IR ν_{max} (film) 3368(OH), 2930 (CHs), 1601 (C=C), 1508 (NCH_2), 1441 (CH_2), 1259 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.93-0.90 (3H, t, J=7.52Hz, H-6, CH_3), 2.41-2.47 (2H, q, J=7.52Hz, H-2), 2.55-2.59 (2H, t, J=8.04Hz, H-1, CH_2), 2.8-2.83 (2H, t, J=8.04Hz, H-2, CH_2), 3.74 (3H, s, OCH_3), 6.65-6.67 (2H, d, H-2''', H-6'''), 6.78-6.80 (2H, d, H-3', H-5'), 6.9-6.96 (4H, t, J=8.52, H-3''', H-5''', H-3'', H-5''), 7.04-7.12 (5H, m, H-2', H-6', H-2'', H-4'', H-6''). ^{13}C NMR δ($CDCl_3$) 12.65 (C-6, CH_3), 26.99 (C-5, CH_2), 33.38 (C-1, CH_2), 36.16 (C-2, CH_2), 54.59 (OCH_3), 112.47 (C-3', C-5'), 114.60 (C-3''', C-5'''), 124.97 (C-4'''), 127.01 (C-2'', C-6''), 129.10 (C-2', C-6'), 129.34 (C-3'', C-5''), 130.47 (C-2''', C-6'''), 133.77 (C-4, C=C), 134.84 (C-3, C=C), 135.39 (C-1'), 139.67 (C-1'''), 142.84 (C-1''), 153.46 (C-4'''), 156.85 (C-4').

5.16.4. Synthesis of 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI182)

As in the general method 5.7, 4-{1-[2-(2-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI095) (0.0033M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.004M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0067M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (80%), (R_f 0.2 acetone). IR ν_{max} (film) 2944, 2929 (CHs), 1597 (C=C), 1511 (NCH_2), 1489 (CH_2), 1236 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.93-0.96 (3H, t, J=7.54Hz, H-6, CH_3), 1.90 (4H, s, H-2'''', H-3''''), 2.39-2.47 (2H, q, J=7.4Hz, H-5, CH_2), 2.55 (2H, m, H-1, CH_2), 2.59 (2H, m, H-2, CH_2), 2.80 (4H, s, H-1'''', H-4''''), 3.02-3.05 (2H, t, J=5.5Hz, H-8), 3.74 (3H, s, OCH_3), 4.17-4.20 (2H, t, J=5.78Hz, H-9), 6.7-6.73 (2H, d, H-3''', H-5'''), 6.77-6.80 (2H, d, H-3'', H-5''), 6.84-6.86 (2H, d, H-3', H-5'), 6.95-6.97 (2H, d, H-2''', H-6'''), 7.03-7.07 (2H, d, H-2'', H-6''), 7.11-7.13 (3H, m, H-2', H-6', H-4''). ^{13}C NMR δ($CDCl_3$) 12.78 (C-6, CH_3), 22.96 (C-2'''', C-3'''', $(CH_2)_2$), 26.31 (C-5, CH_2), 33.32 (C-1, CH_2), 34.98 (C-2, CH_2), 54.15

(OCH_3), 54.44 (C-1'''', C-4'''', $(CH_2)_2$), 54.96 (C-7, CH_2), 65.71 (C-8, CH_2), 110.64 (C-5'''), 113.71 (C-3', C-5'), 119.28 (C-4'''), 125.01 (C-3'''), 125.72 (C-2', C-6'), 126.75 (C-6'''), 127.45 (C-3'', C-5''), 128.90 (C-2'', C-6''), 131.62 (C-4''), 133.47 (C-4, C=C), 134.69 (C-3, C=C), 140.32 (C-1'''), 141.90 (C-1'), 142.86 (C-1''), 156.16 (C-2'''), 156.50 (C-4'). HRMS calculated for $C_{31}H_{38}NO_2$ 456.2903 (M^++1), observed 456.2921.

5.16.5. Synthesis of 1-[2-(4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI179)

The reaction was carried out according to the general method 5.7 by placing 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI094) (0.0033M) in acetone:water 19:1 (10ml) with potassium carbonate (0.004M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0067M). The reaction was refluxed for 5 hours in darkness and then was and extracted using dichloromethane and potassium carbonate solution. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (70%), (R_f 0.2 acetone). IR ν_{max} (film) 2925, 2854 (CHs), 1606 (C=C), 1511 (NCH_2), 1463 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.89-0.92 (3H, t, J=7.52Hz, H-6, CH_3), 1.88 (4H, s, H-2'''', H-3''''), 2.40-2.46 (2H, q, J=7.54Hz, H-5, CH_2), 2.56-2.60 (2H, t, J=8.04Hz, H-1, CH_2), 2.67 (4H, s, 1'''', 4''''), 2.79-2.83 (2H, t, J=8.02Hz, H-2, CH_2), 2.92-2.94 (2H, t, J=6.02Hz, H-7, CH_2), 3.60 (3H, s, OCH_3), 4.11-4.14 (2H, t, J=6.02Hz, H-8, CH_2), 6.50 (H-4'''), 6.57-6.63 (2H, m, H-2''', H-6'''), 6.85-6.87 (2H, d, H-3', H-5'), 6.93-6.94 (2H, d, H-2', H-6'), 6.99-7.01 (1H, d, H-3'''), 7.02-7.04 (1H, m, H-4''), 7.07-7.11 (4H, d, H-2'', H-3'', H-5'', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 12.59 (C-6, CH_3), 23.01 (C-2'''', C-3'''', $(CH_2)_2$), 29.25 (C-5, CH_2), 33.39 (C-1, CH_2), 35.90 (C-2, CH_2), 54.24 (C-1'''', C-4'''', $(CH_2)_2$), 54.56 (C-7, CH_2), 54.64 (OCH_3), 66.48 (C-8, CH_2), 111.03 (C-6'''), 113.98 (C-3', C-5'), 115.12 (C-4'''), 121.88 (C-2'''), 125.12 (C-5'''), 126.98 (C-2'', C-6''), 127.89 (C-4''), 128.91 (C-3'', C-5''), 129.14 (C-2', C-6'), 133.95 (C-4, C=C), 135.85 (C-3, C=C), 140.14 (C-1'), 142.64 (C-1''), 143.90 (C-1'''), 156.53 (C-4'), 158.28 (C-3'''). HRMS calculated for $C_{31}H_{38}NO_2$ 456.2903 (M^++1), observed 456.2923.

5.16.6. Synthesis of 1-(2-{4-[3-(4-methoxyphenyl)-4-phenyl-hex-3-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI109)

The general method 5.7 was used for basic side chain addition at the hydroxy substitutent. 4-[3-(4-Methoxyphenyl)-4-phenyl-hex-3-enyl]-phenol (BRI108) (0.0008M) was placed in acetone:water 19:1 (10ml) with potassium carbonate (0.001M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.00167M) and refluxed for 5 hours in darkness. The reaction mixture was washed and extracted as in the general method and purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (75%), (R_f 0.23 dichloromethane:methanol 9:1). IR ν_{max} (film) 2926 (CHs), 1607 (C=C), 1511 (NCH_2), 1458 (CH_2), 1243 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.86-0.90 (3H, t, J=7.52Hz, H-6, CH_3), 1.9-1.95 (4H, s, H-2'''', H-3''''), 2.38-2.43 (2H, q, J=7.52Hz, H-5, CH_2), 2.52-2.56 (2H, t, J=7.28Hz, H-1, CH_2), 2.75-2.78 (2H, t, J=7.28Hz, H-2, CH_2), 2.96 (4H, s, H-1'''', H-4''''), 3.13-3.16 (2H, t, J=5.28Hz, H-7, CH_2), 3.71 (3H, s, OCH_3), 4.2-4.24 (2H, t, J=5.28Hz, H-8, CH_2), 6.6-6.63 (2H, d, H-2''', H-6'''), 6.81-6.84 (2H, d, H-3', H-5'), 6.88-6.92 (4H, t, J=8.88, H-3''', H-5''', H-3'', H-5''), 7.05-7.08 (5H, m, H-2', H-6', H-2'', H-6'', H-4''). ^{13}C NMR $\delta(CDCl_3)$ 12.62 (C-6, CH_3), 22.87 (C-5, CH_2), 31.29 (C-2'''', C-3'''', $(CH_2)_2$), 33.33 (C-1, CH_2), 36.05 (C-2, CH_2), 53.33 (C-1'''', C-4'''', $(CH_2)_2$), 54.04 (OCH_3), 54.54 (C-7, CH_2), 69.06 (C-8, CH_2), 112.41 (C-3', C-5'), 113.89 (C-3''', C-5'''), 124.94 (C-4''), 127 (C-2'', C-6''), 128.90 (C-3'', C-5''), 129.29 (C-2', C-6'), 130.40 (C-2''', C-6'''), 134.51 (C-4, C=C), 134.69 (C-3, C=C), 135.33 (C-1'), 139.62 (C-1''), 142.77 (C-1'''), 155.90 (C-4'), 156.87 (C-4'''). HRMS calculated for $C_{24}H_{24}O_2$ 456.6310 (M^++1), observed 456.2903.

5.16.7. Attempted synthesis of 4-{1-[2-(3-methoxyphenyl)-vinyl]-2-phenyl-but-1-enyl}-phenol (BRI150)

The general method 5.6 was used in this reaction. Titanium tetrachloride (0.00108M) was added to zinc dust (0.00216M) in THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)-propenone (BRI066) (0.0027M) and propiophenone [26] (0.0054M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml). The filtrate was dried (sodium sulphate) and concentrated. The reaction mixture was separated by column chromatography (eluant: chloroform). It was difficult to achieve separation on the two main products, the self-coupled propiophenone (BRI031) and 4-{1-[2-(3-methoxyphenyl)-vinyl]-2-phenyl-but-1-enyl}-phenol (BRI150), as they appeared to

have the same polarity so an acetylation was performed on the mixture to change the polarity of BRI150.

5.16.8. Attempted synthesis of 1-[2-(4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI151)

The general method for acetylation 5.11.3 was applied here in an attempt to separate 4-{1-[2-(3-methoxyphenyl)-vinyl]-2-phenyl-but-1-enyl}-phenol (BRI150) from the self-coupled propiophenone. The mixture of products (0.7g) was stirred at room temperature for 24 hours with acetic anhydride (5ml) and pyridine (5ml). The reaction was diluted with 10% hydrochloric acid and the product mixture extracted with dichloromethane. No change in polarity occurred when TLC was performed on the mixture and therefore the products could not be separated. This reaction was abandoned.

5.16.9. Attempted synthesis of 4-{1-[2-(4-methoxyphenyl)-vinyl]-2-phenyl-but-1-enyl}-phenol (BRI098)

This coupling was performed according to the general McMurry 3.11 coupling method. To Zinc dust (0.032M) in dry THF (tetrahydrofuran) and titanium tetrachloride (0.016M) were refluxed for 2 hours. Then 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (BRI062) (0.004M) and propiophenone [26] (0.008M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml). The ethyl acetate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: dichloromethane) but once again an inseparable mixture of self-coupled propiophenone (BRI031)and what appeared to be product was recovered. A basic side chain addition reaction was performed this time in an attempt to separate the products.

5.16.10. Attempted synthesis of 1-[2-(4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI100)

As in the general method 5.7, [2-(4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI098) (0.001M) was refluxed for 5 hours in darkness in

acetone:water 19:1 (10ml) with potassium carbonate (0.0012M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.002M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: chloroform). The products were separated but it was discovered that the desired product had not being produced but was actually starting material with basic side chain added and self-coupled propiophenone.

5.16.11. Synthesis of 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol(BRI092)

The general method 5.6 was applied to this reaction. In a round bottom two-neck flask zinc dust (0.00625M) was stirred in dry THF (tetrahydrofuran) (80ml) under a nitrogen atmosphere. Titanium tetrachloride (0.00313M) was added dropwise. The mixture was refluxed for 2 hours in the dark to form the catalyst. 1-(4-Hydroxyphenyl)-3-(3-methoxyphenyl)-propan-1-one (BRI071) (0.00078M) and propiophenone [26] (0.0047M) were dissolved in dry THF (20ml) and added to the reaction in one portion. The reaction was refluxed for 5 hours. The product was washed with 10% K_2CO_3 solution (50ml), brine and extracted with ethyl acetate (2 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and removed under reduced pressure. Column chromatography was performed to purify the crude product (eluant: dichloromethane). The product was obtained as a yellow oil (43%), (R_f 0.7 dichloromethane). IR ν_{max} (film) 3372 (OH), 2951, 2930 (CHs), 1602 (C=C), 1510 (NCH_2), 1441 (CH_2), 1254 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.83-0.79 (3H, t, J=7.5Hz, H-6, CH_3), 2.45-2.47 (2H, q, J=7.48, H-5, CH_2), 2.62-2.64 (2H, t, J=8.84, H-1, CH_2), 2.82-2.84 (2H, t, J=8.88, H-2, CH_2), 3.73 (3H, s, OCH_3), 6.88-6.90 (2H, d, H-3', H-5'), 7.08-7.11 (4H, m, H-2''', H-4''', H-5''', H-6'''), 7.16-7.18 (3H, d, H-2', H-6', H-4''), 7.27-7.28 (2H, d, H-3'', H-5''), 7.33-7.37 (2H, t, J=7.52Hz, H-2'', H-6'') ^{13}C NMR $\delta(CDCl_3)$ 12.65 (C-6, CH_3), 28.13 (C-5, CH_2), 34.52 (C-1, CH_2), 37.14 (C-2, CH_2), 110.54 (C-4'''), 113.38 (C-2'''), 114.57 (C-3', C-5'), 120.47 (C-5'''), 125.79 (C-6'''), 126.99 (C-4''), 127.52 (C-2'', C-6''), 128.24 (C-2', C-6'), 129.34 (C-3'', C-5''), 129.54 (C-3, C=C), 130.63 (C-4, C=C), 134.30 (C-1'), 140.26 (C-1''), 142.26 (C-1'''), 153.70 (C-4',), 158.90 (C-3''').

5.16.12. Synthesis of 4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI099)

Following the general method 5.6, titanium tetrachloride (0.0016M) was added to zinc (0.0032M) in THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (0.004M) and propiophenone [26] (0.008M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: dichloromethane) and yellow oil was obtained (80%), (R_f 0.6 dichloromethane). IR ν_{max} (film) 3391 (OH), 2961 (CHs), 1610 (C=C), 1441 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.78-0.82 (3H, t, J=6.28Hz, H-6, CH_3), 2.21 (4H, m, $(CH_2)_2$), 2.4-2.41 (2H, q, J=7.08, H-5, CH_2), 3.76 (3H, s, OCH_3), 6.57-6.59 (2H, d, H-3', H-5'), 6.72-6.83 (4H, m, H-2''', H-3''', H-5''', H-6'''), 7.06-7.17 (4H, m, H-2', H-6', H-3'', H-5''), 7.28-7.35 (3H, d, H-1'', H-2'', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 12.86 (C-6, CH_3), 28.13 (C-5, CH_2), 33.35 (C-1, CH_2), 37.41 (C-2, CH_2), 54.80 (OCH_3), 113.05 (C-3''', C-5'''), 114.44 (C-3', C-5'), 125.78 (C-4''), 127.51 (C-2''', C-6'''), 128.25 (C-2'', C-6''), 128.76 (C-2', C-6'), 129.53 (C-3'', C-5''), 133.90 (C-4, C=C), 134.34 (C-3, C=C), 135.86 (C-1'), 140.14 (C-1'''), 142.80 (C-1''), 153.70 (C-4'), 157.08 (C-4''').

5.16.13. Synthesis of 1-[2-(4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI101)

The reaction was carried out according to the general method 5.7 by placing 4-{1-[2-(4-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI099) (0.001M) in acetone:water 19:1 (10ml) with potassium carbonate (0.0011M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.002M). The reaction was refluxed for 5 hours in darkness and then was and extracted using dichloromethane and potassium carbonate solution. The product was purified by flash column chromatography (eluant:chloroform). The product was obtained as a light brown oil (38%), (R_f 0.2 chloroform). IR ν_{max} (KBr) 2962 CHs), 1608(C=C), 1510 (NCH_2), 1463 (CH_2), 1265, 1243cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.78-0.80 (3H, t, J=7.52Hz, H-6, CH_3), 1.84-1.90 (4H, m, H-2'''', H-3''''), 2.18-2.24 (2H, m, H-5, CH_2), 2.38-2.46 (4H,

m, H-1, H-2, $(CH_2)_2$), 2.79 (4H, s, H-1'''', H-4''''), 3.0-3.03 (2H, t, J=5.78Hz, H-7, CH_2), 3.76 (3H, s, OCH_3), 4.2-4.24 (2H, t, J=6.02Hz, H-8, CH_2), 6.72-6.74 (2H, d, H-3', H-5'), 6.81-6.84 (2H, d, H-3''', H-5'''), 6.97-6.99 (2H, d, H-2''', H-6'''), 7.11-7.13 (3H, H-2', H-6', H-4''), 7.2-7.22 (2H, d, H-2'', H-6''), 7.34-7.37 (2H, t, J=7.52Hz, H-3'', H-5''). ^{13}C NMR δ($CDCl_3$) 13.20 (C-6, CH_3), 23.41 (C-2'''', C-3'''', $(CH_2)_2$), 28.54 (C-5, CH_2), 33.80 (C-1, CH_2), 37.80 (C-2, CH_2), 54.65 (C-1'''', C-4'''', $(CH_2)_2$), 55.15 (OCH_3), 55.20 (C-7, CH_2), 62.54 (C-8, CH_2), 112.71 (C-3''', C-5'''), 114.08 (C-3', C-5'), 125.36 (C-4''), 127.38 (C-2'', C-6''), 127.89 (C-2', C-6'), 128.64 (C-3'', C-5''), 129.30 (C-2''', C-6'''), 130.82 (C-4, C=C), 134.79 (C-3, C=C), 136.30 (C-1''), 140.47 (C-1'''), 142.50 (C-1'), 158.88 (C-4'), 159.80 (C-4'''). HRMS calculated for $C_{31}H_{37}NO_2$ 456.2876 (M^++1), observed 456.2880.

5.16.14. Synthesis of 1-[2-(4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-piperidine (BRI096)

As in the general method 5.7, 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI092) (0.0002M) was refluxed for 5 hours in darkness in acetone:water 19:1 (20ml) with potassium carbonate (0.00024M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0004M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (34%), (R_f 0.6 dichloromethane:methanol 60:40). IR ν_{max} (film) 2931 (CHs), 1605 (C=C), 1506 (NCH_2), 1458 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.78-0.80 (3H, t, J=7.00Hz, H-6, CH_3), 1.27 (2H, s, H-3''''), 1.65 (4H, s, H-2'''', H-4''''), 2.18 (4H, m, $(CH_2)_2$), 2.42-2.44 (2H, q, J=8.88, H-5, CH_2), 2.56 (4H, s, H-1'''', H-5''''), 2.82-2.85 (2H, t, J=6.26, H-7, CH_2), 3.70 (3H, s, OCH_3), 4.2-4.22 (2H, t, J=6.02Hz, H-8, CH_2), 6.93-6.95 (2H, d, H-3', H-5'), 7.08-7.10 (4H, d, H-2', H-6', H-3'', H-5''), 7.12-7.21 (3H, H-2'', H-6'', H-5'''), 7.27 (1H, s, H-4''), 7.32-7.37 (3H, m, H-2''', H-4''', H-6'''). ^{13}C NMR δ($CDCl_3$) 13.69 (C-6, CH_3), 22.85 (C-2'''', C-4'''', $(CH_2)_2$), 27.85 (C-5, CH_2), 28.14 (C-3'''', CH_2), 32.38 (C-1, CH_2), 37.15 (C-2, CH_2), 54.59 (C-1'''', C-5'''', $(CH_2)_2$), 54.62 (OCH_3), 62.54 (C-7, CH_2), 65.20 (C-8, CH_2), 110.67 (C-4'''), 113.07 (C-6'''), 113.36 (C-3', C-5'), 120.32 (C-2''',), 125.77 (C-1'), 127.50 (C-2', C-6'), 128.24 (C-2'', C-6''), 129.70 (C-4''), 130.40 (C-3'', C-5''), 134.20 (C-3'''), 135.80 (C-3, C=C), 140.18 (C-4, C=C), 142.08 (C-1''), 143.30

(C-1'''), 156.96 (C-4'), 158.90 (C-5'''). HRMS calculated for $C_{32}H_{39}NO_2$ (M^++1) 470.3059, observed 470.3070.

5.16.15. Synthesis of [2-(4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI093)

As in the general method 5.7, 4-{1-[2-(3-methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (BRI092) (0.00056M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.000672M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.00112M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil in a yield 73%. IR ν_{max} (film) 2952, 2928 (CHs), 1606 (C=C), 1508 (NCH_2), 1461 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.82-0.79 (3H, t, J=7.34, H-6, CH_3), 1.88 (4H, s, H-2'''', H-3''''), 2.17-2.23 (4H, m, $(CH_2)_2$), 2.45-2.47 (2H, q, J=8.88, H-5, CH_2), 2.76 (4H, s, 1'''', 4''''), 3-3.04 (2H, t, J=5.86, H-7, CH_2), 3.73 (3H, s, OCH_3), 4.2-4.23 (2H, t, J=6.02Hz, H-8, CH_2), 6.58-6.59 (2H, d, H-3', H-5'), 6.78-6.79 (2H, d, H-3''', H-5'''), 7.02-7.10 (2H, t, J=8.87Hz, H-2'', H-6''), 7.13-7.14 (3H, m, H-2', H-6', H-4''), 7.17-7.23 (4H, d, H-2''', H-6''', H-3'', H-5''). ^{13}C NMR $\delta(CDCl_3)$ 13.69 (C-6, CH_3), 22.85 (C-2'''', C-3'''', $(CH_2)_2$), 29.253 (C-5, CH_2), 29.72 (C-1'''', C-4'''', $(CH_2)_2$), 30.50 (C-1, CH_2), 39.46 (C-2, CH_2), 54.17 (C-7, CH_2), 54.23 (OCH_3), 65.90 (C-8, CH_2), 112.90 (C-2''', C-4'''), 113.70 (C-3', C-5', C-3'', C-4'', C-5''), 115.16 (C-5''', C-6'''), 119.38 (C-1'), 129.16 (C-1''), 129.53 (C-2', C-6'), 128.24 (C-2'', C-6''), 129.54 (C-3, C=C), 130.63 (C-4, C=C), 142.60 (C-1'''), 156.33 (C-4',), 161.86 (C-3'''). HRMS calculated for $C_{31}H_{38}NO_2$ 456.2903 (M^++1), observed 456.2925.

5.17. Synthesis of using 4-{2-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI161)

Following the general method 5.6, titanium tetrachloride (0.0164M) was added to zinc dust (0.0328M) in THF (80ml). The mixture was refluxed at for 2 hours in the dark. 1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (0.0042M) and 4-methoxypropiophenone [20] (0.008M) in THF (20ml) were added to the reaction and refluxing was continued for a further 5 hours. The product was washed with potassium

carbonate solution (100ml), then brine (100ml) and finally extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 40:60) and yellow oil (36%) was obtained (R_f 0.5 hexane:dichloromethane 40:60). IR ν_{max} (film) 3040 (OH), 2876 (CHs), 1607 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.81-0.83 (3H, t, J=7.52Hz, H-6, CH_3), 2.24-2.27 (2H, q, J=7.5Hz, H-5, CH_2), 2.54-2.63 (2H, m, H-7), 2.65 (2H, m, H-2, CH_2), 2.80 (2H, m, H-1, CH_2), 3.78 (6H, s, $(OCH_3)_2$), 6.63-7.78 (12H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.99 (C-6, CH_3), 22.31 (C-5, CH_2), 28.18 (C-1, CH_2), 28.99 (C-2, CH_2), 54.77 ($(OCH_3)_2$), 54.97 (C-7, CH_2), 112.67 (C-3''', C-5'''), 112.97 (C-3'', C-5''), 113.89 (C-3', C-5'), 128.90 (C-2''', C-6'''), 130.71 (C-2'', C-6''), 131.25 (C-2', C-6'), 133.56 (C-4, C=C), 134.77 (C-3, C=C), 136.21 (C-1'''), 137.43 (C-1''), 141.19 (C-1'), 155.74 (C-4'''), 158.45 (C-4''), 172.57 (C-4').

5.18. Synthesis of 1-[2-(4-{2-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI162)

The reaction was carried out according to the general method 5.7 by placing 4-{2-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI161) (0.00026M) in acetone:water 19:1 (10ml) with potassium carbonate (0.0003M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.000515M). The reaction was refluxed for 5 hours and then was and extracted using dichloromethane (2 x 50ml) and potassium carbonate solution (50ml). The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (70%), (R_f 0.2 acetone). IR ν_{max} (film) 2926 (CHs), 1608 (C=C), 1510 (NCH_2), 1462 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.78-0.81 (3H, t, J=7.28Hz, H-6, CH_3), 1.85 (4H, s, H-2'''', H-3''''), 2.27-2.25 (2H, q, J=7.02Hz, H-5, CH_2), 2.5-2.60 (2H, m, H-7), 2.70 (6H, s, H-1'''', H-4'''', H-2, CH_2), 2.95-2.98 (2H, t, J=6.02Hz, H-8, CH_2), 3.85 (6H, s, $(OCH_3)_2$), 4.15-4.18 (2H, t, J=6.04Hz, H-9, CH_2), 6.63-6.66 (2H, m, H-3'', H-5''), 6.86-6.89 (2H, d, H-3''', H-5'''), 6.9-6.94 (4H, d, H-2', H-6', H-2''', H-6'''), 7.14-7.18 (4H, t, H-3', H-5', H-2'', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 13.08 (C-6, CH_3), 22.34 (C-5, CH_2), 23.01 (C-2'''', C-3'''', $(CH_2)_2$), 27.78 (C-1, CH_2), 27.90 (C-2, CH_2), 54.22 ($(OCH_3)_2$), 54.63 (C-7, CH_2), 54.83 (C-1'''', C-4'''', $(CH_2)_2$), 66.12 (C-8, CH_2), 112.46 (C-3''', C-5'''), 112.91 (C-3'', C-5''), 113.69 (C-3', C-5'), 128.60 (C-2''', C-6'''), 129.75 (C-2'', C-6''), 130.26 (C-2', C-6'), 131.62 (C-4, C=C), 134.58 (C-3, C=C), 136.60 (C-1'''), 138.50 (C-1''), 141.16 (C-1'), 156.71 (C-4'''), 157.42

(C-4''), 171.52 (C-4'). HRMS calculated for $C_{32}H_{39}NO_3$ (M^++1) 486.3008, observed 486.3006.

5.19. Nitrostyrene Synthesis – General method

Benzaldehyde [1], nitropropane, potassium fluoride and N,N-dimethyamine.HCl were all placed in a round bottom flask containing 100ml of toluene. A Dean-Stark condenser was attached to the reflux apparatus and the mixture was refluxed at 115°C for 36 hours. The toluene was removed by reduced pressure. The product crystallised overnight and was washed with hexane.

5.19.1. Synthesis of 1-(2-nitrobutyl-1-enyl)-4-methoxy-benzene (BRI001)[195]

This product was prepared using the general method 5.20 with *para*-anisaldehyde [12] (0.05M), nitropropane (0.1M), potassium fluoride (0.04mol) and N,N-dimethyamine.HCl (0.1M) were placed in a round bottom flask containing toluene (100ml). A Dean-Stark condenser was attached to the reflux apparatus and the reaction was refluxed at 115°C for 36 hours. The toluene was evaporated under reduced pressure. The product crystallised overnight. These yellow crystals were washed with hexane (84%), (m.p. 44°C), (R_f 0.78 hexane:diethyl ether 50:50). IR ν_{max} (KBr) 2975, 2935 (CH). 1648 (C=C), 1598 (C-NO_2), 1513 (OCH_3), 1350 (CH_3), 1250 (ArC-NO_2) cm^{-1}, ^1H NMR δ($CDCl_3$) 1.26 (3H, t, J=7.5Hz, CH_3, H-1), 2.87 (2H, q, J=7.5Hz, CH_2 H-2), 3.83 (3H, s, OCH_3), 6.94-6.96 (2H, d, H-3', H-5'), 7.37-7.39 (2H, d, H-2', H-6'), 7.90 (1H, s H-3). ^{13}C NMR δ(CDC l_3) 12.28 (CH_3), 20.75 (CH_2), 55.38 (OCH_3), 114.57 (C-3', C-5'), 131.75 (C-1', C-2', C-6'), 133.10 (C-1), 151.30 (C-2, CNO_2), 161.20 (C-4', C-CH_3).

5.19.2. Synthesis of 1-(3-methoxyphenyl)-2-nitrobut-1-ene (BRI005)[196]

This product was obtained using the general method 5.20. *Meta*-anisaldehyde [13] (0.05M), nitropropane (0.1M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.1M) were all placed in a round bottom flask containing toluene (100ml). A Dean-Stark condenser was attached to the reflux apparatus and the reaction was refluxed at 115°C for 36 hours. The toluene was removed under reduced pressure. The product crystallised overnight (56%) as bright yellow crystals and were washed with hexane (m.p. 43°C), (R_f

0.6 hexane:diethyl ether 4:1). IR ν_{max} (film) 2974, 2932 (CH), 1656 (C=C), 1577 (C-NO_2), 1517 (OCH_3), 1325 (CH_3), 1261 (ArC-NO_2) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.28 (3H, t, J=7.5Hz, CH_3, H-1), 2.89 (2H, q, J=7.5Hz, CH_2, H-2), 3.86 (3H, s, OCH_3), 6.97 (1H, s, H-6'), 6.98-7.0 (1H, d, H-4') 7.0-7.04 (1H, d, H-2'), 7.2-7.40 (1H, t, H-3'), 8.0 (1H, s, H-3). ^{13}C NMR δ(CDC l_3) 12 (CH_3), 20.34 (CH_2), 54.86 (OCH_3), 114.63 (C-3', C-5'), 121.42 (C-1', C-2', C-6'), 129.53 (C-1), 151.30 (C-2, CNO_2), 161.20 (C-4', C-CH_3).

5.19.3. Synthesis of 1-(-2-nitro-but-1-enyl)-2-methoxy-benzene (BRI007)[197]

This product was produced using the general method 5.20. *Ortho*-anisaldehyde [14] (0.05M), nitropropane (0.1M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.1M) were all placed in a round bottom flask containing toluene (100ml). A Dean-Stark condenser was attached to the reflux apparatus and the reaction was refluxed at 115°C for 36 hours. The toluene was removed *in vacuo*. The product crystallised as yellow crystals and were washed with hexane (20%), (m.p. 47°C), (R_f 0.6 hexane:diethyl ether 50:50). IR ν_{max} (film) 2968, 2932 (CH), 1652 (C=C), 1580 (C-NO_2), 1512 (OCH_3), 1334 (CH_3), 1252 (ArC-NO_2) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.27 (3H, t, J=7.5Hz, CH_3, H-1), 2.82 (2H, q, J=7.5Hz, CH_2, H-2), 3.90 (3H, s, OCH_3), 6.95-6.97 (1H, d, H-5'), 6.9-7.05 (1H, t, H-3'), 7.28-7.30 (1H, d, H-4'), 7.31-7.44 (1H, t, H-2'), 8.20 (1H, s, H-3). ^{13}C NMR δ($CDCl_3$) 12.12 (CH_3), 21.50 (CH_2), 54.38 (OCH_3), 114.70 (C-3', C-5'), 128.19 (C-1', C-2', C-6'), 131.44 (C-1), 152.30 (C-2, CNO_2), 160.20 (C-4', C-CH_3).

5.20. Synthesis of 2-methyl-4-methoxy nitrostyrene

5.20.1. Synthesis of 1-(-2-nitro-but-1-enyl)-2-methoxy-benzene (BRI021)

This product was produced using the general method 5.20. 2-Methyl-4-methoxybenzaldehyde (0.0067M), nitropropane (0.0134M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.0134M) were all placed in a round bottom flask containing 50ml of toluene. A Dean-Stark condenser was attached to the reflux apparatus and the reaction was refluxed at 115°C for 2 weeks. The toluene was evaporated under reduced pressure. The product was obtained as a yellow oil (45%), (R_f 0.56 hexane:diethyl ether 1:1). IR ν_{max} (film) 2938(CH), 1644 (C=C), 1603 (C-NO_2), 1513 (OCH_3), 1373 (CH_3), 1259 (ArC-NO_2) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.29-1.32 (3H, t, J=7.52Hz, CH_3, H-1), 2.27

(3H, s, CH_3, H-4), 2.89-2.95 (2H, q, J=7.52Hz, CH_2, H-2), 3.90 (3H, s, OCH_3), 6.9-6.92 (1H, d, H-3'), 7.27 (1H, s, H-1'), 7.31-7.34 (1H, d, H-2'), 8.01 (1H, s, H-3), ^{13}C NMR $\delta(CDCl_3)$ 11.84 (C-1, CH_3), 15.81 (C-6, CH_2), 20.34 (C-2, CH_2), 55.0 (OCH_3), 109.75 (C-2'), 123.76 (C-1'), 127.08 (C-3), 128.99 (C-5'), 132.08 (C-6'), 133.02 (C-3'), 150.59 (C-5), 159.05 (C-4').

5.21. Synthesis of *ortho*, *meta* and *para*-pivaloyl protected benzaldehydes

5.21.1. Synthesis of *meta*-pivaloyloxybenzaldehyde (BRI009)[201]

5.21.1.1. Synthesis of *meta*-pivaloyloxybenzaldehyde (BRI009)

The synthesis of *meta*-pivaloyloxybenzaldehyde (BRI009) was attempted by stirring 3-hydroxybenzaldehyde [16] (0.01M) with sodium hydroxide (0.01M) for 1 hour in acetone (30ml). Trimethylacetyl chloride (0.01M) was added and the solution was left stirring overnight. The reaction produced the oil product in very low yield (6%).

5.21.1.2. Synthesis of *meta*-pivaloyloxybenzaldehyde (BRI009)

This reaction was performed according to the method for pivaloylation General method 5.1. 3-Hydroxybenzaldehyde [16] (0.01M) was stirred with potassium hydroxide (0.01M) in acetone (30ml) for 1 hour. Trimethylacetyl chloride (0.01M) was added when the solution changed colour and the reaction was stirring for 1 hour. The yield was still very low (4.5%).

5.21.1.3. Synthesis of *meta*-pivaloyloxybenzaldehyde (BRI009)

The reaction was attempted again using the general method but this time 3-hydroxybenzaldehyde [16] (0.01M) was stirred with potassium hydroxide (0.01M) in acetone (30ml) for 3 hours and then trimethylacetyl chloride (0.01M) was added. The reaction was left stirring overnight. The reaction mixture was poured into water and the product extracted using dichloromethane (2 x 100ml). The organic phase was dried over sodium sulphate and the solvent removed under reduced pressure to afford a yellow oil product. The product was purified by flash chromatography (eluant: dichloromethane:ethyl

acetate 19:1) and the oil product was produced (10%), (R_f 0.7 dichloromethane:ethyl acetate 19:1). IR v_{max} (film) 3232, 2928 (CHs), 1735 (C=O of pivaloyloxy), 1715 (CHO), 1334 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.40 (9H, s, OPiv), 7.34-7.37 (1H, d, H-4'), 7.55-7.59 (1H, t, H-3'), 7.61 (1H, s, H-6'), 7.75-7.77 (1H, d, H-2'), 10.02 (1H, s, OH). ^{13}C NMR δ($CDCl_3$) 26.61 (($\underline{C}H_3)_3$), 38.69 ($\underline{C}(CH_3)_3$), 121.60 (C-6'), 126.73(C-2'), 127.27 (C-3'), 129.57 (C-4'), 151.28 (C-5'), 176.30 (C=O, OPiv), 190.72 (C=O).

5.21.2. Synthesis of *para*-pivaloyloxybenzaldehyde (BRI010)[202]

5.21.2.1. Synthesis of *para*-pivaloyloxybenzaldehyde (BRI010)

The synthesis of *para*-pivaloyloxybenzaldehyde (BRI010) was attempted by stirring 4-hydroxybenzaldehyde [15] (0.01M) with sodium hydroxide (0.01M) for 1 hour in acetone (30ml). Trimethylacetyl chloride (0.01M) was added and the solution was left stirring overnight. The reaction produced the oil product in very low yield (5%).

5.21.2.2. Synthesis of *para*-pivaloyloxybenzaldehyde (BRI010)

The reaction was carried out according to the general method for pivaloylation 5.1. 4-Hydroxybenzaldehyde [15] (0.01M) was stirred in a solution of potassium hydroxide (0.01M) in acetone (30ml) for 3 hours. Trimethylacetyl chloride (0.01M) was added and the solution was left stirring overnight. The reaction mixture was poured into water and the product extracted using dichloromethane (2 x 100ml). The dichloromethane was dried over sodium sulphate and concentrated under reduced pressure to a white, cloudy oil. The product was purified by flash chromatography (eluant: dichloromethane: ethyl acetate 19:1) and afforded the product as an oil (28%), (R_f 0.72 dichloromethane). IR v_{max} (film) 3353, 2875 (CHs), 1716 (CHO), 1740 (C=O of pivaloyloxy), 1322 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.40 (9H, s, OPiv), 7.18-7.27 (2H, dd, H-3', H -5'), 7.92-8.17 (2H, dd, H-2', H-6'), 10.00 (1H, s, CHO). ^{13}C NMR δ($CDCl_3$) 26.50 (($CH_3)_3$), 38.69 ($C(CH_3)_3$), 121.60 (C-2', C-6'), 130.57 (C-3', C-5'), 133.41 (C-1'), 155.42 (C-4'), 175.80 (C=O, OPiv), 190.32 CHO).

5.21.3. Synthesis of *ortho*-pivaloyloxybenzaldehyde (BRI011)[203]

5.21.3.1. Synthesis of *ortho*-pivaloyloxybenzaldehyde (BRI011)

The synthesis of *ortho*-pivaloyloxybenzaldehyde (BRI011) was attempted by stirring 2-hydroxybenzaldehyde [17] (0.01M) with sodium hydroxide (0.01M) for 1 hour in acetone (30ml). Trimethylacetyl chloride (0.01M) was added and the solution was left stirring overnight. The reaction produced the oil product in very low yield (7.3%).

5.21.3.2. Synthesis of *ortho*-pivaloyloxybenzaldehyde (BRI011)

The desired product was achieved using the general method 5.1 for pivaloylation. 2-Hydroxybenzaldehyde (16) (0.01M) was stirred in a solution of potassium hydroxide (0.01M) in acetone (30ml) for 3 hours. Trimethylacetyl chloride (0.01M) was added and the solution was left stirring overnight. The reaction mixture was poured into water and the product extracted using dichloromethane (2 x 100ml). The dichloromethane was dried over sodium sulphate and concentrated to afford the colourless oil product. The product was purified by flash chromatography (eluant: dichloromethane:ethyl acetate 19:1) to afford the product as an oil (17%), (R_f 0.66 dichloromethane). IR ν_{max} (film) 3451, 2874 (CHs), 1718 (CHO), 1743 (C=O of pivaloyl), 1337 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.40 (9H, s, OPiv), 7.15 -7.17 (1H, d, H-5'), 7.37-7.40 (1H, t, H-3'), 7.61-7.64 (1H, t, H-4'), 7.9-7.93 (1H, d, H-2'), 10.17 (1H, s, COH). ^{13}C NMR δ($CDCl_3$)26.66 (($\underline{C}H_3)_3$), 38.90 ($\underline{C}(CH_3)_3$), 122.80 (C-5', C-3'), 125.37 (C-2'), 129.59 (C-1'), 134 (C-4'), 152.10 (C-6'), 176.20 (C=O, OPiv), 187.83 (CHO).

5.22. Nitrostyrene synthesis from pivaloyl protected benzaldehydes

5.22.1. Synthesis of 1-(-2-nitro-but-1-enyl)-2-pivaloyloxy-benzene (BRI012)

5.22.1.1. Attempted synthesis of 1-(-2-nitro-but-1-enyl)-2-pivaloyloxy-benzene (BRI012)

This reaction was attempted using the reaction carousel by placing 2-pivaloyloxybenzaldehyde (BRI011) (0.05M), nitropropane (0.1M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.1M) in a reaction vessel in toluene (20ml) and leaving to react for 1 week but the reaction was unsuccessful.

5.22.1.2. Synthesis of 1-(-2-nitro-but-1-enyl)-2-pivaloyloxy-benzene (BRI012)

This product was prepared using the general method 5.20. 2-Pivaloyloxybenzaldehyde (BRI011) (0.05M), nitropropane (0.1M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.1M) were all placed in a round bottom flask containing 100ml of toluene. A Dean-Stark condenser was attached to the reflux apparatus and the reaction was refluxed at 115°C for 36 hours. The toluene was evaporated under reduced pressure. The product was obtained as an oil (76%), (R_f 0.55 hexane:diethylether 1:1). IR ν_{max} (film) 3229, 2805 (CHs), 1729 (C=O of pivaloyl), 1646 (C=C), 1578 (C-NO_2), 1264 (ArC-NO_2) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.28 (3H, t, J=7.5Hz, CH_3, , H-1), 1.36 (9H, s, OPiv), 2.86 (2H, q, J=7.5Hz, CH_2, H-2), 7.18-7.20 (2H, d, H-3', H-6'), 7.45-7.47 (2H, d, H-2', H-6'), 8.02 (1H, s, H-3). ^{13}C NMR δ($CDCl_3$) 21.96 (CH_3), 23.42 ((CH_3)$_3$), 32.61 (CH_2), 39.51 (C(CH_3)$_3$), 119.89 (C-3', C-5'), 120.25 (C-1), 122.36 (C-6'), 124.18 (C-4'), 129.86 (C-1', C-NO_2), 145.60 (C-2), 153.64 (C-2', OPiv), 175.70 (C=O, OPiv).

5.22.2. Synthesis of 1-(2-Nitrobutyl-1-enyl)-4-pivaloyloxy-benzene (BRI013)

5.22.2.1. Synthesis of 1-(2-Nitrobutyl-1-enyl)-4-pivaloyloxy-benzene (BRI013)

This reaction was carried out using the general method 5.20. The reaction was repeated by placing 4-pivaloyloxy-benzaldehyde (BRI010) (0.05M), nitropropane (0.1M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.1M) in a reaction vessel on a reaction carousel in toluene (20ml) and left reacting for 1 week. A very low yield of product was produced by the reaction (6%).

5.22.2.2. Synthesis of 1-(2-Nitrobutyl-1-enyl)-4-pivaloyloxy-benzene (BRI013)

This reaction was then attempted using the Dean-Stark apparatus. 4-Pivaloyloxybenzaldehyde (BRI010) (0.05M), nitropropane (0.1M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.1M) were all placed in a round bottom flask containing 100ml of toluene. A Dean-Stark condenser was attached to the reflux apparatus and the reaction was refluxed at 115°C for 36 hours. (R_f 0.64 hexane:diethylether 1:1). The toluene was removed under reduced pressure. This was washed with hexane and oil (52%) was obtained. IR ν_{max} (film) 3309, 2832 (CHs), 1732 (C=O of pivaloyl), 1651 (C=C), 1583

(C-NO_2), 1261 (ArC-NO_2) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.28 (3H, t, J=7.5Hz, CH_3, H-1), 1.40 (9H, s, OPiv), 2.88 (2H, q, J=7.5Hz, CH_2, H-2), 7.18-7.20 (2H, d, H-3', H-5'), 7.45-7.47 (2H, d, H-2', H-6'), 8.02 (1H, s, H-3). ^{13}C NMR δ($CDCl_3$) 11.96 (CH_3), 20.22 ((CH_3)$_3$), 26.61 (CH_2), 38.73 (C(CH_3)$_3$), 121.79 (C-3', C-5'), 129.25 (C-1), 130.36 (C-2', C-6'), 131.72 (C-2), 151.87 (C-1', C-NO_2),152.85 (C-4', OPiv), 174.58 (C=O, OPiv).

5.22.3. Synthesis of 1-(3-pivaloyloxyphenyl)-2-nitrobut-1-ene (BRI014)

5.22.3.1. Attempted synthesis of 1-(3-pivaloyloxyphenyl)-2-nitrobut-1-ene (BRI014)

3-Pivaloyloxybenzaldehyde (BRI009) (0.05M), nitropropane (0.1M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.1M) were all dissolved in toluene (20ml) in a reaction vessel on the reaction carousel and left reacting for 1 week. This reaction was unsuccessful.

5.22.3.2. Synthesis of 1-(3-pivaloyloxyphenyl)-2-nitrobut-1-ene (BRI014)

The reaction was repeated according to the general method 5.20. 3-Pivaloyloxybenzaldehyde (BRI009) (0.05M), nitropropane (0.1M), potassium fluoride (0.04M) and N,N-dimethyamine.HCl (0.1M) were all placed in a round bottom flask containing 100ml of toluene. A Dean-Stark condenser was attached to the reflux apparatus and the reaction was refluxed at 115°C for 36 hours. The toluene was removed under reduced pressure. The product was recovered as an oil (33%), (R_f 0.6 hexane:diethylether 4:1). IR ν_{max} (film) 3334, 2921 (CHs), 1726 (C=O of pivaloyl), 1652 (C=C), 1569 (C-NO_2), 1253 (ArC-NO_2) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.27 (3H, t, J=7.5Hz, CH_3, H-1), 1.40 (9H, s, OPiv), 2.87 (2H, q, J=7.5Hz, CH_2, H-2), 6.61 (1H, s, H-3) 6.76-6.79 (2H, d, H-2'), 7.04-7.06 (1H, dd, H-4'), 7.27-7.28 (1H, s, H-6'), 7.44-7.45 (1H, d, H-3'). ^{13}C NMR δ($CDCl_3$) 21.44 (CH_3), 26.63 ((CH_3)$_3$), 38.60 (CH_2), 40.36 (C(CH_3)$_3$), 119.50 (C-2'), 119.88 (C-4'), 122.52 (C-6'), 126.03 (C-1), 129.46 (C-5'), 131.50 (C-1', NO_2), 143.52 (C-2), 150.90 (C-3', OPiv), 176.58 (C=O, OPiv).

5.23. Synthesis of phenolic nitrostyrenes

5.23.1. Synthesis of 1-(2-nitrobutyl-1-enyl)-benzene (BRI024)[206]

This reaction was carried out using a new method for the synthesis of nitrostyrenes. 1-(2-Nitrobutyl-1-enyl)-benzene (BRI024) was synthesised by refluxing benzaldehyde (0.0189M), nitropropane (0.038M), cyclohexylamine (0.038M) and glacial acetic acid (40ml) in a round bottom flask at 150°C for one day. The reaction was diluted with water (100ml) and extracted with dichloromethane (2 x 100ml). The dichloromethane was then washed with brine solution (100ml). The organic layers were combined, dried on sodium sulphate and evaporated under reduced pressure. The product was purified by chromatographying on silica gel (eluant: dichloromethane:ethyl acetate 19:1). Yellow crystals were obtained (43%), (R_f 0.88 hexane:diethyl ether 1:1). IR ν_{max} (film) 2978 (CH), 1652 (C=C), 1560 (C-NO_2), 1521 (OCH_3), 1330 (CH_3), 1250 (ArC-NO_2) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.28-1.30 (3H, t, J=7.54Hz, CH_3, H-1), 2.86-2.92 (2H, q, J=22.08Hz, CH_2, H-2), 7.43-7.48 (5H, m, H-1', H-2', H-3', H-4', H-5'), 8.04 (1H, s, H-3). ^{13}C NMR $\delta(CDCl_3)$ 11.98 (CH_3, C-1), 20.25 (CH_2, C-2), 128.50 (C-2', C-6'), 129.14 (C-3', C-5'), 129.47 (C-4'), 131.95 (C-1'), 152.90 (C-3, CNO_2).

5.23.2. Synthesis of 1-(-2-nitro-but-1-enyl)-4-hydroxy-benzene (BRI025)[207]

The product was formed using the method 5.24.1 described above. 1-(-2-Nitro-but-1-enyl)-4-hydroxy-benzene (BRI025) was synthesised by refluxing 4-hydroxybenzaldehyde [15] (0.0189M), nitropropane (0.038M), cyclohexylamine (0.038M) and glacial acetic acid (40ml) at 150°C for 24 hours. The reaction was washed with 10% hydrochloric acid (100ml), brine (100ml) and extracted with dichloromethane (2 x 100ml). The organic layers were combined, dried on sodium sulphate and evaporated under reduced pressure. The product was purified by chromatographying on silica gel (eluant: dichloromethane:ethyl acetate 19:1). This reaction produced yellow crystals (8.3%), (R_f 0.21 chloroform). IR ν_{max} (film) 3386 (OH), 2986, 2930 (CH), 1645 (C=C), 1579 (C-NO_2), 1511 (OCH_3), 1332 (CH_3), 1250 (ArC-NO_2) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.30 (3H, t, J=7.5Hz, CH_3, H-1), 2.91 (2H, q, J=7.5Hz, CH_2, H-2), 5.90 (1H, s, OH), 6.95-6.97 (2H, d, H-5', H-3'), 7.37-7.40 (2H, d, H-2', H-6'), 8 (1H, s, H-3). ^{13}C NMR $\delta(CDCl_3)$ 11.86 (CH_3), 20.35 (CH_2), 115.70 (C-3', C-5'), 131.68 (C-2', C-6'), 133.14 (C-1'), 150.70 (C-2, CNO_2), 157.20 (C-4', C-CH_3).

5.23.3. Synthesis of 1-(3-hydroxyphenyl)-2-nitrobut-1-ene (BRI026)

The product was obtained using the reaction outlined in the method 5.24.1. 1-(3-Hydroxyphenyl)-2-nitrobut-1-ene (BRI026) was synthesised by refluxing 3-hydroxybenzaldehyde [16] (0.0189M), nitropropane (0.038M), cyclohexylamine (0.038M) and glacial acetic acid (40ml) at 150°C for one day. The reaction was washed with 10% hydrochloric acid (100ml), brine (100ml) and extracted with dichloromethane (2 x 100ml). The organic layers were combined, dried on sodium sulphate and evaporated under reduced pressure. The product was purified by chromatographying on silica gel (eluant: dichloromethane:ethyl acetate 19:1). Yellow crystals were obtained from this reaction (11%), (R_f 0.45 hexane:diethyl ether 1:1). IR ν_{max} (film) 3394 (OH), 2985, 2932 (CH), 1651 (C=C), 1581 (C-NO_2), 1512 (OCH_3), 1334 (CH_3), 1249 (ArC-NO_2) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.29 (3H, t, J=7.5Hz, CH_3, H-3), 2.88 (2H, q, J=7.5Hz, CH_2, H-2), 5.90 (1H, s, OH), 6.91-6.93 (2H, d, H-2', H-6'), 7.0-7.02 (1H, d, H-4'), 7.33-7.37 (1H, t, H-5'), 8 (1H, s, H-1). ^{13}C NMR $\delta(CDCl_3)$ 12.01 (CH_3), 20.29 (CH_2), 115.70 (C-2'), 116.57 (C-4'), 121.74 (C-6'), 129.80 (C-1), 132.21 (C-5'), 133.48 (C-1'), 145 (C-2, CNO_2), 160.20 (C-3').

5.23.4. Synthesis of 1-(-2-Nitro-but-1-enyl)-2-hydroxy-benzene (BRI027)

The product was formed using the method in section 5.24.1. 1-(-2-Nitro-but-1-enyl)-2-hydroxy-benzene (BRI027) was synthesised by refluxing 2-hydroxybenzaldehyde [17] (0.0189M), nitropropane (0.038M), cyclohexylamine (0.038M) and glacial acetic acid (40ml) at 150°C for one day. The product was extracted with dichloromethane (2 x 100ml) and washed with dilute hydrochloric acid. The organic layers were combined, dried on sodium sulphate and evaporated under reduced pressure. The product was purified by chromatographying on silica gel (eluant: dichloromethane:ethyl acetate 19:1). This reaction produced a yield of yellow crystals (61%), (R_f 0.55 hexane:diethyl ether 1:1). IR ν_{max} (film) 3421 (OH), 2979, 2928 (CH), 1655 (C=C), 1581 (C-NO_2), 1513 (OCH_3), 1328 (CH_3), 1248 (ArC-NO_2) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.27 (3H, t, J=7.5Hz, CH_3, H-1), 2.84 (2H, q, J=7.5Hz, CH_2, H-2), 5.70 (1H, s, OH), 6.89 (1H, d, H-3'), 6.99-7 (1H, t, H-6'), 7.28-7.34 (C-4', C-5'), 8.20 (1H, s, H-3). ^{13}C NMR $\delta(CDCl_3)$ 12.86 (CH_3), 21.35 (CH_2), 113.80 (C-

4', C-5'), 117.98 (C-1'), 121.98 (C-3'), 128.75 (C-6'), 132.20 (C-1), 148.70 (C-2, CNO_2), 160.20 (C-2', C-CH_3).

5.24. Reduction of nitrostyrene to ketone - general method

A suspension of iron powder (0.328M) in glacial acetic acid (150ml) was heated to 100°C for 20 minutes. The nitrostyrene (0.049M) in glacial acetic acid (20ml) was added over 10 minutes. The reaction was stirred for a further 2 hours at 100°C. The reaction mixture was cooled and added to ice-water (100ml) and extracted with dichloromethane (3 x 300ml). The organic layers were washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (eluant: dichloromethane) to yield the product as a colourless oil.

5.24.1. Synthesis of 1-(2-methoxyphenyl)-butan-2-one (BRI117)[200]

As in the general method 5.25, a suspension of iron powder (0.328M) in glacial acetic acid (150ml) was heated to 100°C for 20 minutes. 1-(-2-Nitro-but-1-enyl)-2-methoxy-benzene (BRI007) (0.049M) in glacial acetic acid (20ml) was added over 10 minutes. The reaction was stirred for a further 2 hours at 100°C. The reaction mixture was cooled and added to ice-water (100ml) and extracted with dichloromethane (3 x 300ml). The organic layers were washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (eluant: dichloromethane) to yield the product as a colourless oil (100%), (R_f 0.8 dichloromethane). IR ν_{max} (film) 2975, 2939 (CHs), 1713 (C=O), 1494, 1462 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.04-1.07 (3H, t, J=7.16Hz, H-1, CH_3), 2.44-2.49 (2H, q, J=7.2Hz, H-2, CH_2), 3.68 (2H, s, H-3, CH_2), 3.80 (3H, s, OCH_3), 6.87-6.95 (2H, m, H-1', H-3'), 7.13-7.15 (1H, d, H-2'), 7.24-7.28 (1H, d, H-4'). ^{13}C NMR δ($CDCl_3$) 7.39 (C-1, CH_3), 34.60 (C-2, CH_2), 43.90 (C-5, CH_2), 54.84 (OCH_3), 109.98 (C-3'), 120.16 (C-5'), 123.34 (C-2'), 127.90 (C-6'), 130.70 (C-4'), 156.89 (C-1'), 209.11 (C=O).

5.24.2. Synthesis of 2,2-dimethylpropionic acid 2-(2'-oxo-butyl)-phenyl ester (BRI118)

The general method 5.25 was applied to this reaction. Iron powder (0.328M) was suspended in glacial acetic acid (150ml) and heated to 100°C for 20 minutes. 1-(-2-nitro-but-1-enyl)-2-pivaloyloxy-benzene (BRI012) (0.049M) in glacial acetic acid (20ml) was added over 10 minutes. After stirring for a further 2 hours at 100°C the reaction mixture was cooled and added to ice-water (100ml) and extracted with dichloromethane (3 x 300ml). The organic layers were washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (eluant: dichloromethane) to yield the product as a colourless oil (100%), (R_f 0.7 dichloromethane).IR ν_{max} (film) 2977, 2940 (CHs), 1714 (C=O), 1489, 1455 (CH_2), 1261 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.99-1.03 (3H, t, J=7.16Hz, H-1, CH_3), 1.36 (9H, s, OPiv), 2.38-2.44 (2H, q, J=7.2Hz, H-2, CH_2), 3.58 (2H, s, H-3, CH_2), 7.04-7.06 (1H, d, H-2'), 7.18-7.19 (1H, d, H-3'), 7.21-7.24 (2H, m, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 7.18 (C-1, CH_3), 26.55 (($\underline{C}H_3)_3$, OPiv), 34.38 (C-2, CH_2), 38.74 ($\underline{C}(CH_3)_3$, OPiv), 44.23 (C-3, CH_2), 121.85 (C-3'), 125.60 (C-5'), 126.60 (C-1'), 127.90 (C-4'), 130.99 (C-6'), 149.04 (C-2'), 176.23 (C=O, OPiv), 207.45 (C=O).

5.24.3. Synthesis of 1-(4-methoxyphenyl)-butan-2-one (BRI123)[198]

Following the general method 5.25, a suspension of iron powder (0.328M) in glacial acetic acid (150ml) was heated to 100°C for 20 minutes. To this 1-(2-nitrobutyl-1-enyl)-4-methoxy-benzene (BRI001) (0.049M) in glacial acetic acid (20ml) was added over 10 minutes. The reaction was stirred for a further 2 hours at 100°C. The reaction was allowed to cool and added to ice-water (100ml). Dichloromethane (3 x 300ml) was used to extract the product and were washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash column chromatography on silica gel (eluant: dichloromethane) to yield the product as a colourless oil (88%), (R_f 0.44 dichloromethane). IR ν_{max} (film) 2939 (CHs), 1713 (C=O), 1463 (CH_2), 1249 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.99-1.03 (3H, t, J=7.28Hz, H-1, CH_3), 2.43-2.48 (2H, q, J=7.04Hz, H-2, CH_2), 3.60 (2H, s, H-3, CH_2), 3.77 (3H, s, OCH_3), 6.85-6.87 (2H, d, H-2', H-3'), 7.11-7.13 (2H, d, H-1', H-4'), ^{13}C NMR δ($CDCl_3$) 7.30 (C-1, CH_3), 34.67 (C-2, CH_2), 49.42 (C-3, CH_2), 54.71 (OCH_3), 111.95 (C-3'), 114.53 (C-5'), 121.28 (C-2'), 129.22 (C-6'), 135.49 (C-1'), 159.35 (C-4'), 208.43 (C=O).

5.24.4. Synthesis of 1-(3-methoxyphenyl)-butan-2-one (BRI124)[199]

The general method 5.25 was applied to this reaction. 1-(3-Methoxyphenyl)-2-nitrobut-1-ene (BRI005) (0.049M) in glacial acetic acid (20ml) was added to a suspension of iron powder (0.328M) in glacial acetic acid (150ml), which had being heated to 100°C for 20 minutes. The reaction was stirred for a further 2 hours at 100°C and then allowed to cool. Ice-water (100ml) was added to the reaction mixture and extracted with dichloromethane (3 x 300ml). The organic layers were washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The DCM was removed *in vacuo* and the residue was purified by flash column chromatography on silica gel (eluant: dichloromethane) to yield the product as a colourless oil (100%), (R_f 0.3 dichloromethane). IR ν_{max} (film) 2976, 2940 (CHs), 1714 (C=O), 1490, 1455 (CH_2), 1261 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.01-1.06 (3H, t, J=7.52Hz, H-1, CH_3), 2.48-2.51 (2H, q, J=7.52Hz, H-2, CH_2), 3.66 (2H, s, H-3, CH_2), 3.80 (3H, OCH_3), 6.77-6.83 (3H, d, H-1', H-2', H-3'), 7.25-7.28 (1H, t, J=7.84 H-4'). ^{13}C NMR δ($CDCl_3$) 7.30 (C-1, CH_3), 34.67 (C-2, CH_2), 49.42 (C-3, CH_2), 54.71 (OCH_3), 111.95 (C-4'), 114.53 (C-2'), 121.28 (C-6'), 129.22 (C-5'), 135.49 (C-1'), 159.35 (C-3'), 208.43 (C=O).

5.24.5. Synthesis of 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (BRI125)

The general method 5.25 was used in this reaction. 1-(2-Nitrobutyl-1-enyl)-4- pivaloyloxy-benzene (BRI013) (0.049M) in glacial acetic acid (20ml) was added slowly over 10 minutes to iron powder (0.328M) suspended in glacial acetic acid (150ml) and was heated for 2 hours at 100°C after which the reaction was cooled and added to ice-water (100ml). The product was extracted with dichloromethane (3 x 300ml) and the organic layers were combined and washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and finally dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (eluant: dichloromethane) to yield the product, a colourless oil (100%), (R_f 0.7 dichloromethane). IR ν_{max} (film) 2976, 2940 (CHs), 1713 (C=O), 1491, 1461 (CH_2), 1261 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.87-0.91 (3H, t, J=7.48, H-1, CH_3), 1.26 (9H, s, OPiv), 2.34-2.39 (2H, q, J=7.2Hz, H-2, CH_2), 3.57 (2H, s, H-3, CH_2), 6.9-6.94 (2H, d, H-2', H-3'), 7.1-7.20 (2H, d, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 7.12 (C-1, CH_3), 26.49 (($\underline{C}H_3)_3$, OPiv), 34.54 (C-2, CH_2), 38.42 ($\underline{C}(CH_3)_3$, OPiv),

48.28 (C-3, CH_2), 121.08 (C-2', C-3'), 129.75 (C-5', C-6'), 131.30 (C-1'), 149.51 (C-4'), 176.20 (C=O, OPiv), 184.07 (C=O).

5.24.6. Synthesis of 2,2-dimethylpropionic acid 3-(2'-oxo-butyl)-phenyl ester (BRI126)

The general method 5.25 was applied to this reaction. A suspension of iron powder (0.328M) in glacial acetic acid (150ml) was heated to 100°C for 20 minutes. 1-(3-Pivaloyloxyphenyl)-2-nitrobut-1-ene (BRI014) (0.049M) in glacial acetic acid (20ml) was added and the reaction was heated and stirred for a further 2 hours at 100°C. The reaction mixture was cooled and added to ice-water (100ml) and extracted with dichloromethane (3 x 300ml). The organic layers were combined and washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and finally dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified on by performing flash column chromatography on silica gel (eluant: dichloromethane) to yield the product as a colourless oil (100%), (R_f 0.75 dichloromethane). IR ν_{max} (KBr) 2977 (CHs), 1755 (C=O), 1481, 1454 (CH_2), 1278 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.03-1.07 (3H, t, J=7.48, H-1, CH_3), 1.36 (9H, s, OPiv), 2.47-2.58 (2H, q, J=7.2Hz, H-2, CH_2), 3.70 (2H, s, H-3, CH_2), 6.9-6.99 (2H, d, H-2', H-3'), 7.07-7.09 (1H, d, H-4'), 7.28-7.36 (1H, m, H-1'), ^{13}C NMR δ($CDCl_3$) 7.27 (C-1, CH_3), 26.67 (($\underline{C}H_3)_3$, OPiv), 34.90 (C-2, CH_2), 38.61 ($\underline{C}(CH_3)_3$, OPiv), 48.90 (C-3, CH_2), 119.67 (C-4'), 122.10 (C-2'), 126.07 (C-6'), 129.07 (C-5'), 135.41 (C-1'), 150.81 (C-3'), 176.58 (C=O, OPiv), 208.03 (C=O).

5.24.7. Synthesis of 1'-(2-hydroxyphenyl)-butan-2'-one (BRI127)[208]

The reaction was carried out according to the general method 5.25. Iron powder (0.328M) was suspended in glacial acetic acid (150ml) and 1-(-2-nitro-but-1-enyl)-2-hydroxy-benzene (BRI027) (0.049M) in glacial acetic acid (20ml) was added to the reaction and the reaction was heated for 2 hours at 100°C. The reaction mixture was allowed to cool and ice-water (100ml) added to the reaction mixture. Dichloromethane (3 x 300ml) was used to extract the product. The organic layers were then combined and washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (eluant: dichloromethane) to yield the product as a colourless oil (100%), (R_f 0.67 dichloromethane). IR ν_{max} (KBr) 3370 (OH), 2980 (CHs),

1702 (C=O), 1445 (CH_2), 1226 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 2.15 (3H, s, H-1, CH_3), 2.35 (2H, s, H-2, CH_2), 3.66 (2H, s, H-3, CH_2), 7.10-7.17 (2H, m, H-1', H-2', H-3', H-4'). ^{13}C NMR δ($CDCl_3$) 20.56 (C-1, CH_3), 28.62 (C-2, CH_2), 50.16 (C-3, CH_2), 128.70 (C-3', C-5'), 128.98 (C-4', C-6'), 130.79 (C-1'), 136.18 (C-2'), 206.10 (C=O).

5.24.8. Synthesis of 1'-(3-hydroxyphenyl)-butan-2'-one (BRI128)

As in the general method 5.25, to a suspension of iron powder (0.328M) in glacial acetic acid (150ml), 1-(3-hydroxyphenyl)-2-nitrobut-1-ene (BRI026) (0.049M) in glacial acetic acid (20ml) was added and the reaction was heated for 2 hours at 100°C. The reaction mixture was cooled and added to ice-water (100ml) and extracted with dichloromethane (3 x 300ml). The organic layers were washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by chromatographing on silica gel (eluant: dichloromethane) to yield the product as a colourless oil (100%), (R_f 0.56 dichloromethane). IR v_{max} (film) 3379 (OH), 2978, 2940 (CHs), 1703 (C=O), 1455 (CH_2), 1273 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.00-1.04 (3H, t, J=7.5Hz, H-1, CH_3), 2.47-2.53 (2H, q, J=7.18Hz, H-2, CH_2), 3.64 (2H, s, H-3, CH_2), 6.7-6.78 (2H, m, H-1', H-2'), 7.14-7.18 (1H, t, J=8.18Hz, H-4'), 7.51 (1H, s, H-3'). ^{13}C NMR δ($CDCl_3$) 7.30 (C-1, CH_3), 34.80 (C-2, CH_2), 49.26 (C-3, CH_2), 113.90 (C-4'), 115.95 (C-2'), 120.84 (C-6'), 129.47 (C-5'), 135.15 (C-1'), 156.06 (C-4'), 210.90 (C=O).

5.24.9. Synthesis of 1'-(4-hydroxyphenyl)-butan-2'-one (BRI129)[209]

The general method 5.25 was used in this reaction. Iron powder (0.328M) was stirred and heated in glacial acetic acid (150ml) to 100°C while 1-(2-nitrobutyl-1-enyl)-4-hydroxy-benzene (BRI025) (0.049M) in glacial acetic acid (20ml) was added. The reaction was heated for 2 hours at 100°C. The reaction mixture was allowed to cool and added to ice-water (100ml) and extracted with dichloromethane (3 x 300ml). The DCM phases were combined and washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash column chromatography on silica gel (eluant: dichloromethane) to yield the product as a colourless oil (100%), (R_f 0.5 dichloromethane). IR v_{max} (film) 3386 (OH), 2980, 2940 (CHs), 1698 (C=O), 1446 (CH_2),

1228 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.99-1.03 (3H, t, J=7.5Hz, H-1, CH_3), 2.46-2.52 (2H, q, J=7.5Hz, H-2, CH_2), 3.62 (2H, s, H-3, CH_2), 6.78-6.80 (2H, d, H-2', H-3'), 7.25-7.28 (2H, d, H-1', H-4'), ^{13}C NMR δ($CDCl_3$) 7.30 (C-1, CH_3), 34.75 (C-2, CH_2), 48.42 (C-3, CH_2), 115.34 (C-3', C-5'), 125.17 (C-1'), 130.01 (C-2', C-6'), 154.76 (C-4'), 211.83 (C=O).

5.24.10. Synthesis of 1-(3-methyl-4-methoxyphenyl)-butan-2-one (BRI175)

Following the general method 5.25, to a suspension of iron powder (0.328M) in glacial acetic acid (150ml), 1-(2-nitrobutyl-1-enyl)-3-methyl-4-methoxy-benzene (BRI021) (0.049M) in glacial acetic acid (20ml) was added. The reaction was heated at 100°C for 2 hours. The reaction was allowed to cool and added to ice-water (100ml). Dichloromethane (3 x 300ml) was used to extract the product. The combined DCM phases were washed with saturated sodium bicarbonate solution (100ml), water (100ml), and brine (100ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash column chromatography on silica gel (eluant: dichloromethane) to yield a colourless oil (78%), (R_f 0.54 dichloromethane). IR ν_{max} (film) 2953 (CHs), 1714 (C=O), 1463, 1423 (CH_2), 1248 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.04 (3H, s, H-1, CH_3), 2.46 (3H, s, H-6, CH_3), 2.46 (2H, s, H-2, CH_2), 3.60 (2H, s, H-3, CH_2), 3.82 (OCH_3), 6.77-6.79 (1H, s, H-2'), 6.99 (H-3'). ^{13}C NMR δ($CDCl_3$) 7.30 (C-1, CH_3), 15.74 (C-4, CH_3), 34.47 (C-2, CH_2), 48.50 (C-3, CH_2), 54.81 (OCH_3), 109.57 (C-3'), 125.61 (C-5'), 126.37 (C-1'), 127.11 (C-2'), 131.16 (C-6'), 156.36 (C-4'), 209.16 (C=O).

5.25. Synthesis of 4-pivaloyloxypropiophenone (BRI176)[119]

The reaction was carried out using the general method 5.1 for pivaloylation. 4-hydroxypropiophenone [19] (0.0167M) was stirred with potassium hydroxide (0.0167M) for 3 hours in acetone. Trimethylacetyl chloride (0.0333M) was then added and the reaction was left stirring overnight. The solution was reduced under reduced pressure and the resulting mixture extracted with water (100ml) and washed with dichloromethane (2 x 100ml) to remove any starting material. The water phase was then basified and the product extracted with dichloromethane (2 x 100ml). The dichloromethane was removed *in vacuo* and the product was purified by flash chromatography (eluant: DCM:Hexane 1:1). A yellow oil was obtained (65%), (R_f 0.85 DCM:hexane 1:1). IR ν_{max} (film) 2977 (CHs),

1752 (C=O), 1689 (C=O, OPiv), 1481, 1460, 1412 (CH_2), 1277 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.22-1.26 (3H, t, J=7.02Hz, H-1, CH_3), 1.38 (9H, s, OPiv), 2.98-3.02 (2H, q, J=7.28Hz, H-2, CH_2), 7.16-7.18 (2H, d, H-2', H-3'), 8.01-8.03 (2H, d, H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 7.30 (C-1, CH_3), 26.62 (C-2, CH_2), 31.32 (C-4, CH_2), 38.74 (C-5, CH_2), 121.23 (C-3'', C-5''), 129.08 (C-2'', C-6''), 133.87 (C-1''), 154.25 (C-4''), 176.14 (C=O, OPiv), 199.16 (C=O).

5.26. McMurry Coupling of 1,3-diphenylpropan-1-ones with substituted 1-phenyl-2-butanones

5.26.1. Synthesis of 3,4,6-triphenyl-hex-3-enyl (BRI063)

5.26.1.1. Attempted synthesis of 3,4,6-triphenyl-hex-3-enyl (BRI063)

Following the general method 5.6, this reaction was first attempted using a Reaction Carousel. In a reaction vessel Zinc dust (0.00456M) was suspended in dry THF (tetrahydrofuran) (10ml) under nitrogen and titanium tetrachloride (0.00228M) was added via syringe. The reaction was left reacting in the dark for 2 hours. Then 1,3-diphenylpropan-1-one (BRI051) (0.00057M) and 1-phenyl-2-butanone [3] (0.00114M) were dissolved in dry THF (5ml) and added via syringe to the vessel. The reaction was refluxed for another 5 hours. The reaction mixture was then poured into potassium carbonate solution (50ml) and extracted with ethyl acetate (2 x 100ml) and the ethyl acetate was removed under reduced pressure. A TLC performed on the reaction mixture showed many impurities so flash column chromatography was performed on silica gel (eluant: hexane). The impurities were found to be mainly the self-coupled 1-phenyl-2-butanone (BRI064).

5.26.1.2. Synthesis of 3,4,6-triphenyl-hex-3-enyl (BRI063)

The reaction was repeated using a two-neck round bottom flask and a larger volume of dry THF. Titanium tetrachloride (0.006M) was added dropwise over 5 minutes to zinc dust (0.012M) in dry THF (tetrahydrofuran) under a nitrogen atmosphere. The mixture was refluxed at 100°C for 2 hours in the dark. This formed the catalyst. To this 1,3-diphenylpropan-1-one (BRI051) (0.0015M) and 1-phenyl-2-butanone [3] (0.003M) were

added in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (50ml), brine (50ml) and extracted with ethyl acetate (2 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: hexane). Some self-coupled 1-phenyl-2-butanone (BRI064) was obtained product as a clear oil (73%), (R_f 0.8 hexane). IR ν_{max} (KBr) 2946 (CHs), 1600 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.14-1.18 (3H, t, J=7.18Hz, H-6, CH_2), 2.23-2.85 (2H, q, J=7.8, H-5, CH_3), 2.76-2.81 (2H, t, J=4.12, H-1, CH_2), 2.91-2.99 (2H, t, J=4.12, H-2, CH_2), 3.72 (2H, s, H-7, CH_2), 7.16-7.42 (15H, ArH). ^{13}C NMR $\delta(CDCl_3)$ 13.10 (C-6, CH_3), 24.20 (C-5, CH_2), 34.40 (C-7, CH_2), 36.35 (C-1, CH_2), 37.87 (C-2, CH_2), 125.46 (C-4''', C-4'', C-4'), 127.83-128.28 (ArC), 128.36 (C-4, C=C), 128.63 (C-3, C=C), 134.40 (C-1'), 136.08 (C-1''), 140.71 (C-1'''). HRMS calculated for $C_{20}H_{24}$ 265.0841 (M^++1), observed 265.0850.

5.26.2. Synthesis of 1,4-diphenyl-2,3-diethyl-but-2-ene (BRI064)

5.26.2.1. Attempted synthesis of 1,4-diphenyl-2,3-diethyl-but-2-ene (BRI064)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.0125M) was added dropwise over 5 minutes to zinc dust (0.018M) in dry THF (tetrahydrofuran) (20ml) under a nitrogen atmosphere in a Reaction Carousel vessel. The mixture was refluxed at 100°C for 2 hours in the dark. This formed the catalyst. To this 1,3-diphenylpropenone (BRI050) (0.0024M) and 1-phenyl-2-butanone [3] (0.002M) were added in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (50ml), brine (50ml) and extracted with ethyl acetate (2 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: hexane). Only the self-coupled 1-phenyl-2-butanone (BRI064) and starting material were recovered.

5.26.2.2. Attempted synthesis of 1,4-diphenyl-2,3-diethyl-but-2-ene (BRI064)

The reaction was also attempted in a two neck round bottomed flask using 100ml of dry THF using the method described above. The reaction was washed and extracted as in the

general method but to no avail as only self-coupled 1-phenyl-2-butanone (BRI064) oil was obtained. IR ν_{max} (KBr) 2929 (CHs), 1600 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.03-1.06 (3H, t, J=7.5Hz, H-1, CH_3), 1.09-1.13 (3H, t, J=7.52Hz, H-2, CH_3), 2.18 (4H, s, H-5, H-6, $(CH_2)_2$), 3.61 (4H, s, H-7, H-8, $(CH_2)_2$), 7.2-7.37 (10H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 12.99-13.18 (C-1, C-2, $(CH_3)_2$), 24.13-24.50 (C-5, C-6, $(CH_2)_2$), 36.21-36.65 (C-7, C-8, $(CH_2)_2$), 125.35-125.43 (C-4'', C-4'), 127.69-127.86 (C-3', C-5', C-3'', C-5''), 128.08-128.18 (C-2'', C-6'', C-2', C-6'), 134.3-134.38 (C-3, C-4, $(C=C)_2$), 140.4-140.63 (C-1', C-1'').

5.26.3. Attempted synthesis of 1-(4-methoxyphenyl)-1-(2-phenylvinyl)-2-phenyl-but-1-enyl (BRI065)

The general method 5.7 was used in this reaction. Titanium tetrachloride (0.0095M) was added dropwise over 5 minutes to zinc dust in dry THF (tetrahydrofuran) (10ml) under a nitrogen atmosphere. The mixture was refluxed at 100°C for 2 hours in the dark in the Reaction Carousel. Then 1-(4-methoxyphenyl)-3-phenylpropenone (BRI055) (0.00127M) and 1-phenyl-2-butanone [3] (0.00374M) were dissolved in dry THF (5ml) and added to the reaction vessel and left to reflux for 5 hours. The product was extracted and washed potassium carbonate solution (50ml) and extracted with ethyl acetate (2 x 100ml). The ethyl acetate was concentrated. Column chromatography was performed to purify the crude product (eluant: hexane:diethyl ether 1:1). Only self-coupled 1-phenyl-2-butanone (BRI064) was produced.

5.26.4. Synthesis of 3-phenyl-4,6-(4-methoxyphenyl)-hex-3-enyl (BRI069)

The general method 5.6 was used in this reaction. Titanium tetrachloride (0.0044M) was added to zinc dust (0.0088M) in dry THF (tetrahydrofuran) (80ml). The mixture was refluxed at 100°C for 2 hours in the dark. This formed the catalyst. Then 1,3-bis-(4-methoxyphenyl)-propan-1-one (BRI058) (0.0011M) and 1-phenyl-2-butanone [3] (0.0033M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. Column chromatography was performed to purify the crude product (eluant: hexane:diethyl ether 50:50). The product was obtained as a light yellow oil (50%), (R_f 0.7 hexane:diethyl ether 50:50). IR ν_{max} (KBr) 2944 (CHs), 1606 (C=O), 1510 (NCH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.9-0.96 (3H, t, J=7.52Hz, , H-6, CH_3), 1.88-1.93

(2H, q, J=7.28Hz, H-5, CH_2), 2.55-2.59 (2H, t, H-1, CH_2), 2.73-2.81 (2H, t, H-2, CH_2), 3.37 (2H, s, H-7), 3.86 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.8-7.38 (ArH). ^{13}C NMR $\delta(CDCl_3)$ 13.01 (C-6, CH_2), 23.42 (C-5, CH_2), 33.26 (C-1, CH_2), 35.90 (C-2, CH_2), 37.67 (C-7, CH_2), 113.05 (C-3''', C-5'''), 113.31 (C-3', C-5'), 125.19 (C-4''), 127.70 (C-2''', C-6'''), 128.09 (C-2'', C-6''), 128.25 (C-3'', C-5''), 129.51 (C-2', C-6'), 133.90 (C-3, C=C), 135.00 (C-4, C=C), 135.90 (C-1'), 136.09 (C-1''), 140.70 (C-1'''), 157.30 (C-4'''), 157.55 (C-4').

5.26.5. Synthesis of 1-[2-(4-{2-(4-methoxy-3-methyl-phenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI180)

The general method 5.6 was applied to this reaction. Zinc (0.016M), in dry tetrahydrofuran (100ml), and titanium tetrachloride (0.008M) were refluxed for 2 hours. Then 1-(3-methyl-4-methoxyphenyl)-butan-2-one (BRI175) (0.002M) and 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) (0.002M), dissolved in dry tetrahydrofuran (50ml), were added in one portion to the reaciton and the mixture refluxed for a further 4 hours before being poured into sodium carbonate solution (100ml) and extracted with ethyl acetate (4 x 200ml) and dichloromethane (200ml). The organic phases were dried over anhydrous sodium sulphate, filtered and evaporated to dryness *in vacuo*. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 19:1). The desired product was recovered as a brown oil (85%) (R_f 0.3 dichloromethane:methanol 19:1). IR ν_{max} (film) 2925 (CHs), 1607 (C=C), 1513 (NCH_2), 1462 (CH_2), 1241 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.86-0.89 (3H, t, J=7.54Hz, H-6, CH_3), 1.85 (4H, s, H-2'''', H-3''''), 2.02-2.08 (2H, q, J=7.54Hz, H-5, CH_2), 2.23 (3H, s, CH_3), 2.49-2.51 (2H, m, H-1, CH_2), 2.53-2.55 (2H, m, H-2, CH_2), 2.73 (4H, s, H-1'''', H-4''''), 2.92-2.94 (2H, t, J=6.04Hz, H-8), 3.21 (2H, s, H-7, CH_2), 3.80 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.12-4.18 (2H, t, J=6.04Hz, H-9), 6.70-6.72 (1H, s, H-3''), 6.75-6.78 (2H, d, H-3''', H-5'''), 6.8-6.85 (2H, d, H-3', H-5'), 6.98-7.02 (2H, d, H-2'', H-6''), 7.08-7.10 (2H, d, H-2''', H-6'''), 7.12-7.15 (3H, m, H-2', H-6', H-5''). ^{13}C NMR $\delta(CDCl_3)$ 12.98 (C-6, CH_3), 15.90 (C-8, CH_3), 23.04 (C-2'''', C-3'''', $(CH_2)_2$), 29.65 (C-5, CH_2), 33.36 (C-1, CH_2), 36.63 (C-2, CH_2), 45.17 (C-7, CH_2), 54.25 (OCH_3), 54.28 (OCH_3), 54.69 (C-8, CH_2), 54.89 (C-1'''', C-4'''', $(CH_2)_2$), 66.43 (C-9, CH_2), 109.35 (C-5''), 113.13 (C-3''', C-5'''), 113.62 (C-3'', C-5''), 125.58 (C-6''), 126.07 (C-3, C=C), 128.80 (C-2''', C-6'''), 129.45 (C-2', C-6'), 130.57 (C-2''), 132.14 (C-3''), 133.98 (C-4, C=C), 135.36 (C-1'),

135.57 (C-1''), 136.29 (C-1'''), 155.34 (C-4'), 156.73 (C-4'''), 157.23 (C-4''). HRMS calculated for $C_{33}H_{41}NO_3$ 500.3172 (M^++1), observed 500.3165.

5.27. Synthesis of alternative basic side chain position structures

5.27.1. Synthesis of 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (BRI174)

As in the general method 5.7, 4-hydroxypropiophenone (36) (0.013) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.0156M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.027M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light yellow oil (92%), (R_f 0.3 acetone). IR ν_{max} (film) 2927 (CHs), 1607 (C=O), 1508 (NCH_2), 1458 (CH_2), 1239 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.77 (3H, t, J=7.52Hz, H-1, CH_3), 1.71 (4H, s, H-2'''', H-3''''), 2.22-2.28 (2H, q, J=7.52Hz, H-2, CH_2), 2.58 (4H, s, H-1'''', H-4''''), 2.83-2.86 (2H, t, J=4.5Hz, H-3, CH_2), 4.03-4.05 (2H, t, J=4.5Hz, H-4, CH_2), 6.77-6.79 (1H, s, H-2', H-3'), 6.91-6.93 (H-1', H-4'). ^{13}C NMR δ($CDCl_3$) 21.70 (C-1, CH_3), 23.01 (C-2'''', C-3'''', $(CH_2)_2$), 27.09 (C-2, CH_3), 54.23 (C-1'''', C-4'''', $(CH_2)_2$), 54.69 (C-3, CH_2), 66.38 (C-4, CH_2), 113.37 (C-3', C-5'), 129.49 (C-2', C-6'), 135.89 (C-1'), 156.41 (C-4'), 266.13 (C=O).

5.27.2. Attempted synthesis of 4-[2-ethyl-5-(4-methoxyphenyl)-3-(2-methoxyphenyl)-pent-2-enyl]-phenol (BRI187)

Following the general method 5.6, titanium tetrachloride (0.0022M) was added to zinc dust (0.0044M) in dry THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 3-(4-methoxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI137) (0.011M) and 4-hydroxypropiophenone (36) (0.0054M) were dissolved in THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 40:60). Only self-coupled 4-hydroxypropiophenone (36) was produced by the reaction.

5.27.3. Attempted synthesis of 4-[2-ethyl-5-(4-methoxyphenyl)-3-(3-methoxyphenyl)-pent-2-enyl]-phenol (BRI141)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.008M) was added to zinc (0.016M) in THF (tetrahydrofuran) (80ml). The mixture was refluxed at 100°C for 2 hours. Then 3-(4-methoxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI053) (0.0022M) and 4-hydroxypropiophenone (36) (0.004M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 40:60). Only self-coupled 4-hydroxy propiophenone was produced by the reaction.

5.27.4. Attempted synthesis of 4-[2-ethyl-5-(4-methoxyphenyl)-3-(4-methoxyphenyl)-pent-2-enyl]-phenol (BRI163)

As in the general method 5.6, titanium tetrachloride (0.00323M) was added to zinc dust (0.00646M) in THF (tetrahydrofuran) (80ml) under a. The mixture was refluxed for 2 hours. Then 1,3-bis-(4-methoxyphenyl)-propan-1-one (BRI058) (0.004M) and 4-hydroxypropiophenone (36) (0.008M) were dissolved in THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 40:60). Only self-coupled 4-hydroxypropiophenone (36) was produced by the reaction.

5.27.5. Synthesis of 1-(2-{4-[2-ethyl-5-(4-methoxyphenyl)-3-(2-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI138)

The general method 5.6 was used in this reaction. Titanium tetrachloride (0.0109M) was added to zinc (0.0218M) in THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 3-(4-methoxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (BRI137) (0.0027M) and 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (BRI174) (0.00544M) were dissolved in THF (20ml) and added and left to reflux for 5 hours. Column chromatography was performed to purify the crude product (eluant: dichloromethane:methanol 19:1) and a yellow oil was obtained (30%), (R_f 0.3 dichloromethane:methanol 19:1). IR ν_{max} (film) 2967 (CHs), 1608 (C=C), 1510 (NCH_2),

1463 (CH_2), 1243 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.91-0.94 (3H, t, J=7.28Hz, H-6, CH_3), 1.81 (4H, s, H-2'''', H-3''''), 2.42-2.44 (2H, s, H-5, CH_2), 2.56 (2H, m, H-1, CH_2), 2.65 (4H, s, H-1'''', H-4''''), 2.72 (2H, s, H-2, CH_2), 2.86-2.89 (2H, t, J=6.02Hz, H-7), 3.74 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.9-4.02 (2H, t, J=5.78Hz, H-8), 6.58-6.60 (2H, d, H-3'', H-5''), 6.5-6.68 (1H, t, J=7.28Hz, H-2'), 6.7-6.73 (1H, d, H-4'), 6.76-6.78 (1H, d, H-5'), 6.81-6.86 (4H, m, H-2''', H-3''', H-5''', H-6'''), 7.03-7.07 (1H, t, J=7.76Hz, H-6'), 7.1-7.12 (2H, d, H-2'', H-6''). ^{13}C NMR δ($CDCl_3$) 12.82 (C-6, CH_3), 22.92 (C-5, CH_2), 24.06 (C-2'''', C-3'''', $(CH_2)_2$), 29.50 (C-1, CH_2), 35.10 (C-2, CH_2), 54.11 (OCH_3), 54.16 (OCH_3), 54.66 (C-7, CH_2), 54.80 (C-1'''', C-4'''', $(CH_2)_2$), 65.87 (C-8, CH_2), 109.72 (C-3'), 112.75 (C-3''', C-5'''), 112.98 (C-4'), 113.50 (C-3'', C-5''), 119.38 (C-5'), 126.60 (C-6'), 128.68 (C-2'', C-6''), 129.37 (C-2''', C-6'''), 131.38 (C-1'), 133.18 (C-1''), 134.40 (C-4, C=C), 135.38 (C-3, C=C), 139.70 (C-1'''), 155.96 (C-4'''), 156.52 (C-4''), 157.14 (C-2'). HRMS calculated for $C_{33}H_{41}NO_3$ 500.3143 (M^++1), observed 500.3165.

5.27.6. Synthesis of 1-(2-{4-[2-ethyl-5-(4-methoxyphenyl)-3-(3-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI144)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.009M) was added to zinc dust (0.018M) in dry THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 3-(4-methoxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (BRI053) (0.0023M) and 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (BRI174) (0.00453M) were dissolved in THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. Column chromatography was performed to purify the crude product (eluant: dichloromethane:methanol 19:1) and a yellow oil was obtained (42%), (R_f 0.3 dichloromethane:methanol 19:1). IR ν_{max} (film) 2933 (CHs), 1607 (C=C), 1511 (NCH_2), 1459 (CH_2), 1246 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.88-0.92 (3H, t, J=7.54Hz, H-6, CH_3), 1.82 (4H, s, H-2'''', H-3''''), 2.39-2.44 (2H, q, J=7.52Hz, H-5, CH_2), 2.55-2.59 (2H, t, J=8.02Hz, H-1, CH_2), 2.66 (4H, s, H-1'''', H-4''''), 2.77-2.81 (2H, t, J=8.02Hz, H-2, CH_2), 2.87-2.90 (2H, t, J=6.02Hz, H-7), 3.63 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.0-4.04 (2H, t, J=6.02Hz, H-8), 6.52 (1H, s, H-3'), 6.58-6.65 (4H, m, H-3'', H-5'', H-3''', H-5'''), 6.83-6.85 (4H, d, H-2', H-6', H-2''', H-6'''), 7.01-7.05 (1H, t, J=8.02Hz, H-5'), 7.09-7.12 (2H, d, H-2'', H-6''). ^{13}C NMR δ($CDCl_3$) 12.64 (C-6, CH_3), 22.97 (C-5, CH_2), 30.49 (C-2'''', C-3'''', $(CH_2)_2$), 33.40 (C-1, CH_2), 36.0 (C-2, CH_2), 54.54 (OCH_3), 54.56 (OCH_3), 54.69 (C-7, CH_2), 54.83 (C-1'''', C-4'''', $(CH_2)_2$), 66.12 (C-8, CH_2), 110.53 (C-2'), 113.10 (C-

3''', C-5'''), 113.17 (C-3'', C-5''), 115.11 (C-4'), 121.97 (C-6'), 127.90 (C-5'), 128.63 (C-2''', C-6'''), 130.11 (C-2'', C-6''), 133.27 (C-3, C=C), 134.14 (C-4, C=C), 135.50 (C-1''), 142.60 (C-1'''), 147.55 (C-1'), 151.76 (C-4''), 155.69 (C-4'''), 157.28 (C-3'). HRMS calculated for $C_{33}H_{41}NO_3$ 500.3189 (M^++1), observed 500.3165.

5.27.7. Synthesis of 1-(2-{4-[2-ethyl-5-(4-methoxyphenyl)-3-(4-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (BRI169)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.0325M) was added to zinc (0.0646M) in THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 1,3-bis-(4-methoxyphenyl)-propan-1-one (BRI058) (0.004M) and 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (BRI174) (0.008M) were dissolved in THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. Column chromatography was performed to purify the crude product (eluant: dichloromethane:methanol 19:1) and a yellow oil was obtained (57%), (R_f 0.3 dichloromethane:methanol 19:1). IR ν_{max} (film) 2944 (CHs), 1606 (C=C), 1510 (NCH_2), 1463 (CH_2), 1286, 1244 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.87-0.91 (3H, t, J=7.54Hz, H-6, CH_3), 1.87 (4H, s, H-2'''', H-3''''), 2.37-2.43 (2H, q, J=7.28Hz, H-5, CH_2), 2.54-2.58 (2H, t, J=8.04Hz, H-1, CH_2), 2.79 (4H, s, H-1'''', H-4''''), 2.91-2.95 (2H, t, J=7.28Hz, H-2, CH_2), 2.97-3.01 (2H, t, J=6.28Hz, H-7), 3.74 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.07-4.09 (2H, t, J=5.52Hz, H-8), 6.6-6.62 (2H, d, H-3''', H-5'''), 6.65-6.67 (2H, d, H-3', H-5'), 6.82-6.86 (4H, m, H-2'', H-6'', H-3'', H-5''), 6.9-6.92 (2H, d, H-2''', H-6'''), 7.09-7.11 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 12.68 (C-6, CH_3), 22.87 (C-2'''', C-3'''', $(CH_2)_2$), 26.98 (C-5, CH_2), 30.80 (C-1, CH_2), 33.38 (C-2, CH_2), 54.06 (OCH_3), 54.39 (OCH_3), 54.58 (C-7, CH_2), 54.83 (C-1'''', C-4'''', $(CH_2)_2$), 65.39 (C-8, CH_2), 112.05 (C-3''', C-5'''), 113.05 (C-3', C-5'), 113.18 (C-3'', C-5''), 128.90 (C-2', C-6'), 130.33 (C-2''', C-6'''), 130.45 (C-4'', C-6''), 134.24 (C-3, C=C), 134.96 (C-4, C=C), 135.08 (C-1''), 135.37 (C-1'), 138.95 (C-1'''), 155.73 (C-2''), 156.79 (C-4''') 157.26 (C-4'). HRMS calculated for $C_{33}H_{41}NO_3$ 500.3150 (M^++1), observed 500.3165.

5.28. Synthesis of pivaloyl-substituted coupled compounds

5.28.1. Synthesis of 2,2-dimethylpropionic acid 2-[2-ethyl-3-(4-hydroxyphenyl)-5-(4-methoxyphenyl)-pent-2-enyl]-phenyl ester (BRI120)

Using the general method 5.6, titanium tetrachloride (0.008M) was added to zinc (0.016M) in THF (tetrahydrofuran) (80ml) and the reaction was refluxed. 1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (0.002M) and 2,2-dimethylpropionic acid 2-(2'-oxo-butyl)-phenyl ester (BRI118) (0.004M) were dissolved in dry THF (20ml) and added to the reaction vessel. The reaction was refluxed for a further 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (2 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (44%), (eluant: hexane:dichloromethane 3:2), (R_f 0.5 hexane:dichloromethane 3:2). IR ν_{max} (film) 3390 (OH), 2964 (CHs), 1731 (C=O, OPiv), 1633, 1610 (C=C), 1455 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.02-1.05 (3H, t, J=7.04Hz, H-6, CH_3), 1.38 (9H, s, OPiv), 2.48-2.49 (2H, q, J=7.44Hz, H-5, CH_2), 2.5-2.57 (2H, t, J=8.6Hz, H-1, CH_2), 2.6-2.63 (2H, t, J=8.5Hz, H-2, CH_2), 3.62 (2H, s, H-7, CH_2), 3.80 (3H, s, OCH_3), 6.77-6.78 (2H, d, H-3', H-5'), 6.81-6.83 (2H, d, H-3''', H-5'''), 6.98-7.0 (2H, d, H-2', H-6'), 7.06-7.09 (2H, d, H-2''', H-6'''), 7.1-7.30 (4H, m, H-2'', H-3'', H-5'', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 7.22 (C-6, CH_3), 21.78 (C-5, CH_2), 26.15 (($\underline{C}H_3)_3$, OPiv), 33.15 ($\underline{C}(CH_3)_3$, OPiv), 34.46 (C-1, CH_2), 36.47 (C-2, CH_2), 44.20 (C-7, CH_2), 54.80 (OCH_3), 113.15 (C-3', C-5'), 114.35 (C-3''', C-5'''), 122.02 (C-3'', C-5''), 125.44 (C-2''', C-6'''), 127.33 (C-4'', C-6''), 128 (C-3, C=C), 129.58 (C-4, C=C), 129.73 (C-2', C-6'), 134.23 (C-1'), 135.29 (C-1''), 149.03 (C-1'''), 153.62 (C-2''), 156.33 (C-4'''), 157.13 (C-4''), 176.41 (C-4'''), 191.00 (C=O, OPiv).

5.28.2. Synthesis of 2,2-dimethylpropionic acid 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI121)

The reaction was carried out according to the general method 5.7 by placing 2,2-dimethylpropionic acid 2-[2-ethyl-3-(4-hydroxyphenyl)-5-(4-methoxyphenyl)-pent-2-enyl]-phenyl ester (BRI120) (0.0006M) in acetone:water 19:1 (10ml) with potassium carbonate (0.00072M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0012M). The reaction was

refluxed for 5 hours in darkness and was then extracted using dichloromethane (2 x 50ml) and potassium carbonate solution (50ml). The product was purified by flash column chromatography (eluant: dichloromethane:methanol 19:1). The product was obtained as a light brown oil (37%), (R_f 0.3 dichloromethane:methanol 19:1). IR ν_{max} (KBr) 2959, 2872 (CHs), 1747 (C=O, OPiv), 1611 (C=C), 1512 (NCH_2), 1454 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.81-0.85 (3H, t, J=7.5Hz, H-6, CH_3), 1.36 (9H, s, OPiv), 1.78-1.86 (4H, s, H-2'''', H-3''''), 2.48-2.49 (2H, q, J=7.5Hz, H-5, CH_2), 2.63-2.68 (4H, m, H-1, H-2 $(CH_2)_2$), 2.72 (4H, s, H-1'''', H-4''''), 2.95-3.0 (2H, t, J=6.12Hz, H-8), 3.38-3.41 (2H, s, H-7, CH_2), 3.74-3.80 (3H, s, OCH_3), 4.11-4.17 (2H, t, J=6.12Hz, H-9), 6.7-7.20 (13ArH). ^{13}C NMR δ($CDCl_3$) 12.93 (C-6, CH_3), 22.24 (C-5, CH_2), 23.32 ($\underline{C}(CH_3)_3$, OPiv), 29.25 (C-2'''', C-3'''', $(CH_2)_2$), 30.50 ($(\underline{C}H_3)_3$, OPiv), 33.33 (C-1, CH_2), 36.70 (C-2, CH_2), 40.31 (C-7, CH_2), 54.07 (C-1'''', C-4'''', $(CH_2)_2$), 54.15 (C-8, CH_2), 54.80 (OCH_3), 65.58 (C-9, CH_2), 113.15 (C-3''', C-5'''), 113.58 (C-3', C-5'), 114.60 (C-3'', C-4''), 128.60 (C-2', C-6'), 129 (C-5'', C-6''), 129.44 (C-2''', C-6'''), 130.60 (C-3, C=C), 132.05 (C-4, C=C), 133.90 (C-1'), 135.40 (C-1''), 136.14 (C-1'''), 153.67 (C-2''), 156.33 (C-4'''), 157.22 (C-4''), 206.60 (C=O). HRMS calculated for $C_{37}H_{48}NO_4$ 570.3583 (M^++1), observed 570.3598.

5.28.3. Synthesis of 2,2-dimethylpropionic acid 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI148)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.0057M) was added to zinc dust (0.01132M) in dry THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) (0.00142M) and 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (BRI125) (0.00283M) were dissolved in dry THF (20ml) and added to the reaction and to refluxed for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml). The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: dichloromethane:methanol 19:1) and yellow oil was obtained (32%), (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 2946 (CHs), 1748 (C=O, OPiv), 1607 (C=C), 1511 (NCH_2), 1462 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.82-0.86 (3H, t, J=7.52Hz, H-6, CH_3), 1.36 (9H, s, OPiv), 2.05-2.07 (4H, s, H-2'''', H-3''''), 2.46-2.50 (2H, q, J=7.52Hz, H-5, CH_2), 2.52-2.56 (2H, t, J=6.16Hz, H-1, CH_2), 2.65-2.70 (2H, t,

J=6.12Hz, H-2, CH_2), 3.24 (4H, s, H-1'''', H-4''''), 3.34-3.37 (2H, t, J=6.12Hz, H-8), 3.38 (2H, s, H-7, CH_2), 3.77 (3H, s, OCH_3), 4.4-4.42 (2H, t, J=5.12, H-9), 6.77-6.79 (2H, d, H-3', H-5'), 6.82-6.86 (2H, d, H-3'', H-5''), 6.9-6.92 (2H, d, H-2''', H-6'''), 6.96-7.0 (2H, m, H-2'', H-6''), 7.06-7.09 (2H, m, H-3''', H-5'''), 7.16-7.18 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 12.99 (C-6, CH_3), 22.82 (C-5, CH_2), 26.69 (($\underline{C}H_3)_3$, OPiv), 29.90 (C-2'''', C-3'''', $(CH_2)_2$), 33.33 (C-1, CH_2), 34.50 ($\underline{C}(CH_3)_3$, OPiv), 36.70 (C-1, CH_2), 40.31 (C-2, CH_2), 54.07 (C-1'''', C-4'''', $(CH_2)_2$), 54.15 (C-8, CH_2), 54.80 (OCH_3), 65.58 (C-9, CH_2), 113.17 (C-3''', C-5'''), 113.65 (C-3', C-5'), 120.39 (C-3'', C-5''), 120.70 (C-2', C-6'), 128.60 (C-2'', C-6''), 129.11 (C-2''', C-6'''), 130.60 (C-3, C=C), 132.05 (C-4, C=C), 133.90 (C-1'), 135.40 (C-1''), 136.14 (C-1'''), 153.67 (C-4''), 156.33 (C-4'''), 157.22 (C-4''), 207.60 (C=O). HRMS calculated for $C_{37}H_{47}NO_4$ 569.7734 (M^++1), observed 570.3583.

5.28.4. Synthesis of 2,2-dimethylpropionic acid 3-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI155)

Using the general method 5.6, titanium tetrachloride (0.008M) was added to zinc dust (0.016M) in THF (tetrahydrofuran) (20ml) and the reaction was refluxed for 2 hours. Once the catalyst had formed, 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) (0.002M) and 2,2-dimethylpropionic acid 3-(2'-oxo-butyl)-phenyl ester (BRI126) (0.004M) were dissolved in dry THF (80ml) and added to the reaction vessel and the reaction was refluxed for a further 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (2 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (44%), (eluant: hexane:dichloromethane 3:2), (R_f 0.5 hexane:dichloromethane 3:2). IR ν_{max} (film) 2946 (CHs), 1755 (C=O, OPiv), 1609 (C=C), 1512 (NCH_2), 1459 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.93-0.97 (3H, t, J=7.52Hz, H-6, CH_3), 1.38 (9H, s, OPiv), 2.04 (4H, s, H-2'''', H-3''''), 2.06-2.08 (2H, q, J=7.52Hz, H-5, CH_2), 2.53-2.57 (2H, t, J=8.04Hz, H-1, CH_2), 2.67-2.71 (2H, t, J=7.78Hz, H-2, CH_2), 2.85 (4H, s, H-1'''', H-4''''), 3.46 (2H, s, H-7, CH_2), 3.67-3.69 (2H, t, J=8.04Hz, H-8), 3.80 (3H, s, OCH_3), 4.20-4.24 (2H, t, J=8.78, H-9), 6.75-6.93 (2H, m, H-3', H-5', H-3'', H-5'', H-2''', H-6'''), 6.98-7.13 (4H, m, H-2'', H-6'', H-3''', H-5'''), 7.21-7.25 (1H, t, J=7.78Hz, H-2''). ^{13}C NMR δ($CDCl_3$) 12.94 (C-6, CH_3), 22.93 (C-5, CH_2), 25.85 (($\underline{C}H_3)_3$, OPiv), 29.26 (C-2'''', C-

3'''', $(CH_2)_2$), 33.12 (C-1, CH_2), 35.18 ($\underline{C}(CH_3)_3$, OPiv), 37.39 (C-1, CH_2), 39.59 (C-2, CH_2), 54.01 (C-1'''', C-4'''', $(CH_2)_2$), 54.25 (C-8, CH_2), 54.81 (OCH_3), 67.67 (C-9, CH_2), 113.16 (C-3''', C-5'''), 113.63 (C-3', C-5'), 118.45 (C-3'', C-5''), 121.09 (C-2', C-6'), 125.05 (C-2'', C-6''), 128.47 (C-2''', C-6'''), 129.42 (C-3, C=C), 133.77 (C-4, C=C), 135.20 (C-1'), 135.48 (C-1''), 141.87 (C-1'''), 150.52 (C-4''), 156.40 (C-4'''), 157.26 (C-4''), 176.69 (C=O). HRMS calculated for $C_{37}H_{47}NO_4$ 570.3583 (M^++1), observed 570.3542.

5.29. Synthesis of methoxy substituted compounds

5.29.1. Synthesis of 4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI119)

The general method 5.6 was applied to this reaction. Zinc dust (0.0656M) in dry THF (tetrahydrofuran) (80ml) and titanium tetrachloride (0.0328M) were refluxed for 2 hours. 1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (0.0047M) and 1-(2-methoxyphenyl)-butan-2-one (BRI117) (0.0047M) were dissolved in dry THF (20ml) and added to the reaction vessel and refluxed for 5 hours. The reaction mixture was poured into a solution of K_2CO_3 (100ml) and extracted with ethyl acetate (3 x 100ml). The filtrate was dried (sodium sulphate) and concentrated. Flash Column chromatography was performed to purify the crude product (49%), (eluant: hexane:dichloromethane 40:60), (R_f 0.4 hexane:dichloromethane 40:60). IR v_{max} (film) 3396 (OH), 2946 (CHs), 1610 (C=C), 1463 (CH_2), 1242 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.95-0.99 (3H, t, J=7.52Hz, H-6, CH_3), 2-2.05 (2H, q, J=7.16Hz, H-5, CH_2), 2.55-2.59 (2H, t, J=6.14Hz, H-1, CH_2), 2.7-2.75 (2H, t, J=6.48Hz, H-2, CH_2), 3.24 (2H, s, H-7, CH_2), 3.82 (3H, s, OCH_3), 6.74-6.76 (2H, d, H-3', H-5'), 6.78-6.80 (2H, d, H-3''', H-5'''), 6.84-6.88 (4H, m, H-3'', H-4'', H-5'', H-6''), 7.03-7.05 (2H, d, H-2''', H-6'''), 7.08-7.10 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 13.05 (C-6, CH_3), 23.81 (C-5, CH_2), 31.10 (C-1, CH_2), 33.32 (C-2, CH_2), 35.63 (C-7, CH_2), 54.80 (OCH_3), 109.30 (C-3''), 113.16 (C-3''', C-5'''), 114.32 (C-3', C-5'), 119.74 (C-1''), 126.10 (C-4''), 128.65 (C-5''), 128.90 (C-2', C-6'), 128.99 (C-6''), 129 (C-2''', C-6'''), 133.90 (C-3, C=C), 135.11 (C-4, C=C), 135.30 (C-1'''), 135.90 (C-1'), 153.30 (C-4'), 157.11 (C-2''), 157.25 (C-4''').

Synthesis of 4-{2-(3-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI131)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.0229M) was added to zinc dust (0.0458M) in dry THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (0.0057M) and 1-(3-methoxyphenyl)-butan-2-one (BRI124) (0.01147M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml). The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: hexane:dichloromethane 40:60) and yellow oil was obtained (80%). IR ν_{max} (KBr) 3401 (OH), 2945 (CHs), 1609 (C=C), 1454 (CH_2), 1246 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.96-0.99 (3H, t, J=7.52Hz, H-6, CH_3), 2.04-2.10 (2H, q, J=7.18Hz, H-5, CH_2), 2.56-2.59 (2H, t, J=6.14Hz, H-1, CH_2), 2.7-2.75 (2H, t, J=6.48Hz, H-2, CH_2), 3.29 (2H, s, H-7, CH_2), 3.82 (3H, s, OCH_3), 6.65-6.66 (3H, s, H-2'', H-4'', H-5''), 6.80-6.82 (2H, d, H-3''', H-5'''), 6.84-6.86 (2H, d, H-3', H-5'), 7.07-7.11 (4H, m, H-2', H-6', H-2''', H-6''', H-6''). ^{13}C NMR $\delta(CDCl_3)$ 12.99 (C-6, CH_3), 23.35 (C-5, CH_2), 33.37 (C-1, CH_2), 35.98 (C-2, CH_2), 37.61 (C-7, CH_2), 54.66 (OCH_3), 54.84 (OCH_3), 110.24 (C-2''), 113.24 (C-3''', C-5'''), 114.11 (C-4''), 114.54 (C-3', C-5'), 120.74 (C-6''), 128.60 (C-1'), 128.90 (C-2', C-6'), 129.61 (C-2''', C-6'''), 134.0 (C-4, C=C), 135 (C-3, C=C), 135.60 (C-5''), 136 (C-1'''), 142.40 (C-1''), 153.68 (C-4'''), 157.20 (C-4'), 158.99 (C-3'').

5.29.2. Synthesis of 4-{2-(4-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI132)

Following the general method 5.6, titanium tetrachloride (0.016M) was added dropwise to zinc dust (0.032M) in THF (80ml). The mixture was refluxed for 2 hours. 1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (0.0042M) and 1-(4-methoxyphenyl)-butan-2-one (BRI123) (0.008M) in THF (20ml) were added to the reaction and refluxed for a further 5 hours. The product was washed with potassium carbonate solution (100ml), then brine (100ml) and finally extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude

product (eluant: hexane:dichloromethane 40:60) and yellow oil was (95%). IR ν_{max} (KBr) 3368 (OH), 2933 (CHs), 1610 (C=C), 1455 (CH_2), 1246 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.94-0.98 (3H, t, J=7.33Hz, H-6, CH_3), 2.55-2.60 (2H, t, J=6.12Hz, H-1, CH_2), 2.60-2.65 (2H, q, J=8.18Hz, H-5, CH_2), 2.68-2.72 (2H, t, J=6.48Hz, H-2, CH_2), 3.24 (2H, s, H-7, CH_2), 3.81 (3H, s, OCH_3), 6.79-6.80 (2H, d, H-3'', H-5''), 6.80-6.83 (2H, d, H-3', H-5'), 6.86-6.87 (2H, d, H-2'', H-6''), 7.05-7.07 (2H, d, H-3''', H-5'''), 7.08-7.10 (2H, d, H-2''', H-6'''), 7.15-7.18 (2H, d, H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 12.90 (C-6, CH_3), 23.27 (C-5, CH_2), 28.90 (C-1, CH_2), 33.32 (C-2, CH_2), 42.17 (C-7, CH_2), 54.76 (OCH_3), 54.82 (OCH_3), 113.07 (C-3''', C-5'''), 113.55 (C-3'', C-5''), 1144.51 (C-3', C-5'), 128.72 (C-2', C-6'), 129.0 (C-2'', C-6''), 130.05 (C-2''', C-6'''), 132.70 (C-4, C=C), 133.99 (C-3, C=C), 134.70 (C-1''), 135.57 (C-1'), 135.90 (C-1'''), 153.91 (C-4'), 157.10 (C-4'''), 158.99 (C-4'').

5.29.3. Synthesis of 1-[2-(4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI133)

As in the general method 5.7, 4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI119) (0.0006M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.00072M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0012M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 19:1). The product was obtained as a light brown oil (34%), (R_f 0.1 dichloromethane:methanol 19:1). IR ν_{max} (film) 2930 (CHs), 1609 (C=C), 1510 (NCH_2), 1463 (CH_2), 1244 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.98-0.99 (3H, t, J=7.5Hz, H-6, CH_3), 1.87-1.89 (4H, s, H-2'''', H-3''''), 2.47-2.49 (2H, q, J=7.52Hz, H-5, CH_2), 2.54-2.61 (4H, m, H-1, H-2, $(CH_2)_2$), 2.72-2.79 (4H, s, H-1'''', H-4''''), 3.02-3.04 (2H, t, J=5.8Hz, H-8), 3.49 (2H, s, H-7, CH_2), 3.75 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 4.2-4.23 (2H, t, J=5.8Hz, H-9), 6.74-6.78 (2H, d, H-3'', H-5''), 6.82-6.89 (2H, d, H-3', H-5'), 6.91-6.97 (4H, m, H-2'', H-6'', H-3''', H-5'''), 7.06-7.10 (2H, d, H-2''', H-6'''), 7.12-7.18 (2H, d, H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 13.22 (C-6, CH_3), 22.98 (C-2'''', C-3'''', $(CH_2)_2$), 23.79 (C-5, CH_2), 33.15 (C-1, CH_2), 35.70 (C-2, CH_2), 36.71 (C-7, CH_2), 53.73 (C-1'''', C-4'''', $(CH_2)_2$), 54.16 (C-8, CH_2), 54.70 (OCH_3), 54.82 (OCH_3), 65.95 (C-9, CH_2), 109.47 (C-3''), 113.07 (C-3''', C-5'''), 113.58 (C-3', C-5'), 119.70 (C-5''), 126.08 (C-4''), 128.23 (C-6''), 128.66 (C-2', C-6'), 129.46 (C-2''', C-6'''), 133.90

(C-3, C=C), 134.09 (C-4, C=C), 135.20 (C-1'), 135.30 (C-1''), 135.64 (C-1'''), 156.53 (C-2''), 156.78 (C-4'''), 157.11 (C-4''). HRMS calculated for $C_{33}H_{41}NO_3$ 499.3102 (M^++1), observed 500.3165.

5.29.4. Synthesis of 1-[2-(4-{2-(3-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI134)

The reaction was carried out according to the general method 5.7 by placing 4-{2-(3-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI131) (0.0006M) in acetone:water 19:1 (10ml) with potassium carbonate (0.00072M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0012M). The reaction was refluxed for 5 hours in darkness and then was and extracted using dichloromethane (2 x 50ml) and potassium carbonate solution (50ml). The product was purified by flash column chromatography (eluant: dichloromethane:methanol 19:1). The product was obtained as a light brown oil (43%), (R_f 0.1 dichloromethane:methanol 19:1). IR ν_{max} (film) 2931 (CHs), 1606 (C=C), 1510 (NCH_2), 1490, 1463 (CH_2), 1241 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.84-0.88 (3H, t, J=7.5Hz, H-6, CH_3), 1.81-1.85 (4H, s, H-2'''', H-3''''), 2.04-2.06 (2H, q, J=7.52Hz, H-5, CH_2), 2.46-2.49 (2H, t, J=5.44Hz, H-1, CH_2), 2.53-2.57 (2H, t, J=5.44Hz, H-2, CH_2), 2.63-2.68 (4H, s, H-1'''', H-4''''), 2.91-2.97 (2H, t, J=6.16Hz, H-8), 3.45 (2H, s, H-7, CH_2), 3.77 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.1-4.17 (2H, t, J=6.48Hz, H-9), 6.79-6.81 (2H, d, H-2'', H-5''), 6.76-6.78 (4H, d, H-3', H-5', H-3''', H-5'''), 6.97-6.99 (2H, d, H-4'', H-6''), 7.09-7.13 (4H, m, H-2''', H-6''', H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 12.90 (C-6, CH_3), 23.05 (C-5, CH_2), 25.40 (C-2'''', C-3'''', $(CH_2)_2$), 29.25 (C-1, CH_2), 35.90 (C-2, CH_2), 37.59 (C-7, CH_2), 54.21 (OCH_3), 54.25 (OCH_3), 54.64 (C-8, CH_2), 54.77 (C-1'''', C-4'''', $(CH_2)_2$), 66.38 (C-9, CH_2), 110.27 (C-2''), 113.12 (C-3''', C-5'''), 113.61 (C-3', C-5'), 113.90 (C-4''), 120.68 (C-6''), 128.50 (C-5''), 128.74 (C-2', C-6'), 129.39 (C-2''', C-6'''), 133.87 (C-4, C=C), 133.92 (C-3, C=C), 135.0 (C-1'), 135.20 (C-1'''), 135.56 (C-1''), 156.70 (C-4'), 157.20 (C-3''), 159.18 (C-4'''). HRMS calculated for $C_{33}H_{41}NO_3$ 499.31 (M^++1), observed 500.3165.

5.29.5. Synthesis of 1-[2-(4-{2-(4-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI135)

As in the general method 5.7, 4-{2-(4-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI132) (0.0006M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.00072M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0012M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 19:1). The product was obtained as a light brown oil (22%), (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 2932 (CHs), 1608 (C=C), 1511 (NCH_2), 1462 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.83-0.87 (3H, t, J=7.5Hz, H-6, CH_3), 1.81-1.85 (4H, s, H-2'''', H-3''''), 2.47-2.51 (2H, q, J=7.52Hz, H-5, CH_2), 2.53-2.56 (2H, t, J=7.84Hz, H-1, CH_2), 2.59-2.61 (2H, t, J=7.52Hz, H-2, CH_2), 2.69-2.73 (4H, s, H-1'''', H-4''''), 2.95-3.00 (2H, t, J=5.8Hz, H-8), 3.42 (2H, s, H-7, CH_2), 3.79 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.13-4.19 (2H, t, J=5.8Hz, H-9), 6.77-6.79 (2H, d, H-3'', H-5''), 6.8-6.86 (H-3', H-5'), 6.91-6.93 (2H, d, H-3''', H-5'''), 6.98-7.01 (2H, d, H-2'', H-6''), 7.06-7.13 (4H, m, H-2''', H-6''', H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 13.0 (C-6, CH_3), 23.25 (C-5, CH_2), 25.40 (C-2'''', C-3'''', $(CH_2)_2$), 29.26 (C-1, CH_2), 34.40 (C-2, CH_2), 36.81 (C-7, CH_2), 54.17 (OCH_3), 54.57 (OCH_3), 54.75 (C-8, CH_2), 54.80 (C-1'''', C-4'''', $(CH_2)_2$), 66.17 (C-9, CH_2), 113.05 (C-3'', C-5''), 113.25 (C-3''', C-5'''), 113.62 (C-3', C-5'), 128.85 (C-2''', C-6'''), 129.01 (C-2'', C-6''), 129.45 (C-2', C-6'), 133.90 (C-4, C=C), 135.35 (C-3, C=C), 135.70 (C-1'), 135.90 (C-1'''), 136.18 (C-1''), 156.60 (C-4'), 157.20 (C-4'''), 159.31 (C-4''). HRMS calculated for $C_{33}H_{41}NO_3$ 499.31 (M^++1), observed 500.3165.

5.30. McMurry coupling of phenolic 1-phenyl-2-butanones with 1,3-diphenylpropan-1-ones

5.30.1. Attempted synthesis of 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI157)

Following the general method 5.6, titanium tetrachloride (0.0057M) was added dropwise over 5 minutes to zinc dust (0.0113M) in THF (tetrahydrofuran) (80ml). The mixture was refluxed for 2 hours. Then 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) (0.00142M) and 1'-(4-hydroxyphenyl)-butan-2'-one (BRI129) (0.00284M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine

(100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: dichloromethane:methanol 19:1). Self-coupled hydroxy1-phenyl-2-butanone and starting material were recovered from the reaction.

5.30.2. Attempted synthesis of 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI158)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.0057M) was added to zinc dust (0.01132M) in dry THF (tetrahydrofuran) (80ml). The mixture was refluxed at 100°C for 2 hours. Then 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) (0.00142M) and 1'-(2-hydroxyphenyl)-butan-2'-one (BRI127) (0.00283M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml). The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: dichloromethane:methanol 19:1). Only starting material and self-coupled hydroxy1-phenyl-2-butanone were recovered from the reaction.

5.30.3. Attempted synthesis of 1-[2-(4-{2-(3-methoxy-benzyl)-1-[2-(4-hydroxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI149)

Using the general method 5.6, titanium tetrachloride (0.0057M) was added dropwise over 5 minutes to zinc dust (0.0113M) in dry THF (tetrahydrofuran) (80ml). The mixture was refluxed at 100°C for 2 hours. 3-(4-Methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI145) (0.00142M) and 1'-(3-hydroxyphenyl)-butan-2'-one (BRI128) (0.00284M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: dichloromethane:methanol 19:1). Only self-coupled hydroxy1-phenyl-2-butanone was produced by this reaction.

5.30.4. Attempted synthesis of 4-{2-(4-methoxy-benzyl)-1-[2-(4-hydroxyphenyl)-ethyl]-but-1-enyl}-phenol (BRI156)

As in the general method 5.6, titanium tetrachloride (0.008M) was added to zinc (0.016M) in dry THF (tetrahydrofuran). The mixture was refluxed at 100°C for 2 hours. 1'-(4-hydroxyphenyl)-butan-2'-one (BRI129) (0.004M) and 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (BRI072) (0.002M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated under reduced pressure to afford an oil. Separation of the products was achieved by flash column chromatography (eluant: DCM:hexane 6:4) and it was discovered that only self-coupled hydroxy 1-phenyl-2-butanone was produced by the reaction.

5.31. Synthesis of phenolic analogues via deprotection reaction

5.31.1. Attempted synthesis of 2-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI164)

5.31.1.1. Attempted deprotection of 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI158)

Using the general method 5.4.6, boron trifluoride-dimethyl sulphide (0.0005M) was added dropwise over 30 min to 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI158) (0.0001M) in dichloromethane (10ml). Reaction was only stirring for 12 hours and not overnight as normal. A nitrogen purge was used to remove the reagent and solvent. The remaining residue was washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) while using ethyl acetate (200ml) to extract the product. The ethyl acetate was then dried over Na_2SO_4 and removed under reduced pressure to leave an oil residue, which was chromatographed, on silica gel (eluant: dichloromethane:methanol 19:1). The starting material disintegrated so no product was produced.

5.31.1.2. Attempted deprotection of 1-[2-(4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI133)

Using general method 5.4.6, boron trifluoride-dimethyl sulphide (0.0005M) was added dropwise over 30 min to 1-[2-(4-{2-(2-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI133) (0.0001M) in dichloromethane (50ml). Stirring was continued for a further 10 hours at room temperature. The solvent was removed using a nitrogen purge and the remaining residue was dissolved in ethyl acetate (200ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield once again disintegrated starting material and no product.

5.31.2. Attempted synthesis of 1-[2-(4-{2-(3-hydroxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI152)

As in the general method 5.4.6, boron trifluoride-dimethyl sulphide (0.0015M) (15 equivalents) was added dropwise over 30 min to 1-[2-(4-{2-(3-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI134) (0.0001M) in dichloromethane (10ml). Stirring was continued overnight at room temperature. The solvent was removed using a nitrogen purge and the remaining residue was dissolved in ethyl acetate (200ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) and was dried (sodium sulphate) over Na_2SO_4. The solvent was removed under reduced pressure and residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1).

5.31.3. Synthesis of 4-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI184)

5.31.3.1. Attempted deprotection of 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI157)

Using the general method 5.4.6, 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI157) (0.0001M) was stirred in dichloromethane (10ml) and to this boron trifluoride-dimethyl sulphide (0.001M) (10 equivalents) was added dropwise over 20 min. Stirring was stirred overnight at room temperature. The solvent was removed using a nitrogen purge and the remaining residue was dissolved in ethyl acetate (200ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) and was dried (sodium sulphate) over Na_2SO_4. The solvent was removed under reduced pressure and the residue was separated by chromatography on silica gel (eluant: dichloromethane:methanol 19:1). No product was recovered from this reaction. The starting material disintegrated in the reaction.

5.31.3.2. Attempted deprotection of 1-[2-(4-{2-(4-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI135)

The general method 5.4.6 was applied to this reaction. Boron trifluoride-dimethyl sulphide (0.0005M) (5 equivalents) was added dropwise over 30 min to 1-[2-(4-{2-(4-methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (BRI135) (0.0001M) in dichloromethane (10ml). The reaction was then stirred overnight at room temperature. The solvent and reagent were purged by nitrogen and the residue was dissolved in ethyl acetate (200ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) and was dried (sodium sulphate). The solvent was removed under reduced pressure and residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1), but the starting material disintegrated during the reaction process.

5.32. Synthesis of phenolic compounds

5.32.1. Synthesis of 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI157)

The general depivaloylation method 5.7.3.2 was used in this reaction. 2,2-Dimethylpropionic acid 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI148) (0.000664M) was stirred with sodium hydroxide (0.00332M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours. The reaction mixture was acidified with 10% HCl (10ml) and extracted with dichloromethane (4 x 40ml). The combined organic layers were washed with brine (20ml) and dried over sodium sulphate. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield the product as a yellow oil (33%) (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 3403 (OH), 2927 (CHs), 1608 (C=C), 1511 (NCH_2), 1462 (CH_2), 1243 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.92-0.95 (3H, t, J=7.5Hz, H-6, CH_3), 1.90 (4H, s, H-2'''', H-3''''), 2.01-2.06 (2H, q, J=7.84Hz, H-5, CH_2), 2.51-2.55 (2H, t, J=8.26, H-1, CH_2), 2.65-2.70 (2H, t, J=7.52Hz, H-2, CH_2), 2.86 (4H, s, H-1'''', H-4''''), 3.03-3.17 (2H, t, J=5.46Hz, H-8), 3.79 (2H, s, H-7, CH_2), 3.79 (3H, s, OCH_3), 4.2-4.20 (2H, t, J=5.46Hz, H-9), 6.68-6.70 (2H, d, H-3', H-5'), 6.80-6.84 (6H, m, H-3'', H-5'', H-2''', H-6''', H-3''', H-5'''), 7.05-7.08 (4H, m, H-2'', H-6'', H-3', H-5'). ^{13}C NMR $\delta(CDCl_3)$ 12.93 (C-6, CH_3), 22.80 (C-2'''', C-3'''', $(CH_2)_2$), 23.28 (C-5, CH_2), 33.31 (C-1, CH_2), 35.85 (C-2, CH_2), 36.70 (C-7, CH_2), 54.15 (OCH_3), 54.43 (C-1'''', C-4'''', $(CH_2)_2$), 54.80 (C-8, CH_2), 65.33 (C-9, CH_2), 113.46 (C-3''', C-5'''), 113.57 (C-3', C-5'), 114.70 (C-3'', C-5''), 128.90 (C-2', C-6'), 129.10 (C-2'', C-6''), 129.45 (C-2''', C-6'''), 131.75 (C-3, C=C), 133.92 (C-4, C=C), 135.41 (C-1'), 135.60 (C-1''), 136.14 (C-1'''), 153.67 (C-4''), 156.21 (C-4'''), 157.20 (C-4''). HRMS calculated for $C_{32}H_{40}NO_3$ 486.3008 (M^++1), observed 486.3020.

5.32.2. Synthesis of 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI158)

The general depivaloylation method 5.7.3.2 was applied to this reaction. 2,2-Dimethylpropionic acid 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI121) (0.000664M) was stirred with sodium

hydroxide (0.00332M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours. The reaction mixture was acidified with 10% HCl (10ml) and extracted with dichloromethane (4 x 40ml). The combined organic layers were washed with brine (20ml) and dried over sodium sulphate. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield the product as a yellow oil (21%) (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 2926, 2855 (CHs), 1610 (C=C), 1511 (NCH_2), 1459 (CH_2), 1245 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.81-0.84 (3H, t, J=7.5Hz, H-6, CH_3), 1.88 (4H, s, H-2'''', H-3''''), 2.45-2.49 (2H, q, J=7.5Hz, H-5, CH_2), 2.62-2.65 (4H, m, H-1, H-2, $(CH_2)_2$), 2.79 (4H, s, H-1'''', H-4''''), 3.01-3.04 (2H, t, J=5.46Hz, H-8), 3.38 (2H, s, H-7, CH_2), 3.77 (3H, s, OCH_3), 4.16-4.19 (2H, t, J=5.8Hz, H-9), 6.74-6.78 (4H, d, H-3''', H-5''', H-3', H-5'), 6.84-6.86 (2H, d, H-4'', H-6''), 6.9-6.99 (2H, d, H-2''', H-6'''), 7.01-7.03 (2H, d, H-2', H-6'), 7.07-7.09 (2H, d, H-2'', H-5''). ^{13}C NMR $\delta(CDCl_3)$ 13.0 (C-6, CH_3), 22.93 (C-2'''', C-3'''', $(CH_2)_2$), 25.16 (C-5, CH_2), 30.50 (C-1, CH_2), 33.11 (C-2, CH_2), 36.81 (C-7, CH_2), 54.18 (OCH_3), 54.59 (C-8, CH_2), 54.79 (C-1'''', C-4'''', $(CH_2)_2$), 66.81 (C-9, CH_2), 113.15 (C-3''', C-5'''), 113.54 (C-3', C-5'), 114.85 (C-3'', C-4''), 128.70 (C-2', C-6'), 128.85 (C-2''', C-6'''), 129.88 (C-5'', C-6''), 130.40 (C-3, C=C), 133.88 (C-4, C=C), 135.10 (C-1'), 136.30 (C-1''), 136.37 (C-1'''), 153.67 (C-4''), 156.85 (C-4'''), 165.22 (C-4''). HRMS calculated for $C_{32}H_{40}NO_3$ 486.3008 (M^++1), observed 486.3010.

5.32.3. Synthesis of 2,2-dimethylpropionic acid 4-{1-ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl ester (BRI130)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.003M) was added dropwise over 5 minutes to zinc dust (0.006M) in dry THF (tetrahydrofuran) and the mixture was refluxed. Then 3-(4-hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI177) (0.0007M) and 4-pivaloyloxypropiophenone (BRI176) (0.0015M) were dissolved in dry THF (20ml) and added to the reaction and refluxed for a further 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. The product was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 19:1). The desired product was recovered as a brown oil (43%) (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (KBr) 3290 (OH), 2965, 2930 (CHs), 1747 (C=O, OPiv), 1606 (C=C), 1509

(NCH_2), 1460 (CH_2), 1240 (CH_3) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.88-0.91 (3H, t, J=7.28Hz, H-6, CH_3), 1.32 (9H, s, OPiv), 1.88 (4H s, H-1'''', H-4''''), 2.41-2.43 (2H, q, J=7.44Hz, H-5, CH_2), 2.51-2.56 (2H, t, J=7.78Hz, H-1, CH_2), 2.75-2.77 (2H, t, J=7.78Hz, H-2, CH_2), 2.82 (4H, s, H-2'''', H-3''''), 3.0 (2H, s, H-8, CH_2), 4.10 (2H, s, H-9, CH_2), 6.57-6.59 (2H, d, H-3', H-5'), 6.70-6.72 (2H, d, H-3''', H-5'''), 6.77-6.80 (2H, d, H-2', H-6'), 6.83-6.85 (2H, d, H-2''', H-6'''), 6.9-6.93 (2H, d, H-2'', H-6''), 6.96-6.98 (2H, d, H-3'', H-5''). ^{13}C NMR δ($CDCl_3$) 12.64 (C-6, CH_3), 22.87 (C-5, CH_2), 26.95 (($\underline{C}H_3)_3$, OPiv), 29.27 (C-2'''', C-3'''', $(CH_2)_2$), 33.45 ($\underline{C}(CH_3)_3$, OPiv), 36.15 (C-1, CH_2), 38.50 (C-2, CH_2), 54.10 (C-1'''', C-4'''', $(CH_2)_2$), 54.28 (C-7, CH_2), 65.22 (C-8, CH_2), 113.17 (C-3', C-5'), 114.85 (C-3''', C-5'''), 119.97 (C-3'', C-5''), 128.94 (C-2'', C-6''), 130.17 (C-2', C-6'), 130.46 (C-2''', C-6'''), 132.70 (C-3, C=C), 135.73 (C-1'), 136.01 (C-4, C=C), 138.64 (C-1''), 140.14 (C-1'''), 148.21 (C-4'), 154.26 (C-4''), 155.69 (C-4'''), 176.71 (C-4'''), 191.00 (C=O, OPiv). HRMS calculated for $C_{35}H_{44}NO_4$ 542.3270 (M^++1), observed 542.3280.

5.32.4. Synthesis of 2,2-dimethylpropionic acid 4-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI181)

Using the general method 5.6, titanium tetrachloride (0.003M) was added to zinc dust (0.006M) in dry THF (tetrahydrofuran) (100ml). The mixture was refluxed for 2 hours. 3-(4-Hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (BRI177) (0.0074M) and 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (BRI125) (0.0015M) were dissolved in dry THF (20ml) and added to the reaction vessel and left to reflux for 5 hours. The product was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml) by filtering the product though a Buchner funnel. The filtrate was dried (sodium sulphate) and concentrated. Column chromatography was performed to purify the crude product (eluant: dichloromethane:methanol 19:1) and oil was obtained (80%). (R_f 0.27 dichloromethane:methanol 19:1). IR v_{max} (film) ^1H NMR δ($CDCl_3$) 0.92-0.95 (3H, t, J=7.54Hz, H-6, CH_3), 1.36 (9H, s, OPiv), 1.84 (4H, s, H-2'''', H-3''''), 1.98-1.99 (2H, q, J=7.52Hz, H-5, CH_2), 2.50-2.55 (2H, t, J=7.78Hz, H-1, CH_2), 2.68-2.70 (6H, m, H-2, CH_2, H-1'''', H-4''''), 2.95-2.97 (2H, t, J=6.00Hz, H-8), 3.24 (2H, s, H-7, CH_2), 4.1-4.14 (2H, t, J=6.02Hz, H-9), 6.69-6.71 (2H, d, H-3''', H-5'''), 6.80-6.83 (2H, d, H-3'', H-5''), 6.88-6.92 (2H, d, H-3', H-5'), 6.94-6.96 (2H, m, H-2''', H-6'''), 6.98-7.01 (2H, m, H-2'', H-6''), 7.03-7.05 (2H, d, H-2', H-6'). ^{13}C NMR δ($CDCl_3$) 12.88 (C-6, CH_3), 22.94 (C-5,

CH_2), 23.19 (($\underline{C}H_3)_3$, OPiv), 26.70 ($\underline{C}(CH_3)_3$, OPiv), 29.25 (C-1, CH_2), 30.49 (C-2'''', C-3'''', $(CH_2)_2$), 33.26 (C-2, CH_2), 35.64 (C-7, CH_2), 54.22 (C-1'''', C-4'''', $(CH_2)_2$), 54.58 (C-8, CH_2), 66.20 (C-9, CH_2), 113.65 (C-3'', C-5''), 114.78 (C-3''', C-5'''), 120.69 (C-3', C-5'), 128.72 (C-2', C-6'), 129.95 (C-2'', C-6''), 129.44 (C-2''', C-6'''), 131.90 (C-4, C=C), 135.10 (C-1, C=C), 135.90 (C-1'), 137.90 (C-1''), 148.52 (C-1'''), 153.70 (C-4'), 156.53 (C-4''), 157.80 (C-4'''), 203.80 (C=O).

5.32.5. Synthesis of 4-{1-ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (BRI185)

The general depivaloylation method 5.7.3.2 was used in this reaction. 2,2-Dimethylpropionic acid 2,2-dimethylpropionic acid 4-{1-ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl ester (BRI130) (0.00037M) was stirred with sodium hydroxide (0.0018M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours. The reaction mixture was acidified with 10% HCl (10ml) and extracted with dichloromethane (4 x 40ml). The combined organic layers were washed with brine (20ml) and dried over sodium sulphate. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (eluant: acetone) to yield the product (31%) as a yellow oil (R_f 0.2 acetone). IR ν_{max} (film) 3340 (OH), 2928 (CHs), 1609 (C=C), 1511 (NCH_2), 1463 (CH_2), 1241 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.87-0.91 (3H, t, J=7.52Hz, H-6, CH_3), 1.89 (4H, s, H-2'''', H-3''''), 2.07-2.15 (2H, q, J=6.26, H-5, CH_2), 2.54 (2H, m, H-1, CH_2), 2.61 (2H, m, H-2, CH_2), 2.82 (4H, s, H-1'''', H-4''''), 3.03-3.06 (2H, t, J=5.5Hz, H-7), 4.16-4.19 (2H, t, J=5.76Hz, H-8), 6.6-6.55 (2H, d, H-3''', H-5'''), 6.71-6.73 (2H, d, H-2''', H-6'''), 6.77-6.79 (2H, d, H-3'', H-5''), 6.82-6.84 (2H, d, H-3', H-5'), 6.95-6.93 (2H, d, H-2'', H-6''), 7.07-7.09 (2H, d, H-2', H-6'). ^{13}C NMR $\delta(CDCl_3)$ 12.94 (C-6, CH_3), 22.89 (C-5, CH_2), 29.26 (C-2'''', C-3'''', $(CH_2)_2$), 31.29 (C-1, CH_2), 33.64 (C-2, CH_2), 54.03 (C-1'''', C-4'''', $(CH_2)_2$), 54.76 (C-7, CH_2), 65.94 (C-8, CH_2), 113.62 (C-3''', C-5'''), 114.49 (C-3', C-5'), 114.58 (C-3'', C-5''), 128.94 (C-2'', C-6''), 129.31 (C-2', C-6'), 129.37 (C-2''', C-6'''), 131.90 (C-4, C=C), 135.01 (C-3, C=C), 135.90 (C-1'), 137.90 (C-1''), 139.60 (C-4''), 148.52 (C-1'''), 153.70 (C-4'), 156.50 (C-4'''). HRMS calculated for $C_{30}H_{36}NO_3$ 458.2695 (M^++1), observed 458.2690.

5.32.6. Synthesis of 4-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (BRI184)

The general depivaloylation method 5.7.3.2 was applied to this reaction. 4-{2-Ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (BRI181) (0.00037M) was stirred with sodium hydroxide (0.00018M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours. The reaction mixture was acidified with 10% HCl (10ml) and extracted with dichloromethane (4 x 40ml). The DCM layers were combined and washed with brine (20ml) and dried over sodium sulphate. The solvent was then removed *in vacuo* and the residue separated by chromatography on silica gel (eluant: dichloromethane:methanol 19:1) to yield the product as a yellow oil (42%) (R_f 0.1 dichloromethane:methanol 19:1). IR ν_{max} (film) 3308 (OH), 2924, 2854 (CHs), 1609 (C=C), 1511 (NCH_2), 1462 (CH_2), 1240 (CH_3) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.85-0.90 (3H, m, H-6, CH_3), 1.85 (4H, s, H-2'''', H-3''''), 2.34-2.38 (2H, m, H-5, CH_2), 2.49-2.52 (4H, m, H-1, H-2, $CH_2)_2$), 2.72 (4H, s, H-1'''', H-4''''), 2.97-2.98 (2H, m, H-8, CH_2), 3.52 (2H, s, H-7), 4.12-4.15 (2H, m, H-9, CH_2), 6.67-7.18 (13H, m, ArH). ^{13}C NMR $\delta(CDCl_3)$ 13.68 (C-6, CH_3), 22.90 (C-5, CH_2), 29.26 (C-2'''', C-3'''', $(CH_2)_2$), 30.50 (C-1, CH_2), 33.64 (C-2, CH_2), 36.59 (C-7, CH_2), 54.17 (C-1'''', C-4'''', $(CH_2)_2$), 66.59 (C-8, CH_2), 113.56 (C-3''', C-5'''), 114.55 (C-3', C-5'), 114.77 (C-3'', C-5''), 115.96 (C-2'', C-6''), 116.54 (C-2', C-6'), 129.00 (C-2''', C-6'''), 129.14 (C-4, C=C), 129.37 (C-1, C=C), 140.90 (C-1'), 141.90 (C-1''), 143.60 (C-4''), 147.52 (C-1'''), 151.70 (C-4'), 154.50 (C-4'''). HRMS calculated for $C_{31}H_{37}NO_3$ 472.2852 (M^++1), observed 472.2872.

6. References

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Publication

"Synthesis, Structure-Activity Relationships and Antagonistic Effects in Human MCF-7 Breast Cancer Cells of Flexible Estrogen Receptor Modulators", David G. Lloyd, Helena M. Smith, Timothy O'Sullivan, Daniella M. Zisterer, and Mary J. Meegan, Journal of Medicinal Chemistry, in press 2005.

Synthesis, Structure-Activity Relationships and Antagonistic Effects in Human MCF-7 Breast Cancer Cells of Flexible Estrogen Receptor Modulators

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Abstract: Estrogen receptors are therapeutic intervention targets for diseases such as osteoporosis and breast cancer with both tamoxifen and raloxifene established as clinical estrogen receptor antagonists. We report a series of novel selective estrogen receptor modulators (SERMs) whose structures are based on a flexible core scaffold differing from the triphenylethylene of tamoxifen analogues through the insertion of a benzylic methylene group as a flexible spacer between the aryl ring C and the ethylene group. A facile synthesis of the target compounds utilises the titanium tetrachloride/zinc mediated McMurry coupling reaction. Successive introduction onto the parent scaffold of hydroxyl functional groups afforded a series of increased potency ligands for the ER – essentially exploring the predicted *in vivo* metabolic activiation of such aromatic SERM ligands. This second generation compound series demonstrated high antiproliferative potency against the MCF-7 human breast cancer cell line, with low cytotoxicity. High ER binding affinity (IC_{50} 20nM) together with up to 12 fold ERα/β selectivity was also observed. In addition, the compounds displayed antiestrogenic effects at 40nM when evaluated in the Ishikawa cell line with little estrogenic stimulation. Representative ligands were shown to be pro-apoptotic in human MCF-7 cells in a FACS based assay. Comparison of the docked structure obtained for the most active compound with the X-ray crystal structure reported for the complex of ERα and 4-hydroxytamoxifen, predict that these ligands bind in an antiestrogenic manner with some differences being observed in the benzylic Ring C orientation, as expected. This work further demonstrates the tolerance of the estrogen receptor towards flexible modulators.

Key Words: Flexible Estrogen Receptor Modulators, Antiestrogens, Structure Activity Relationships, Drug Design.

INTRODUCTION

Tamoxifen (**1a**) is one of the most widely used drugs for the treatment of hormone sensitive breast cancer in pre and post-menopausal women. It is classified as a selective estrogen receptor modulator (SERM) in that it also provides the beneficial effects of estrogen (i.e. maintains bone density and decreases the levels of low density lipoprotein cholesterol) without the associated risks [1]. The antiestrogenic effects of such compounds are related to their ability to compete with estrogen binding sites in target tissues such as breast tissue with obvious beneficial effect; SERMs also show positive estrogenic effects in other tissues such as bone and there is much speculation as to the possible positive beneficial effects of SERMs on memory and cognition [2-4]. Other SERMs in clinical use include raloxifene (**2**) used in the treatment and prevention of osteoporosis [5] and toremifene (**1b**), useful in breast cancer treatment, Fig. (**1**). Lasofoifene, fulvestrant and EM652 are currently in clinical trials for the treatment of breast cancer, osteoporosois and other indications [6].

Recent protein X-ray structural studies on several estrogen receptor (ER)-ligand complexes such as raloxifene (**2**) and 4-hydroxytamoxifen (**1c**) have demonstrated that SERMs exert their physiological effects through the specific manner of their binding to the Ligand Binding Domain (LBD) of the estrogen receptor [7,8,9]. On binding an antagonist, the resulting orientation of ER helix 12 in the LBD prevents the formation of a critical coactivator recruitment site on the protein surface, and inhibits the downstream transcription regulation and cell proliferation.

A number of diverse disease processes are known to be regulated by the estrogen receptor e.g. estrogen receptor positive breast cancers, osteoporosis, cardiovascular disease, together with control of menopausal symptoms, and there is considerable interest in the discovery and development of new ligands and therapeutic agents which modulate the estrogen receptor and contribute to our understanding of the complexity of the physiological function of the estrogen receptor [10].

Many diverse structures have been investigated for ER modulation properties including steroid, triarylethylenes [11,12] and numerous conformationally constrained analogues based on benzothiophene [13], benzocycloheptene [14], benzopyran [15] and more recently isoquinoline [16] ring systems such as structure (**3**), further demonstrating the eclectic liganding behaviour of the ER. We have previously reported the synthesis and biological activities of a number of both flexible and non-isomerisable estrogen receptor

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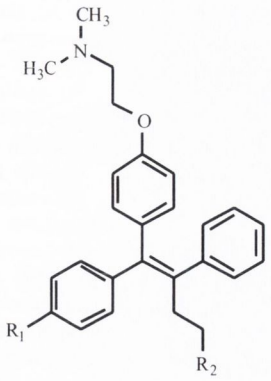
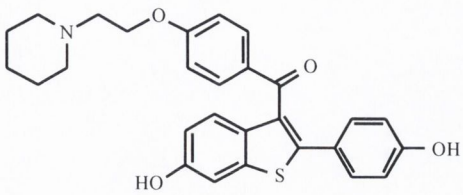
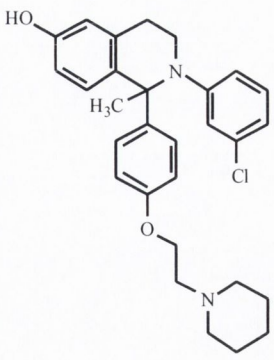


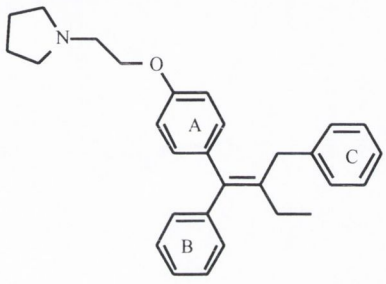
1a R_1=R_2=H, Tamoxifen
1b R_1=H, R_2=Cl, Toremiphene
1c R_1=OH, R_2=H, 4-Hydroxytamoxifen



2 Raloxifene



3



4

Fig. (1). Structures of SERMs and lead compound (4).

modulator analogues of tamoxifen [17,18,19]. Of the flexible ligand structures we have introduced, compound (**4**) was identified as a novel potent lead compound, demonstrating an IC_{50} against MCF-7 cell line of 12.5μM [17]. We now report a second generation compound series based on this flexible parent, as part of our strategy to improve the anti-proliferative and receptor binding activity of triarylethylene-derived SERMs.

This work describes the rational design, synthesis and biochemical studies carried out on a series of compounds which are structurally related to tamoxifen in that they possess three aryl rings, but the scaffold is modified through insertion of an additional methylene group, positioned between the aryl ring C and the vinylic carbon of the more typical tamoxifen type antiestrogens. The assessment of the chemotherapeutic and antiestrogenic potential of the new products prepared is achieved by appropriate human MCF-7 breast cancer cell-based biochemical assays and binding experiments in recombinant human estrogen receptors ERα and ERβ. Through computational modeling with the reported resolved crystal structures of the estrogen receptor α (ERα), the actual and theoretical interactions for the new estrogenic and antiestrogenic materials prepared with the LBD of the ER can be examined. To rationalize the antiestrogenic activity observed for the new ligands prepared and provide an insight into the structural basis for the observed activity, the predicted orientation and interaction of the prepared compounds within the LBD of the human ERα is presented and the various ligand-receptor interactions examined. The ability of the ERα to accommodate structurally diverse ligands is demonstrated together with the ability of the flexible ligands to function as ER modulators.

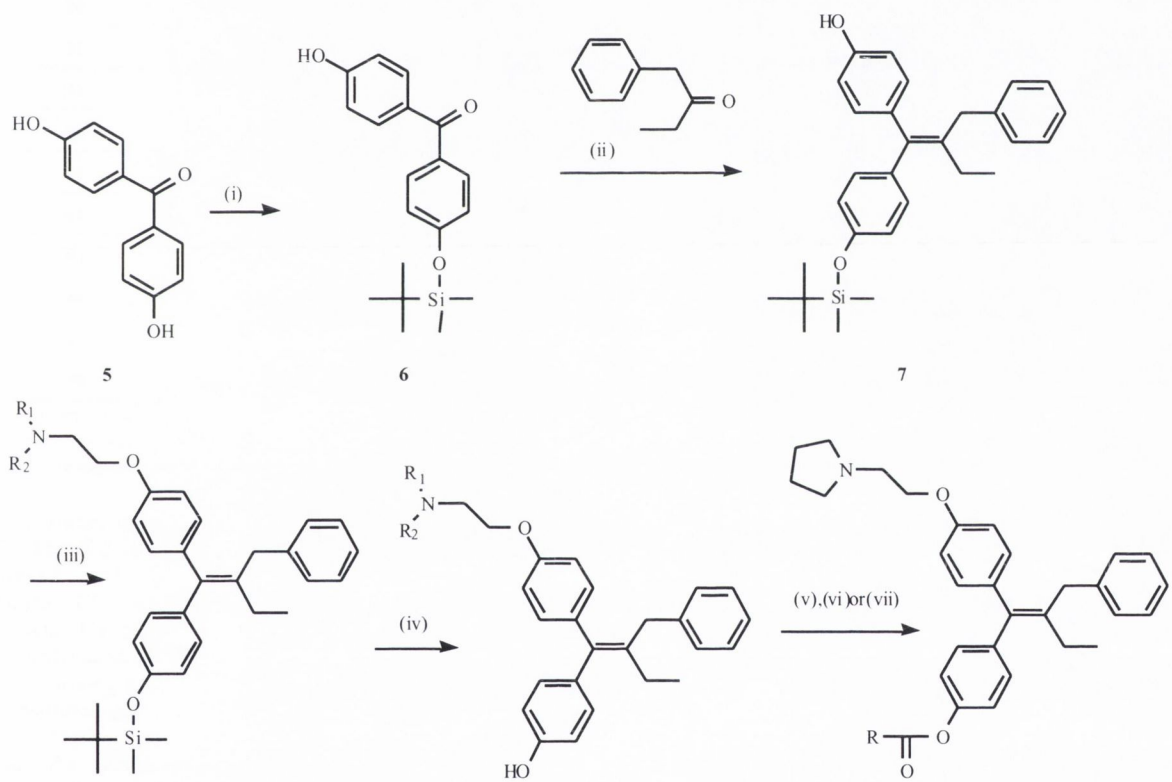
RATIONALE

Given that it is known that aromatic estrogen receptor modulators such as tamoxifen are metabolically activated through *para*-hydroxylation in ring C, and the resultant phenolic compounds exhibit increased potency and binding affinity, a logical starting point for our investigation of second generation derivatives of compound (**4**) was through the introduction of hydroxyl groups on ring B. The biological disadvantages of such *para*-hydroxylated ligands arise through subsequent metabolic glucuronidation and rapid elimination of the metabolized ligand. We purported to explore alternate substitution positions for the hydroxyl substituent and also the introduction of other oxygen containing species on this ring, such as esters and carbamates. To ameliorate the effect of metabolic glucuoronidation, the potential of the pivaloyl ester group as an intrinsically active pro-drug of the hydroxylated ligands was explored. In addition, the efficacy of putative anti-metabolic *para*-halogenated derivatives of the parent compounds was examined.

CHEMISTRY

The initial group of target compounds (**14-23**) containing a free phenolic group in ring B, was prepared by means of

the titanium tetrachloride/zinc mediated McMurry type reductive coupling procedure [20,21,22] as outlined in Scheme **1**. The required benzophenone (**6**) was obtained by monoprotection of the 4,4'-dihydroxybenzophenone (**5**) as the *tert*-butyldimethylsilyl ether. The use of the TBDMS protecting group was found to be most efficient for the subsequent chemical transformations and was used routinely for the preparation of this series of products.(Scheme **1**) Reductive coupling of benzophenone (**6**) with phenylbutanone afforded the alkene product (**7**) which appeared to be formed as the major *E* isomer. It is known that in the McMurry coupling reaction, the use of phenolic benzophenones favours the formation of the product having the *trans* arrangement of the phenolic aryl group relative to the ethyl vinylic substituent [23,24]. The mechanism of the (*E*)-stereocontrol in this McMurry olefination reaction has not been fully explained. However, it is possible that there is a preferred interaction between the phenolic substituted ring of the ketone **6** and the phenyl ring of the phenylbutanone in the proposed titanium-bound pinacol intermediate initially formed in the reaction, leading to predominant (*E*) products [22]. The basic side chains were introduced by Mitsunobu reaction using the appropriate amino alcohol, triphenylphosphine and diisopropylazodicarboxylate (DIAD) to afford the products (**8-13**) which appeared as single *E* isomers in the case of compounds (**10**), (**11**) and (**12**), as indicated from the ^{1}H NMR spectra where the benzylic CH_2 group protons appear as a single signal in the region δ3.50-3.59, while alkene products (**8**), (**9**) and (**13**) were obtained as *E/Z* mixtures displaying two signals for the benzylic methylene group protons, (major isomers only shown in Scheme **1**). Confirmation of the predominant isomer identity was obtained by examination of the NOE spectra. Removal of the TBDMS protecting group in the compounds with tetrabutylammonium fluoride(TBAF) afforded the required products cleanly and in good yield. The products were isolated as *E/Z* mixtures as indicated in Table **1** due to the partial conversion of initially formed phenolic *Z* isomer to



8 R_1=R_2=CH_3
9 R_1=R_2=CH_2CH_3
10 R_1=R_2= -$CH_2CH_2CH_2CH_2$-
11 R_1=R_2=-$CH_2CH_2CH_2CH_2CH_2$-
12 R_1=R_2= -$CH_2CH_2OCH_2CH_2$-
13 R_1=R_2= -CO-$CH_2CH_2CH_2$-

14 R_1=R_2=CH_3
15 R_1=R_2=CH_2CH_3
16 R_1=R_2=-$CH_2CH_2CH_2CH_2$-
17 R_1=R_2= -$CH_2CH_2CH_2CH_2CH_2$-
18 R_1=R_2=-$CH_2CH_2OCH_2CH_2$-
19 R_1=R_2= -CO-$CH_2CH_2CH_2$-

20 R=CH_3 **21** R=$N(CH_3)_2$ **22** R=NH-CH_2CH_3 **23** R=NH-C_6H_5

Scheme reagents and conditions: (i) TBDMSCl, imidazole, DMF (ii) $TiCl_4$, Zn, THF, reflux (iii) $R_1R_2NCH_2CH_2Cl$, K_2CO_3, Acetone/H_2O or $R_1R_2NCH_2CH_2OH$, DIAD, PPh_3, CH_2Cl_2 (iv) $(n$Bu$)_4$NF, THF (v) $(CH_3CO)_2O$, pyridine (vi) $(CH_3)_2NCOCl$, Et_3N (vii) RNCO, CH_2Cl_2

Scheme 1.

Table 1. Yield an Isomeric Ratio Data for Compounds 14-23,30-32,40-44,49,50

Compound	Yield (%)	Isomer ratio[a]
14	81	65:35
15	76	68:32
16	33	64:36
17	72	67:33
18	91	65:35
19	62	64:36
20	76	61:39
21	39	62:38
22	54	65:35
23	7	76:24
30	44	67:33
31	36	80:20
32	36	50:50
40	19	58:42
41	87	66:34
42	40	66:34
43	75	75:25
44	30	86:14
49	80	>100:1
50	95	75:25

[a] Ratio is determined as major:minor isomer present .

Z isomer for compounds **14-19**, **30-32**,**43**, **44**

E isomer for compounds **20-23**, **40-42**, **49**, **50**

the *E* isomer once the protecting group has been removed [25]. The *E/Z* isomeric ratio for the products (**14**)-(**19**) was assigned on the basis of the relative peak heights of the benzylic CH_2 group in the ^1H NMR spectrum of the products. The major/minor *Z/E* isomer composition can be assigned based on the relative positions of the aryl proton signals arising from the A_2B_2 *para* substituted system of the 4-substituted phenyl ring and/or the relative chemical shifts of the OCH_2 signal assigned to the protons in the basic side chain. As previously reported, compounds were assayed biologically as *E/Z* mixtures as indicated [29].

A number of structural modifications of the initial target phenol product were also investigated. Diethyl carbamate ester (**21**) was obtained by reaction of the phenol (**16**) with dimethylcarbamoyl chloride. The ethyl carbamate (**22**) and phenyl carbamate (**23**) were prepared by treatment with ethyl isocyanate and phenyl isocyanate respectively.

The second group of products investigated were the methoxy ether and pivaloyloxy ester modifications of the phenolic compound (**16**) above and the synthetic route is illustrated in Scheme **2**. The required benzophenones (**24**)-(**26**), (**33**)-(**36**) were prepared by modifications of known literature procedures [23,24,40,41,42,43]. 4-Hydroxy-2'-pivaloyloxybenzophenone (**34**) was obtained by reacting 2-hydroxy-4'-methoxybenzophenone with pivaloyl chloride and sodium hydroxide to form the ester (**33**) which was then treated with boron trifluoride-dimethyl sulphide to afford the demethylated product ester (**34**) in 65% yield, (Scheme **3**). Reductive coupling of the phenolic benzophenones (**24**)-(**26**), (**33**)-(**36**) with 1-phenylbutanone resulted in the formation of the alkene products (**27**)-(**29**), (**37**)-(**39**) in good yield. Alkylation of the phenols was achieved by treatment with 1-(2-chloroethyl)pyrrolidine in aqueous acetone to give the required products (**30**)-(**32**), (**40**)-(**42**); major isomers only are shown in Scheme **2**. Conversion of the methoxy ethers (**31**) and (**32**) to the corresponding phenolic products (**44**) and (**16**) was achieved by demethylation with boron trifluoride-dimethyl sulphide, while the pivaloyl ester (**40**) was hydrolysed to afford the phenol (**43**).

The preparation of the related fluoro and bromo substituted alkenes (**47**), (**48**) is also illustrated in Scheme **2** and consists of the McMurry type reductive coupling of the appropriate benzophenones (**45**) and (**46**) with 1-phenylbutanone. Alkylation of the resulting phenols (**47**) and (**48**) was achieved using 2-chloroethylpyrrolidine hydrochloride in potassium carbonate or by the Mitsunobu procedure using the appropriate amino alcohol, PPh_3 and DEAD to give the required alkenes (**49**) and (**50**) respectively. All products were isolated and tested as oils containing the free base.

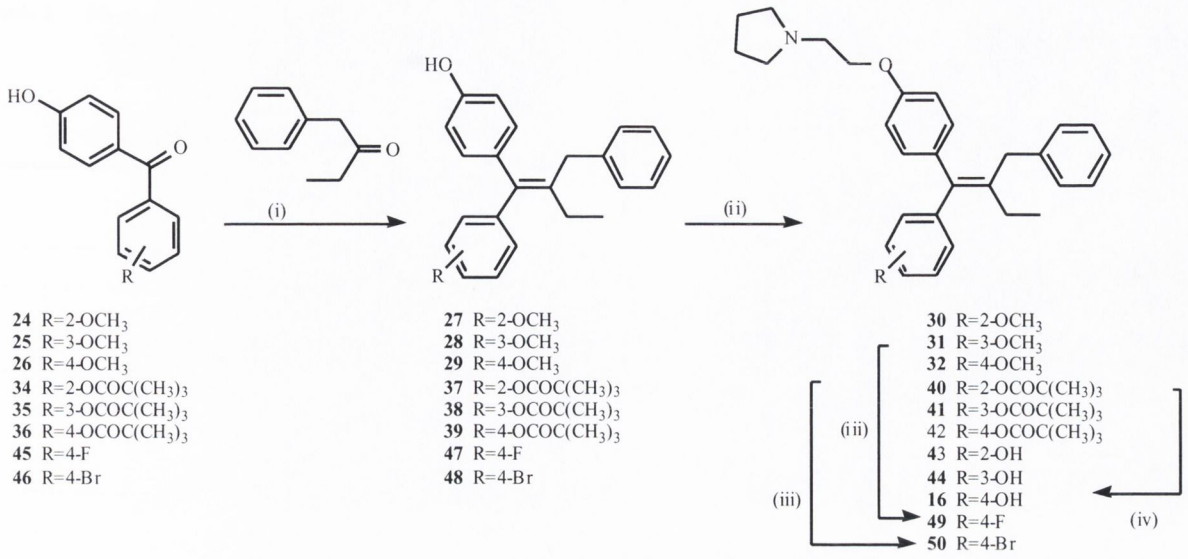
BIOCHEMISTRY

Inhibition of Proliferation – Structure-Activity Relationships

The ability of the compounds prepared above to inhibit the proliferation of the human MCF-7 breast cancer was investigated using the standard MTT assay in order to initially assess their chemotherapeutic potential. The IC_{50} values for the products are presented in Table **2** using tamoxifen(**1a**) as a reference standard. The data can be considered by grouping into the following five series for the exploration of structure-activity relationships:

1. Ring B hydroxylated compounds (**14**)-(**19**), (**43**), (**44**)
2. Ring B methoxy ethers (**30**), (**31**), (**32**)
3. Ring B pivalate and acetate esters (**20**), (**30**)-(**32**), (**40**)-(**42**)
4. Ring B carbamates (**21**)-(**23**)
5. Ring B halogenated compounds (**49**), (**50**)

The ring B hydroxylated products (compounds (**14**)-(**19**)) all contain a phenolic group in the *para* position of the aromatic ring B, while the basic side chain includes a number if structural variations (e.g. N,N-dimethyl, N,N-diethyl, pyrrolidinyl, piperidinyl, morpholinyl, and pyrrolidin-2-onyl) with IC_{50} activities ranging from 27.1 to 0.174μM. Since the pyrrolidine base is a common feature of many SERMs, [13,28] we also wished to investigate the effect on activity of the replacement of the pyrrolidine in **4** with structurally diverse basic side chain components. By



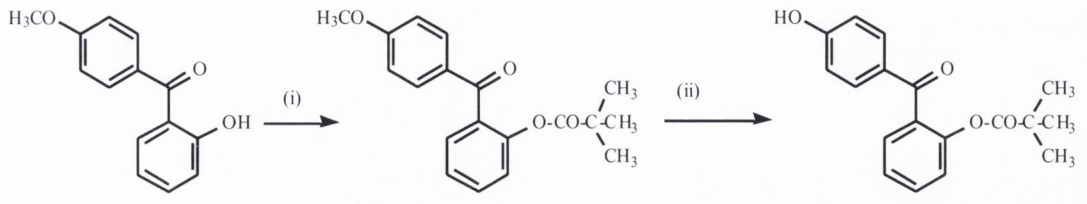
Scheme reagents and conditions: (i) $TiCl_4$, Zn, THF, reflux (ii) $R_1R_2NCH_2CH_2Cl$, K_2CO_3, Acetone/H_2O or $R_1R_2NCH_2CH_2OH$, DIAD, PPh_3, CH_2Cl_2 (iii) $BF_3.(CH_3)_2S$, CH_2Cl_2 (iv) NaOH, EtOH

Scheme 2.

comparison to the activity of the lead compound (**4**) (IC_{50}12.5μM) it can be seen that the introduction of a *para* positioned hydroxyl group results in a marked improvement in antiproliferative activity, with the most active compounds (**14**), (**15**), (**17**) and (**19**) having IC_{50} values in the low micromolar and submicromolar ranges. This is of course consistent with experimental observations of increased activity in SERM ligands on metabolic activation through *para*-hydroxylation. Varying the hydroxyl substituent location in ring B leads to a slight decrease in ligand activity. When the phenolic group is positioned at the *meta* position as for compound (**44**), (which could be considered a flexible analogue of known SERM droloxifene [2]) a reduced IC_{50} of 1.3μM is observed, while there is further loss in activity for the *ortho* positioned hydroxylated compound (**43**). It is also possible that this structural class may differently recruit cofactors which may also influence the structural activity of the compounds [48] and explain the differences in activity when compared to the traditional triphenylethylenes.

The ring B methoxy ethers (**30**), (**31**), (**32**) exhibit a interesting reversal in the activity pattern seen for the corresponding phenolic series with the most active compound (**31**) being the *meta* substituted product(IC_{50} 7.56μM) while the least active was the *para* substituted compound (**32**), IC_{50} 35.9μM. Examination of the activities for the *ortho*- and *meta*-pivaloyloxy esters (**40**), (**41**) revealed that esterification of the phenol resulted in decrease in antiproliferative activity of at least 10-fold. However the *para* substituted pivaloyloxy ester (**42**) displays increased antiproliferative activity relative to the parent lead structure with IC_{50} 5.90μM, and suggests the potential utility of this group as a pro-drug of **16** with intrinsic antiestrogenic potency. It is known that pivalic esters are very slowly hydrolysed *in vivo* liberating the corresponding phenols [26] The superior suitability of the *para*-pivaoloyloxy group for a pro-drug role is highlighted when compared to the corresponding acetate ester (**20**), IC_{50} 32.7μM or methyl ether (**32**), IC_{50} 35.9μM.

Ring B dimethyl, ethyl and phenylcarbamates (**21**), (**22**), (**23**) all displayed improved activities over the corresponding methyl ethers and *ortho* and *meta* pivaloyloxy esters with IC_{50} values in the range 3.9-6.5μM. These products might



33 **34**

Scheme reagents and conditions: $(CH_3)_3C$-COCl, acetone, KOH (ii) $BF_3.(CH_3)_2S$, CH_2Cl_2

Scheme 3.

Table 2. Mean IC_{50} values of Compounds 14-23,30-32,40-44, 49-58 for Antiprolifreative Effects on Human MCF-7 Breast Cancer Cell Line[a,b]

Compound	$IC_{50}(\mu M)$
14	1.62
15	15.0
16	0.174
17	1.57
18	27.1
19	2.14
20	32.7
21	4.38
22	6.52
23	3.90
30	12.1
31	7.56
32	35.9
40	29.0
41	23.7
42	5.90
43	16.5
44	1.30
49	20.5
50	14.0
1a	3.75
4	12.5

[a]The values are an average of at least three experiments.

[b]The value recorded for tamoxifen(**1a**) in this work is in good agreement with IC_{50} values in other works using the MTT assay in human MCF-7 cells[27].

not be expected to undergo facile hydrolysis to release the parent phenol. Finally, Ring B halogenated products compounds (**49**) and (**50**) were examined to study the effect on activity of the introduction of the halogens, bromide and fluoride on the Ring B of the lead compound structure (**4**). The halo substituted products (**49**) and (**50**), which can be considered as flexible analogues of idoxifene [2] displayed similar biological activity to the parent lead compound with IC_{50} values in the range 14.0-20.5μM, however the metabolism of these compounds would not include initial *para*-hydroxylation of Ring B, and so an increased biological half-life of activity would be expected for such ligands.

Cytotoxicity Profile

All compounds assayed for antiproliferative effects were also tested to determine their cytotoxicity using the LDH assay as presented in the Experimental section. With a view to therapeutic potential, the potency of a new investigational compound in the context of its inhibition of cellular proliferation should not be due to its ability to promote cellular necrosis. The primary objective in the development of these new flexible ligands was to make available compounds which could achieve cytostasis in therapeutic use. The clinically useful antiestrogenic drug tamoxifen is known to produce its antiproliferative effects due to a combination of cellular actions including its cytotoxic effects and also its ability to induce apoptosis in cell lines [30,31].

The flexible compounds demonstrated low cytotoxicity in the LDH assay as well as their antiproliferative effects. Many of the compounds displayed remarkably low cytotoxicity profiles, suggesting that their mode of action to be truly cytostatic rather than cytotoxic. For compound (**16**) the cytotoxicity observed remains almost constant across a full range of concentrations examined (1mM-1nM). For compounds (**14**), (**17**) and (**19**), the observed cytotoxicity at 10μM concentration of the test compounds was 0% thus indicating that the activity of the compounds is not due to cytotoxic effects at the concentrations studied. For tamoxifen a sharp increase in cytotoxicity is observed at concentrations above 20mM, with a value of 24% at 10μM [17].

Estrogen Receptor Binding Studies

A series of receptor binding studies were carried out for selected compounds prepared representing the most potent antiproliferative products, (**14**), (**16**), (**17**), (**19**), (**21**), (**31**) and (**42**). The procedure was based on a competitive binding assay using a fluorescence polarization protocol (from Invitrogen) in which the displacement of fluorescently labeled estradiol (fluoromone®) from human recombinant full length receptor proteins ERα and ERβ was measured. ERα and ERβ were expressed from baculovirus-infected insect cells and purchased from Invitrogen [32,33]. In this assay, the polarization values decrease with the addition of competitors. The obtained sigmoidal inhibition curves can be used to obtain the 50% inhibition (IC_{50}). Ligand binding affinity of the specified compounds for ERα and ERβ is determined as the IC_{50} value and is illustrated in Table **3**. Each of the compounds examined demonstrated good binding affinity for the ER, superior to tamoxifen(**1a**), indicating competitive inhibition. Compounds (**14**), (**16**), (**17**), (**19**) and (**21**) all demonstrate IC_{50} values for ERα of less than 120 nM, with compound (**16**) being the most potent, having an IC_{50} value of 20nM, which is more potent than that obtained for 4-hydroxytamoxifen(**1c**) in this assay. For many SERMs, the base change does not dramatically effect the binding affinity, but has a more significant effect on functional activity. When the binding data is correlated with the antiproliferative activity, (Table **2**), it is apparent that the most active compound (**16**) in the antiproliferative assay, also is the compound with the highest binding affinity for the ERα. The increase in lipophilicity for compound (**16**) (when compared to 4-hydroxytamoxifen) due to the introduction of the benzylic methylene group may facilitate increased binding to the ER. Compounds (**31**) and (**42**), the least active of the compounds selected for further investigation, also demonstrate the lowest binding affinity

Table 3. Estrogen Receptor Binding Data For Compounds 12, 16, 17, 19, 21, 31, 42

Compound Number	ER Binding assay IC_{50}[a] (μM)		ERα : β Ligand Binding ratio
	α	β	
14	0.107	0.891	8.33:1
16	0.020	0.160	8:1
17	0.119	1.014	8.52:1
19	0.078	0.464	5.95:1
21	0.118	1.490	12.63:1
31	1.735	3.995	2.30:1
42	0.814	0.168	1:4.85
1c	0.040	0.024	1:2.66

[b]Values are an average of at least three experiments for ERα and two experiments for ERβ with standard errors below 15%

for the ERα, with IC_{50} values of 1.735μM and 0.814μM respectively. Affinity for the ERβ in the series was found to be in the region 0.16-3.99μM, with the highest value observed for compound (**16**), containing the *para* phenolic substituent in the Ring B and the pyrrolidine basic side chain substituent on Ring A, together with the *para*-phenolic Ring B, (IC_{50} 160nM). The *para*- pivaloyloxy substituted Ring B compound (**42**) is also shown to have a similar binding affinity of IC_{50} 168nM for ERβ. The ERα/β ligand binding ratio for the compounds assayed is illustrated in Table **3** and shows that with the exception of compound (**42**), there is a degree of selectivity for the ERα observed for these flexible ligands which for many of the compounds is considerable greater that that observed for 4-hydroxytamoxifen. The highest ERα/β selectivity of 12.6:1 is observed for compound (**21**), with compounds (**14**),(**16**) and (**17**) displaying ER α/β selectivities of 8.3:1, 8.1:1 and 8.52:1 respectively. These ratio values differ from the ER α/β selectivity of 1:1.67 obtained for 4-hydroxytamoxifen (**1c**) in this assay, and are comparable to reported ERα/β selectivity ratio of 8-20:1 for various phenanthrene [34] and pyrazole [35] ER antagonist ligands. For *para*-pivaloyloxy substituted Ring B compound (**42**), a reversal in selectivity was observed with ratio of ER α/β obtained as 1:5. Compound (**42**) is unique among the flexible ligands investigated in this study in demonstrating a five fold selectivity for the ERβ.

There is therefore a clear relationship between binding affinity and the antiproliferative potency observed for the series of compounds prepared which may be of use in the development of related flexible ER modulators.

Estrogenic Stimulation and Apoptotic Potential

In any study of potential estrogen receptor modulators it is essential that there is no associated adverse estrogenic effect observed in other ER-expressing tissues. The potential uterine estrogen stimulating properties of two representative compounds from the parent series, (**14**) and (**17**) was determined using a simple and sensitive estrogen bioassay which is based on the stimulation of alkaline phosphatase (AlkP) in an Ishikawa human endometrial adenocarcinoma cell line [36]. Ishikawa cells are extremely sensitive to estrogen as demonstrated by estrogen stimulation of AlkP at concentrations as low as 10^{-12}M. Since the stimulatory effect of estradiol on AlkP is dramatically inhibited by antiestrogens, we can determine the modulatory activity of the compounds by measuring their ability to antagonize the stimulatory effect of estradiol 10^{-9}M in a dose dependent manner. Compound (**14**) antagonised the estradiol effect with an IC_{50} of 53nM, while (**17**) exhibited an IC_{50} of 40nM, compared to an IC_{50} of 170nM for tamoxifen(**1a**). The estrogenic effect of the compounds can be determined by monitoring the estrogen stimulatory effect on the Ishikawa cell of the compounds administered in the absence of estradiol. Compound (**14**) exhibited 6% estrogenic stimulation – comparable to the 4% level observed for tamoxifen(**1a**), while compound (**17**) exhibited a zero level of observed estrogenic stimulation. These data clearly highlight the utility of this compound series as candidate SERM ligands.

In a further mechanistic investigation, compounds (**14**) and (**17**) were assayed for the induction of apoptosis in MCF-7 cells by measurement of poly-adenosine diphosphate ribose polymerase (PARP) cleavage. The DNA repair enzyme PARP is a substrate for the apoptosis effector caspase 9 and its functions include the dissolution of macromolecular synthesis and cellular repair mechanisms. PARP cleavage confirms that apoptosis has been induced in the cell line *via* caspase activation and tamoxifen has been previously shown to induce apoptosis in this manner [9,31,37,38]. Western Blot analysis may therefore be used to detect PARP cleavage as seen by the disappearance of the 116kDa PARP protein band and the concurrent appearance of the 87kDa cleavage product. The compounds induced apoptosis at 50μM in MCF-7 cells of the order of 16%, 11%, 15%, 13% and 37% (subG1 Phase) for compounds (**14**), (**16**), (**17**), (**19**) and (**21**) respectively after 24 hours, while tamoxifen was active at 20.4%. These values are comparable to the values determined for other antiestrogenic compounds and cytotoxic products [37] and demonstrate the antiproliferative mechanism of action of the compound series is associated with the cell cycle effects.

COMPUTATIONAL MODELLING

To gain insight into the mode of binding of the second generation compounds a brief computational docking study was undertaken using compound (**16**) which is the most potent ligand of the series prepared. The crystal structure used in the docking studies was that obtained from the cocrystallisation of ERα with 4-hydroxytamoxifen(**1c**) (OHT) as found in the PDB database (reference code 3ERT) [39]. After removal of 4-hydroxytamoxifen from the ligand binding site, FlexX was used to provide thirty putative docking poses for compound (**16**), from which the top ranked binding mode scored by the DrugScore function was selected. The docked geometry for compound (**16**) in comparison to the experimental binding mode for OHT(**1c**)

is illustrated in Fig. (**2**). It is evident that incorporation of the additional methylene spacing group, while facilitating extra flexibility in the structure, also allows the compounds to adopt the required arrangement for liganding in an established antiestrogenic mode. The overlap of the docked geometries for (**16**) and OHT is high, differing only in the areas of the structure where the benzylic methylene is introduced, and in the orientation of the basic side chain, which in compound (**16**) is better oriented for hydrogen bonding interactions with Asp 351 in the active site than the OHT basic nitrogen orientation.

LIGAND PROTEIN CONTACTS

To quantify the relative interactions predicted for the ligand (**16**) in comparison to the experimental results observed for 4-hydroxytamoxifen and raloxifene, a simple ligand-protein contact (LPC) analysis was carried out, referring to the following specific residues: Glu 353 and Arg 394 (which are known to be crucial in the binding of Ring B of the ligands to the active site), His 524, (known to be an important estrogenic residue in the binding of diethylstilbestrol and estradiol) and Asp 351which is well recognized as an important antiestrogenic residue associated with the binding of the basic side chain nitrogen.

Table **4** illustrates the key predicted LPC data for the compound (**16**) with the specific residues chosen together with the residue contacts calculated from the existing crystal structures of 4-hydroxytamoxifen and raloxifene. It can be seen that the flexible ligand series as exemplified by compound (**16**) are predicted be accommodated in the receptor LBD in a very similar orientation to the known antiestrogenic compounds, relative to the residues Asp 351, Arg 394 and Glu 353. The primary divergence from receptor interactions associated with known ER ligands occurs in the region of His 524, where the flexibility of benzylic portion of compound (**16**) orients ring C of the structure differently. Interaction with His 524 is not essential for antiproliferative potency, but may increase receptor binding affinity, and the potential interactions of our flexible scaffold with this receptor will be investigated in future work.

CONCLUSIONS

In summary, we have investigated the design and synthesis of substituted 2-benzyl-1-phenyl-1-[4-(pyrrolidinyl-ethoxy)phenyl]but-2-ene type structures as estrogen receptor ligands. These are conformationally flexible compounds which differ from the structure of the traditional triphenyl-ethylene structure of the known antiestrogen tamoxifen by the inclusion of a benzylic methylene spacing group at the vinylic system. The compounds demonstrate good antiestrogenic potency by inhibition of proliferation of MCF-7 breast cancer cell line. From the cytotoxic assessment, the compounds demonstrate low cytotoxicity, indicating their mode of action to be cytostatic. High ER binding affinity together with 12 fold ERα/β selectivity was also observed for examples of compounds in the series. In addition, the compounds displayed an antiestrogenic effect at 40nM when evaluated in the Ishikawa cell line with little estrogenic

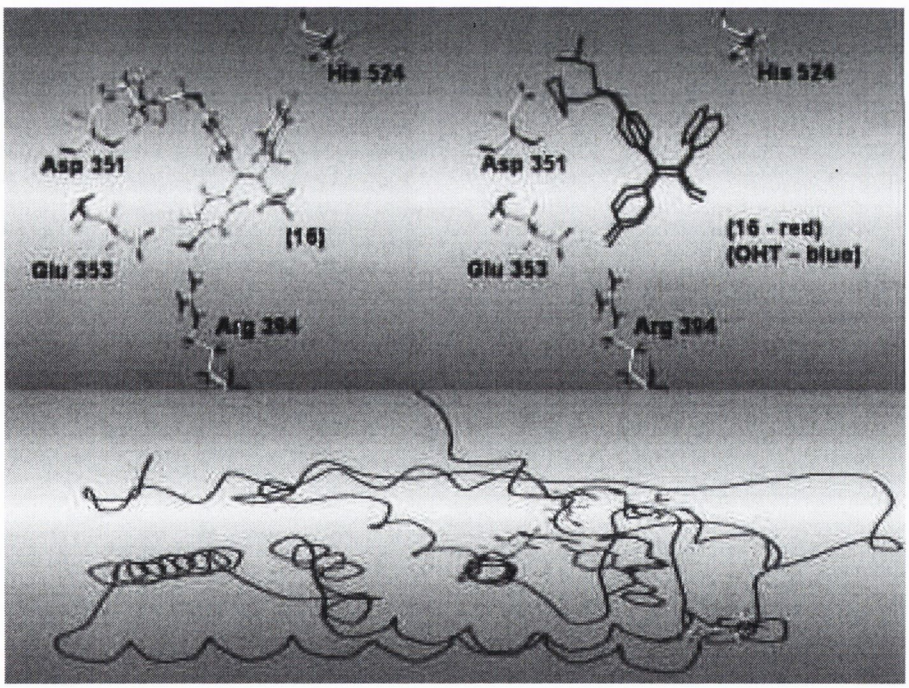


Fig. (2). FlexX docked structure for **16.** Top Left - **16** in the context of the ER active site, hydrogen bonding to Arg394, Glu353 and Asp 351. Top Right – docked mode for **16** overlaid with experimental binding mode of 4-hydroxytamoxifen (**OHT**). Bottom – **16** orientation in the human ER alpha ligand binding domain.

Table 4. Summary of Key Protein-Ligand Contacts

Ligand	Residue	Distance[d]	Surface[e]	HB[f]
RAL[a]	351 Asp	2.7	30.9	+
OHT[b]	351 Asp	3.2	29.6	+
16[c]	351 Asp	2.7	24.7	+
RAL[a]	353 Glu	2.4	32.7	+
OHT[b]	353 Glu	2.4	34.0	+
16[c]	353 Glu	2.9	30.8	+
RAL[a]	394 Arg	3.0	22.0	+
OHT[b]	394 Arg	3.0	23.0	+
16[c]	394 Arg	2.9	23.6	+
RAL[a]	524 His[g]	3.0	22.0	+
OHT[b]	524 His	3.0	23.0	-
16[c]	524 His	3.5	9.4	-

[a]RAL : raloxifene(**2**), from PBD 1ERR[47]

[b]OHT : 4-hydroxytamoxifen(**1c**), from PDB 3ERT[39]

[c]**16** : from FlexX docked structure above

[d]Distance - nearest distance (Angstoms) between atoms of the ligand and the residue

[e]Surf - contact surface area (Angstroms^2) between the ligand and the residue

[f]HB - hydrophilic-hydrophilic contact (hydrogen bond)

[g] - Hydrogen bonding to His 524 is a key feature in molecules with an estrogenic core and is not predicted for OHT or **16** as they do not possess a second hydrogen bonding feature on ring C, as found in Raloxifene.

stimulation. In a further investigation of the mechanism of action, the most active products were shown to promote apoptosis in MCF-7 cells in a FACS based assay. Investigation of the ability of the synthesized compounds to inhibit estrogen mediated ERE promoter based transcription using a luciferase reporter assay will be developed in future studies to elucidate the precise biochemical mechanism of action of these flexible ligands.

To rationalise the observed biochemical results, computational docking and ligand -protein contact studies were carried out on the most active examples which predict the compound series bind in the expected region of the ER in an antiestrogenic mode with interactions with the same key residues as known antiestrogens. These results further demonstrate the liganding tolerance of the estrogen receptor and the potential utility of this class of flexible modulators as chemotherapeutics against hormone dependent breast cancers.

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EXPERIMENTAL

All reagents used were commercial grade chemicals from freshly opened containers. IR spectra were recorded as thin films on NaCl plates on a Perkin-Elmer Paragon 100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DPX 400 instrument at 20 C, 400.13MHz for ^{1}H spectra, 100.61MHz for ^{13}C spectra, in either $CDCl_3$ (internal standard tetramethylsilane) or CD_3OD . All J values are quoted in Hz. Low resolution mass spectra were run on a Hewlett-Packard 5973 MSD GC–MS system in an electron impact mode, while high resolution accurate mass determinations for selected compounds were obtained on a Micromass Time of Flight mass spectrometer (TOF) equipped with electrospray ionization(ES) interface operated in the positive ion mode at the High Resolution Mass Spectrometry Laboratory in the Department of Chemistry, Trinity College Dublin. Flash chromatography was carried out using standard silica gel 60 (230-400 mesh) obtained from Merck. All products isolated were homogenous on TLC. Analytical HPLC work was performed on a chromatographic system comprising a Waters 501 pump (flow-rate 2 ml/min / sample loop 20 µl) and a Waters Spherisorb® S5 ODS2 (4.6x250mm) reversed phase C18 analytical column. Detection was on a Waters 486 Tunable

Absorbance Detector with λ=241, chart recorder speed at 1/6 cm per min. The mobile phase used was prepared from HPLC grade solvents and comprised ACN:H_2O:THF:18M NH_3 Buffer 30:12.5:7.5:2 respectively. Retention times are given in minutes. Unless otherwise stated all reactions were carried out under a nitrogen atmosphere. The benzophenones (**24**),(**25**),(**26**),(**35**),(**36**) were prepared following the reported literature procedures [23,24,40-43].

4-(*tert*-Butyldimethylsilanyloxy)-4'-hydroxybenzophenone (6)

4,4'-Dihydroxybenzophenone (10.0 g, 46.73 mmol) and imidazole (3.495 g, 51.40 mmol) were dissolved in DMF (40 mL). *tert*-butyldimethylsilyl chloride (7.042 g, 46.73 mmol) was added in 6 portions over 6 hours and stirring was continued for a further 16 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and quenched with 10% HCl (50 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane : diethyl ether = 6:1) to yield the product **6** (5.84 g, 38%) as a colourless oil. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3256 (OH), 2955, 2931, 2886 (CH), 1652 (C=O), 1599 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.27 (s, 6H, $(CH_3)_2$-Si), 1.02 (s, 9H, $(CH_3)_3$-C), 6.91-6.97 (m, 4H, ArH), 7.73-7.74 (m, 4H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ -4.79 $(CH_3)_2$-Si), 17.80 (C-$(CH_3)_3$), 25.16 $(CH_3)_3$-C), 114.77-113.25 (ArC), 159.17 (ArC-OSi), 160.14 (ArC-OH), 194.92 (C=O). HRMS Found 351.1390 (M^++Na); $C_{19}H_{24}O_3NaSi$ requires 351.1392.

4-{2-Benzyl-1-[4-(*tert*-butyldimethylsilanyloxy)-phenyl]-but-1-enyl}-phenol (7)

7 was prepared from **6** and 1-phenyl-2-butanone as follows: zinc powder (602 mg, 9.26 mmol) was placed in a 3-necked flask into which dry THF (10 mL) was added under nitrogen. Titanium tetrachloride (509 μL, 4.63 mmol) was slowly added via syringe over 5 min in the dark. The reaction mixture was maintained at reflux for 1.5 hours, after which time **7** (430 mg, 1.32 mmol) and 1-phenyl-2-butanone (235 mg, 1.32 mmol) in THF (10 mL) were added via syringe. After a further 4 hours at reflux, the solution was cooled to room temperature, diluted with ethyl acetate (50 mL) and quenched with 10% K_2CO_3 solution (20 mL). The aqueous layer was extracted with ethyl acetate (3 x 40 mL). The combined organic layers were washed with 10% K_2CO_3 solution (20 mL), water (20 ml) and brine (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane : diethyl ether = 4:1) to yield the pure product **7** as a colourless oil (76%) yield following chromatography on silica gel and used in the following reaction without further purification. LRMS (*m/z*): 444 (M^+, 100%).; IR: ν_{max} ($CHCl_3$) cm^{-1}: 3392 (OH), 3015, 2931, 2858 (CH), 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.23 (s, 3H, CH_3-Si), 0.26 (s, 3H, CH_3-Si), 0.98 (t, 3H, *J* = 7.5 Hz, CH_3), 1.01 (s, 9H, $(CH_3)_3$-C), 2.08 (q, 2H, *J* = 7.5 Hz, CH_2), 3.57 (s, 2H, CH_2-Ar), 6.73-6.81 (m, 4H, ArH), 7.06-7.12 (m, 4H, ArH), 7.21-7.33 (m, 5H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ -4.85 $(CH_3)_2$-Si), 12.81 (CH_3), 17.71 (C-$(CH_3)_3$), 24.29 (CH_2), 25.22 $(CH_3)_3$-C), 36.83 (CH_2-Ar), 114.41-140.42 (ArC), 153.46 (ArC-OSi), 153.52 (ArC-OH). HRMS(EI) Found. 385.1450; $C_{27}H_{29}O_2$ requires 385.2167.

[2-(4-{2-Benzyl-1-[4-(*tert*-butyl-dimethyl-silanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-dimethylamine (8)

8 was prepared from **7** and *N,N*-dimethylethanolamine in the manner described for **10** above. The pure product was isolated as a colourless oil in 49% yield following chromatography on silica gel (dichloromethane : methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2957, 2943, 2938, 2858, 2772 (CH), 1604 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.19 (s, 3H, CH_3-Si), 0.21 (s, 3H, CH_3-Si), 0.98 (t, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 9H, $(CH_3)_3$-C), 2.07 (q, 2H, *J* = 7.5 Hz, CH_2), 2.36 (s, 3H, CH_3N), 2.38 (s, 3H, CH_3N), 2.76 (m, 2H, CH_2N), 3.57 and 3.58 (2 x s, 2H, CH_2-Ar), 4.08 (m, 2H, CH_2O), 6.74-6.88 (m, 4H, ArH), 7.06-7.31 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ -4.85 $(CH_3)_2$-Si), 12.88 (CH_3), 17.72 (C-$(CH_3)_3$), 24.25 (CH_2), 25.23 $(CH_3)_3$-C), 36.78 (CH_2-Ar), 45.36 $(CH_3)_2N$), 57.81 (CH_2N), 65.21 (CH_2O), 113.49-140.43 (ArC), 153.47 (ArC-OSi), 156.71 (ArC-OCH_2). HRMS (EI): Found 516.3290 (M^++H), $C_{33}H_{46}NO_2Si$ requires 516.3298.

[2-(4-{2-Benzyl-1-[4-(*tert*-butyldimethylsilanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-diethylamine (9)

9 was prepared from **7** in the manner described for **10** above. The pure product was isolated as a colourless oil in 47% yield following chromatography on silica gel (dichloromethane : methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2969, 2933, 2868 (CH), 1604 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.19 (s, 3H, CH_3-Si), 0.22 (s, 3H, CH_3-Si), 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 9H, $(CH_3)_3$-C), 1.09 (t, 6H, *J* = 7.3 Hz, 2 x CH_2), 2.06 (q, 2H, *J* = 7.5 Hz, CH_2), 2.67 (q, 4H, *J* = 7.3 Hz, 2 x CH_2), 2.90 (m, 2H, CH_2N), 3.56 and 3.57 (2 x s, 2H, CH_2-Ar), 4.05 (m, 2H, CH_2O), 6.73-6.87 (m, 4H, ArH), 7.06-7.30 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ -4.84 $(CH_3)_2$-Si), 11.30 (CH_3), 12.88 (CH_3), 17.72 (C-$(CH_3)_3$), 24.28 (CH_2), 25.22 $(CH_3)_3$-C), 36.77 (CH_2-Ar), 47.34 (CH_2), 51.27 (CH_2N), 65.75 (CH_2O), 113.45-140.43 (ArC), 153.46 (ArC-OSi), 156.73 (ArC-OCH_2). HRMS (EI): Found 514.3635 (M^++H), $C_{35}H_{50}NO_2Si$ requires 544.3611.

1-[2-(4-{2-Benzyl-1-[4-(*tert*-butyldimethylsilanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (10)

7 (450 mg, 1.01 mmol), 1-(2-hydroxyethyl)pyrrolidine (590 μL, 5.05 mmol) and triphenylphosphine (1.058 g, 4.04 mmol) were dissolved in dry dichloromethane (10 mL) under nitrogen atmosphere. Diispropylazodicarboxylate (DIAD) (795 μL 4.04 mmol) was added dropwise via syringe over 10 min. Stirring was continued at room temperature for 4 hours. The reaction mixture was diluted with dichloromethane (100 ml) and quenched with saturated NH_4Cl solution (20 ml). The organic layer was washed with brine (30 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane : methanol = 15:1) to yield the product **10** (361 mg, 66%) as a colourless oil. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3015, 2968, 2921, 2858, 2785 (CH), 1601 (C=C). ^{1}H-NMR

(400MHz, $CDCl_3$): δ 0.20 (s, 3H, CH_3-Si), 0.23 (s, 3H, CH_3-Si), 0.99 (t, 3H, *J* = 7.5 Hz, CH_3), 1.02 (s, 9H, $(CH_3)_3$-C), 1.83 (m, 4H, CH_2), 2.08 (q, 2H, *J* = 7.5 Hz, CH_2), 2.65 (m, 4H, $(CH_2)_2N$), 2.91 (m, 2H, CH_2N), 3.59 (s, 2H, CH_2-Ar), 4.11 (m, 2H, CH_2O), 6.74-6.89 (m, 4H, ArH), 7.06-7.31 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ -4.85 $(CH_3)_2$-Si), 12.84 (CH_3), 17.70 (C-$(CH_3)_3$), 23.05 (CH_2), 24.30 (CH_2), 25.22 $(CH_3)_3$-C), 36.85 (CH_2-Ar), 54.20 $(CH_2)_2N$), 54.69 (CH_2N), 66.50 (CH_2O), 113.59-140.43 (ArC), 153.50 (ArC-OH), 156.85 (ArC-OCH_2). LRMS (*m/z*): 541 (M^+, 4%). HRMS (EI): Found 542.3416 (M^++H), $C_{35}H_{48}NO_2Si$ requires 542.3454.

1-[2-(4-{2-Benzyl-1-[4-(*tert*-butyldimethylsilanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-piperidine (11)

11 was prepared from **7** in the manner described for **10** above. The pure product was isolated as a colourless oil in 81% yield following chromatography on silica gel (dichloromethane : methanol = 10:1) and was used in following reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3009, 2932, 2856, 2783 (CH), 1604 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.19 (s, 3H, CH_3-Si), 0.22 (s, 3H, CH_3-Si), 0.98 (t, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 9H, $(CH_3)_3$-C), 1.47 (m, 2H, CH_2), 1.63 (m, 4H, CH_2), 2.07 (q, 2H, *J* = 7.5 Hz, CH_2), 2.53 (m, 4H, $(CH_2)_2N$), 2.80 (t, 2H, *J* = 6.0 Hz, CH_2N), 3.58 (s, 2H, CH_2-Ar), 4.11 (t, 2H, *J* = 6.0 Hz, CH_2O), 6.73-6.87 (m, 4H, ArH), 7.06-7.33 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ -4.83 $(CH_3)_2$-Si), 12.88 (CH_3), 17.72 (C-$(CH_3)_3$), 23.69 (CH_2), 24.25 (CH_2), 25.23 $(CH_3)_3$-C), 25.42 (CH_2), 36.77 (CH_2-Ar), 54.57 $(CH_2)_2N$), 57.52 (CH_2N), 65.23 (CH_2O), 113.51-140.38 (ArC), 153.47 (ArC-OSi), 156.71 (ArC-OCH_2).

4-[2-(4-{2-Benzyl-1-[4-(*tert*-butyldimethylsilanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-morpholine (12)

12 was prepared from **7** and 4-(2-hydroxyethyl) morpholine in the manner described for **10** above. The pure product was isolated as a colourless oil in 70% yield following chromatography on silica gel (dichloromethane : methanol = 10:1) and used in following reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2992, 2958, 2858 (CH), 1604(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.13 (s, 3H, CH_3-Si), 0.15 (s, 3H, CH_3-Si), 0.94 (t, 3H, *J* = 7.5 Hz, CH_3), 0.94 (s, 9H, $(CH_3)_3$-C), 1.99 (q, 2H, *J* = 7.5 Hz, CH_2), 2.54 (m, 4H, $(CH_2)_2N$), 2.76 (m, 2H, CH_2N), 3.50 (s, 2H, CH_2-Ar), 3.67 (m, 4H, $(CH_2)_2O$), 4.05 (m, 2H, CH_2O), 6.67-6.81 (m, 4H, ArH), 6.99-7.28 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ -4.88 $(CH_3)_2$-Si), 12.81 (CH_3), 17.67 (C-$(CH_3)_3$), 24.18 (CH_2), 25.17 $(CH_3)_3$-C), 36.71 (CH_2-Ar), 53.53 $(CH_2)_2N$), 57.18 (CH_2N), 65.08 $(CH_2)_2O$), 66.38 (CH_2O), 113.48-140.26 (ArC), 153.42 (ArC-OSi), 156.57 (ArC-OCH_2). HRMS (EI): Found 558.3395 (M^++H), $C_{35}H_{48}NO_3Si$ requires 558.3403.

1-[2-(4-{2-Benzyl-1-[4-(*tert*-butyldimethylsilanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidin-2-one (13)

13 was prepared from **7** and 1-(2-hydroxyethyl)-2-pyrrolidinone in the manner described for **10** above. The pure product was isolated as a colourless oil in 14% yield following chromatography on silica gel (dichloromethane : methanol = 10:1) and used in following reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2967, 2932 (CH), 1686 (CO), 1621, 1603 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.14 (s, 3H, CH_3-Si), 0.16 (s, 3H, CH_3-Si), 0.93 (t, 3H, *J* = 7.5 Hz, CH_3), 0.95 (s, 9H, $(CH_3)_3$-C), 1.94-2.04 (m, 4H, 2 x CH_2), 2.33 (t, 2H, *J* = 8.0 Hz, CH_2CO), 3.50 and 3.52 (2 x s, 2H, CH_2-Ar), 3.54 (m, 2H, CH_2N), 3.66 (m, 2H, CH_2N), 4.05 (m, 2H, CH_2O), 6.68-6.80 (m, 4H, ArH), 7.01-7.28 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ -4.88 $(CH_3)_2$-Si), 12.82 (CH_3), 17.64 (C-$(CH_3)_3$), 21.53 (CH_2), 24.19 (CH_2), 25.18 $(CH_3)_3$-C), 30.33 (CH_2CO), 36.71 (CH_2-Ar), 41.94 (CH_2), 48.43 (CH_2N), 66.05 (CH_2O), 113.39-140.29 (ArC), 153.45 (ArC-OSi), 156.33 (ArC-OCH_2), 174.84 (C=O). HRMS(EI): Found 517.2865. $C_{33}H_{45}NO_2Si$ requires 517.3376.

4-{2-Benzyl-1-[4-(2-dimethylaminoethoxy)-phenyl]-but-1-enyl}-phenol (14)

14 was prepared from **8** in the manner described for **16** above. The pure product was isolated as a colourless oil in 81% yield following chromatography on silica gel (dichloromethane : methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3028 (OH), 2963, 2875, 2824, 2774 (CH), 1607(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.93 (t, 3H, *J* = 7.5 Hz, CH_3), 2.03 (q, 2H, *J* = 7.5 Hz, CH_2), 2.38 (s, 3H, CH_3N), 2.40 (s, 3H, CH_3N), 2.81 (m, 2H, CH_2N), 3.52 and 3.54 (2 x s, 2H, CH_2-Ar), 4.06 (m, 2H, CH_2O), 6.70-7.28 (m, 13H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.87 (CH_3), 24.22 (CH_2), 36.74 (CH_2-Ar), 44.96 $(CH_3)_2N$), 58.28 (CH_2N), 64.62 (CH_2O), 113.39-140.45 (ArC), 155.33 (ArC-OH), 156.40 (ArC-OCH_2). HRMS (EI): Found 402.2443 (M^++H), $C_{27}H_{32}NO_2$ requires 402.2433.

4-{2-Benzyl-1-[4-(2-diethylaminoethoxy)-phenyl]-but-1-enyl}-phenol (15)

15 was prepared from **9** in the manner described for **16** above. The pure product was isolated as a colourless oil in 76% yield following chromatography on silica gel (dichloromethane : methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3171(OH), 2964, 2929, 2872 (CH), 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.95 (t, 3H, *J* = 7.5 Hz, CH_3), 1.10 (t, 6H, *J* = 7.3 Hz, 2 x CH_2), 2.04 (q, 2H, *J* = 7.5 Hz, CH_2), 2.69 (q, 4H, *J* = 7.3 Hz, 2 x CH_2N), 2.92 (m, 2H, CH_2N), 3.54 and 3.57 (2 x s, 2H, CH_2-Ar), 4.05 (m, 2H, CH_2O), 6.72-6.79 (m, 4H, ArH), 7.01-7.30 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 10.73 (CH_3), 12.88 (CH_3), 24.24 (CH_2), 36.76 (CH_2-Ar), 47.93 (CH_2), 51.07 (CH_2N), 65.17 (CH_2O), 113.39-140.42 (ArC), 154.74 (ArC-OSi), 156.54 (ArC-OCH_2). HRMS (EI): Found 430.2763 (M^++H), $C_{29}H_{36}NO_2$ requires 430.2746.

4-{2-Benzyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (16)

Tetrabutylammonium fluoride (1M, 870 μL) was added to **10** (313 mg, 0.58 mmol) in THF (5 mL) and stirred for 1.5 hours at room temperature. The reaction mixture was diluted with ethyl acetate (50 mL) and quenched with 10% HCl (10 mL). The layers was separated and the aqueous layer was extracted with ethyl acetate (2 x 50 mL). The combined

organic layers were washed with water (20 mL) and brine (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane : methanol = 15:1) to yield the product **16** (220 mg, 89%) as a colourless oil. HPLC: t_R = 3.1, 3.2 min. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3057, 2952, 2868, 2785 (CH), 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.96 (t, 3H, *J* = 7.5 Hz, CH_3), 1.91 (m, 4H, CH_2), 2.07 (q, 2H, *J* = 7.5 Hz, CH_2), 2.85 (m, 4H, $(CH_2)_2N$), 3.03 (m, 2H, CH_2N), 3.56 and 3.58 (2 x s, 2H, CH_2-Ar), 4.13 (m, 2H, CH_2O), 6.65-6.78 (m, 4H, ArH), 7.01-7.29 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.83 (CH_3), 22.81 (CH_2), 24.30 (CH_2), 36.82 (CH_2-Ar), 53.94 ($(CH_2)_2N$), 54.39 (CH_2N), 64.99 (CH_2O), 113.44-140.41 (ArC), 154.69 (ArC-OH), 156.24 (ArC-OCH_2). LRMS (*m/z*): 427 (M^+, 2%). HRMS (EI): Found 428.2597 (M^++H), $C_{29}H_{34}NO_2$ requires 428.2590.

4-{2-Benzyl-1-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (17)

17 was prepared from **11** in the manner described for **16** above. The pure product was isolated as a colourless oil in 72% yield following chromatography on silica gel (dichloromethane : methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3027, 2935 (CH), 1606 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.50 (m, 2H, CH_2), 1.71 (m, 4H, CH_2), 2.12 (q, 2H, *J* = 7.5 Hz, CH_2), 2.63 (m, 4H, $(CH_2)_2N$), 2.85 (t, 2H, *J* = 6.0 Hz, CH_2N), 3.57 and 3.60 (2 x s, 2H, CH_2-Ar), 4.07 (m, 2H, CH_2O), 6.56-6.76 (m, 4H, ArH), 7.01-7.22 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.90 (CH_3), 23.38 (CH_2), 24.33 (CH_2), 25.52 (CH_2), 36.76 (CH_2-Ar), 54.36 ($(CH_2)_2N$), 57.48 (CH_2N), 63.72 (CH_2O), 113.22-140.48 (ArC), 154.74 (ArC-OH), 156.39 (ArC-OCH_2). HRMS (EI): Found 442.2738 (M^++H), $C_{36}H_{50}NO_2$ requires 442.2746.

4-{2-Benzyl-1-[4-(2-morpholin-4-ylethoxy)-phenyl]-but-1-enyl}-phenol (18)

18 was prepared from **12** in the manner described for **16** above. The pure product was isolated as a colourless oil in 91% yield following chromatography on silica gel (dichloromethane : methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3284 (OH), 2978, 2935, 2858, 2815 (CH), 1606 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.92 (t, 3H, *J* = 7.5 Hz, CH_3), 2.02 (q, 2H, *J* = 7.5 Hz, CH_2), 2.56 (m, 4H, $(CH_2)_2N$), 2.77 (m, 2H, CH_2N), 3.51 and 3.53 (2 x s, 2H, CH_2-Ar), 3.69 (m, 4H, $(CH_2)_2O$), 4.05 (m, 2H, CH_2O), 6.69-6.79 (m, 4H, ArH), 6.98-7.28 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.86 (CH_3), 24.19 (CH_2), 36.73 (CH_2-Ar), 53.47 ($(CH_2)_2N$), 57.19 (CH_2N), 64.87 ($(CH_2)_2O$), 66.33 (CH_2O), 113.46-140.3 (ArC), 154.76 (ArC-OH), 156.48 (ArC-OCH_2). HRMS (EI): Found 444.2549 (M^++H), $C_{29}H_{34}NO_3$ requires 444.2539.

1-(2-{4-[2-Benzyl-1-(4-hydroxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidin-2-one (19)

19 was prepared from **13** in the manner described for **16** above. The pure product was isolated as a colourless oil in 62% yield following chromatography on silica gel (dichloromethane : methanol = 10:1).). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3296(OH), 2970, 2934, 2871 (CH), 1665 (C=O), 1606 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.93 (t, 3H, *J* = 7.5 Hz, CH_3), 1.95-2.06 (m, 4H, 2 x CH_2), 2.34 (t, 2H, *J* = 8.0 Hz, CH_2CO), 3.51 and 3.52 (2 x s, 2H, CH_2-Ar), 3.56 (m, 2H, CH_2N), 3.63 (m, 2H, CH_2N), 4.05 (m, 2H, CH_2O), 6.72-6.79 (m, 4H, ArH), 6.98-7.24 (m, 9H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.85 (CH_3), 17.64 (CH_2), 24.20 (CH_2), 30.35 (CH_2CO), 36.73 (CH_2-Ar), 41.96 (CH_2), 48.46 (CH_2N), 66.05 (CH_2O), 113.37-134.33 (ArC), 154.93 (ArC-OH), 156.26 (ArC-OCH_2), 174.91 (C=O) HRMS (EI): Found 442.2390 (M^++H), $C_{29}H_{32}NO_3$ requires 442.2382.

Acetic acid 4-{2-benzyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl ester (20)

16 (160 mg, 0.37 mmol) and triethylamine (104 µL, 0.75 mmol) were dissolved in THF (5 ml). Acetyl chloride (40 µL, 0.56 mmol) was added and stirring was continued for 30 min at room temperature. The reaction mixture was diluted with ethyl acetate (40 mL) and quenched with 10% HCl (10 ml). The organic layer was separated, washed with and brine (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane : methanol = 10:1) to yield the product **20** (133 mg, 76%) as a colourless oil. HPLC: t_R = 6.0, 6.4 min. LRMS (*m/z*): 469 (M^+, 2%). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2959, 2917, 2865, 2781 (CH), 1757 (C=O), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.98 (t, 3H, *J* = 7.5 Hz, CH_3), 1.87 (m, 4H, CH_2), 2.07 (q, 2H, *J* = 7.5 Hz, CH_2), 2.28 and 2.29 (2 x s, 3H, CH_3CO), 2.78 (m, 4H, $(CH_2)_2N$), 3.02 (m, 2H, CH_2N), 3.57 and 3.58 (2 x s, 2H, CH_2-Ar), 4.17 (m, 2H, CH_2O), 6.82-6.89 (m, 2H, ArH), 6.99-7.32 (m, 11H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.81 (CH_3), 20.69 (CH_3CO), 22.96 (CH_2), 24.27 (CH_2), 36.77 (CH_2-Ar), 53.93 ($(CH_2)_2N$), 54.21 (CH_2N), 65.78 (CH_2O), 113.74-140.43 (ArC), 148.59 (ArC-OAc), 156.69 (ArC-OCH_2), 168.91 (C=O). HRMS (EI): Found 428.2597; $C_{29}H_{34}NO_2$ requires 428.2590.

Dimethyl-carbamic acid 4-{2-benzyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl ester (21)

16 (22 mg, 0.056 mmol) was dissolved in triethylamine (500 µL, 3.59 mmol) and added dropwise over 5 min to dimethylcarbamyl chloride (500 µL, 5.46 mmol). Stirring was continued for 1.5 hours at room temperature. The reaction mixture was diluted with 50 ml of dichoromethane and washed with 10% HCl (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and following removal of the solvent under reduced pressure, the residue was chromatographed on silica gel (dichloromethane : methanol = 20:1) to yield the product **21** (10 mg, 39%) as a colourless oil. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3025, 2963, 2921, 2785 (CH), 1722 (C=O), 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.87 (m, 4H, CH_2), 2.06 (q, 2H, *J* = 7.5 Hz, CH_2), 2.76 (m, 4H, $(CH_2)_2N$), 3.02-3.10 (m, 8H, CH_2N, $(CH_3)_2N$), 3.56 and 3.57 (2 x s, 2H, CH_2-Ar), 4.16 (m, 2H, CH_2O), 6.81-6.86 (m, 2H, ArH), 7.01-7.30 (m, 11H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.78 (CH_3), 22.97 (CH_2), 24.25 (CH_2), 35.95 (CH_3N), 36.19 (CH_3N), 36.77 (CH_2-Ar), 54.08 ($(CH_2)_2N$), 54.37 (CH_2N), 65.81 (CH_2O), 113.70-140.17 (ArC), 149.43 (ArC-OCO), 154.43

(C=O), 156.61 (ArC-OCH_2). HRMS (EI): Found 499.2965 (M^++H), $C_{36}H_{39}N_2O_3$ requires 499.2961.

Ethyl-carbamic acid 4-{2-benzyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl ester (22)

Ethyl isocyanate (110 µL, 1.40 mmol) was added dropwise over 10 min to **16** (300 mg, 0.070 mmol) in dichloromethane (10 mL). Stirring was continued for 16 hours at room temperature. The reaction mixture was diluted with dichoromethane (50 mL) and washed with 10% HCl (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and following removal of the solvent under reduced pressure, the residue was chromatographed on silica gel (dichloromethane : methanol = 15:1) to yield the product **22** (188 mg, 54%) as a colourless oil. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3028, 2968, 2932, 2874, 2803 (CH), 1739 (C=O), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.96 (t, 3H, *J* = 7.5 Hz, CH_3), 1.21 (t, 3H, *J* = 7.0 Hz, CH_3), 1.85 (m, 4H, CH_2), 2.05 (q, 2H, *J* = 7.5 Hz, CH_2), 2.71 (m, 4H, $(CH_2)_2N$), 2.96 (m, 2H, CH_2N), 3.30 (q, 2H, *J* = 7.0 Hz, CH_2), 3.55 and 3.56 (2 x s, 2H, CH_2-Ar), 4.14 (m, 2H, CH_2O), 6.80-6.86 (m, 2H, ArH), 7.01-7.28 (m, 11H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.83 (CH_3), 14.68 (CH_3), 22.97 (CH_2), 24.21 (CH_2), 35.64 (CH_2), 36.76 (CH_2-Ar), 54.14 (CH_2)$_2$N), 54.45 (CH_2N), 65.98 (CH_2O), 113.62-140.15 (ArC), 148.93 (ArC-OCO), 154.06 (C=O), 156.67 (ArC-OCH_2). HRMS (EI): Found 499.2952 (M^++H), $C_{32}H_{39}N_2O_3$ requires 499.2961.

Phenyl-carbamic Acid 4-{2-benzyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl Ester (23)

23 was prepared from **16** in the manner described for **22** above using phenyl isocyanate (110 µL, 1.40 mmoL). The pure product was isolated as a colourless oil in 7% yield following chromatography on silica gel (dichloromethane : methanol = 15:1). IR: ν_{max} ($CHCl_3$) cm^{-1}:2964, 2918, 2875 (CH), 1737 (C=O), 1603 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.89 (m, 4H, CH_2), 2.05 (q, 2H, *J* = 7.5 Hz, CH_2), 2.84 (m, 4H, $(CH_2)_2N$), 3.04 (m, 2H, CH_2N), 3.53 and 3.55 (2 x s, 2H, CH_2-Ar), 4.19 (m, 2H, CH_2O), 6.74-7.09 (m, 12H, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.83 (CH_3), 22.90 (CH_2), 24.21 (CH_2), 36.73 (CH_2-Ar), 54.06 (CH_2)$_2$N), 54.19 (CH_2N), 65.38 (CH_2O), 113.48-156.40 (ArC), 157.22 (C=O), 157.22 (ArC-OCO), 157.47 (ArC-OCH_2). HRMS (EI): Found 429.2709, $C_{29}H_{35}NO_2$ requires 429.2667.

2 - Benzyl-1-(4-hydroxyphenyl)-1-(2-methoxyphenyl)-but-1-ene (27)

Following the general method above for **7** using titanium tetrachloride (11.75 mmol), zinc dust (37 mmol), 4-hydroxy-2'-methoxy benzophenone [42] **24** (4.7 mmol) and 1-phenyl-2-butanone (14.1mmol) in dry THF(10 mL). The crude product was purified by flash chromatography (eluant: hexane:dichloromethane 40:60) to afford the title compound (18%) was achieved as orange-yellow oil (R_f 0.15 dichloromethane:hexane 6:4). IR ν_{max} (film) 3490 (OH), 3023-2854 (CH), 1606 (C=C) cm^{-1}. ^{1}H-NMR (400MHz, $CDCl_3$):0.92 (3H, t, CH_3), 1.91 (2H, q, CH_2), 3.62 (2H, s, CH_2), 3.78 (3H, s, OCH_3), 4.79 (H, s, OH), 6.70-6.97 (5H, m, ArH), 7.19-7.24 (6H, m, ArH), 7.31-7.32 (2H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 11.79 (CH_3), 25.6 (CH_2), 28.7 (CH_2), 56.84 (OCH_3), 113.72- 135.29 (ArC), 155 (ArC-OH), 156.4 (ArC-OCH_3). HRMS(EI) : Found 342.1697, $C_{24}H_{22}O_2$ requires 342.1619.

2 - Benzyl - 1-(4-hydroxyphenyl)-1-(3-methoxyphenyl)-but-1-ene (28)

Following the general method above for **7** using titanium tetrachloride (11.75 mmol), zinc dust (37 mmol), 4-hydroxy-3'-methoxybenzophenone [43]**25** (4.7 mmol) and 1-phenyl-2-butanone (14 mmol) in dry THF(10 mL). The crude product was purified by flash chromatography (eluant: hexane:dichloromethane 40:60) to afford the title compound (80%) was achieved as light orange oil (R_f 0.2 dichloromethane:hexane 6:4). IR ν_{max} (film) 3346 (OH), 3027-2904 (CH), 1607 (C=C) cm^{-1}. ^{1}H-NMR (400MHz, $CDCl_3$):1.07 (3H, t, CH_3), 2.54 (2H, q,CH_2), 3.75 (2H, s,CH_2), 3.84 (3H, s, OCH_3) 5.78 (1H, s, OH) 6.80-6.94 (5H, m, ArH), 7.16-7.19 (2H, m, ArH) 7.29-7.30 (6H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.79 (CH_3), 24.3 (CH_2), 28.36 (CH_2), 55.7 (OCH_3), 111.68-140.07 (ArC), 156.96 (ArC-OH), 158.55 (ArC-OCH_3). HRMS(EI) Found 342.0465, $C_{24}H_{22}O_2$ requires 342.1619.

2 - Benzyl - 1-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-but-1-ene (29)

Following the general method above for **7** using titanium tetrachloride (11.75 mmol), zinc dust (37.6 mmol), 4-hydroxy-4'-methoxy-benzophenone [41] **26** (4.7 mmol) and 1-phenyl-2-butanone (14.1 mmol) in dry THF(100 mL). The crude product was purified by column chromatography (eluant: hexane:dichloromethane 40:60) to afford the title compound (88%) as a light yellow oil, (R_f 0.26 dichloromethane:hexane 2:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3402 (OH), 3000-2963 (CH), 1606 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): 1.04 (3H, t, CH_3), 2.16 (2H, q, CH_2), 3.66 (2H, s, CH_2), 3.82 (3H, s, OCH_3) 6.18 (H, s, OH), 6.80-6.95 (4H, m, ArH) 7.15 –7.32 (7H, m,ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 11.8 (CH_3), 24.12 (CH_2), 25.24 (CH_2), 53.82 (OCH_3), 113.11-140.1 (ArC), 154.96 (ArC-OH), 162.58 (ArC-OCH_3). HRMS(EI) Found 367.1659 (M^++Na).$C_{24}H_{24}O_2Na$ requires 367.1674.

2 - Benzyl - 1-(2-methoxyphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (30)

Method 1: **27** (1.74 mmol) was stirred in dichloromethane (10 mL) with triphenylphosphine (3.48 mmol) and 1-(2-hydroxyethyl)pyrrolidine (5.22 mmol) at room temperature. Diisopropylazodicarboxylate (4.35 mmol) was added slowly over 20 minutes. The reaction was stirred for two days. then washed with ammonium chloride solution, extracted with dichloromethane and dried (sodium sulphate). The solvent was removed under reduced pressure to give a brown oil. which was purified by flash column chromatography on silica gel (eluant: dichloromethane:methanol 80:20) to afford the product as a yellow oil (20%) (R_f 0.2 dichloromethane:methanol 80:20). *Method 2:* **27** (0.45 mmol) was

suspended in in acetone:water 19:1 (10 mL) with potassium carbonate (0.54 mmol), and 1-(2-chloroethyl)pyrrolidine. HCl (0.9 mmol). The reaction mixture was refluxed for 5 hours in darkness, cooled, extracted with dichloromethane and washed with potassium carbonate solution. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20) and obtained as a light brown oil (44%). (R_f 0.2 dichloromethane:methanol 80:20). IR ν_{max} (film) 2954, 2786 (CH), 1604 (C=C) cm^{-1}. ^{1}H-NMR (400MHz, $CDCl_3$):0.90 (3H, t, CH_3), 1.85 (2H, q, CH_2), 1.93 (4H, s, CH_2), 2.91 (4H, s, CH_2), 3.09 (2H, m, CH_2), 3.6 (2H, s, CH_2), 3.8 (3H, s, OCH_3), 4.19-4.23 (2H, m, CH_2), 6.77-6.95 (5H, m, ArH), 7.16- 7.30 (8H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.25 (CH_3), 23.90 (CH_2), 24.60 (CH_2), 29.00 (CH_2), 36.6 (CH_2), 53.9 (CH_2), 54.84 (OCH_3), 65.30 (CH_2), 113.37-135.29 (ArC), 156 (ArC-OCH_2), 157.55 (ArC-OCH_3). HRMS(EI) Found 442.1002 (M^++H). $C_{30}H_{36}NO_2$ requires 442.2746.

2 - Benzyl - 1-(3-methoxyphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (31)

Method 1 Following the procedure for **16**, using diisopropylazodicarboxylate (2.3 mmol), **28** (9.29 mmol), triphenylphosphine (1.86 mmol) and 1-(2-hydroxyethyl) pyrrolidine (2.79 mmol) in dichloromethane (10 mL). The product was purified using flash column chromatography on silica gel (eluant: dichloromethane:methanol 80:20) and recovered as yellow oil (15%) (R_f 0.15 dichloromethane:methanol 80:20). *Method 2* Following the procedure above for **30** using **28** (0.45 mmol), potassium carbonate (0.54 mmol), acetone:water 19:1 (10 mL) and 1-(2-chloroethyl)pyrrolidine.HCl (0.9 mmol). The product was obtained using flash column chromatography (eluant: dichloromethane:methanol 80:20) as a light brown oil (36%), (R_f 0.15 dichloromethane:methanol 80:20). IR ν_{max} (film) 2964, 2785 (CH), 1604 (C=C), cm^{-1}. ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.90 (3H, t, CH_3), 2.01-2.03 (4H, s, CH_2), 2.04-2.06 (2H, q, CH_2), 3.15 (4H, s, CH_2), 3.29-3.33 (2H, m, CH_2), 3.56 (2H, s, CH_2), 3.77-3.8 (2H, m, CH_2), 3.86 (3H, s, OCH_3), 6.81-6.92 (5H, m, ArH), 7.11-7.14 (6H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.80 (CH_3), 22.90 (CH_2), 24.27 (CH_2), 29.26 (CH_2), 36.82 (CH_2)$_2$, 53.85 (CH_2), 54.7 (OCH_3), 64.98 (CH_2), 112.68-140.07 (ArC), 156.96 (ArC-OCH_2), 157.55 (ArC-OCH_3). HRMS(EI) Found 442.2772 (M^++H), $C_{30}H_{36}NO_2$ requires 442.2746.

2 - Benzyl - 1-(4-methoxyphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (32)

2-Benzyl-1-(4-hydroxyphenyl)-1-(4-methoxyphenyl) - but-1-ene **29** (0.45 mmol) was dissolved in acetone:water 19:1 (10 mL) with potassium carbonate (0.54 mmol) and 1-(2-chloroethyl)pyrrolidine.HCl (0.9 mmol) added and the mixture was refluxed for 5 hours in darkness. The solvent was removed and the reaction residue purified by flash column chromatography (eluant: dichloromethane:methanol 80:20). The product was obtained as a light brown oil (36%), (R_f 0.2 dichloromethane:methanol 19:1). IR ν_{max} (film) 2932, 2963 (CH), 1606 (C=C) cm^{-1}. ^{1}H-NMR (400MHz, $CDCl_3$): 0.95 (3H, t, CH_3), 1.77 (4H, s,CH_2), 2.03 (2H, q,CH_2), 2.61-2.63 (4H, s, CH_2), 3.06-3.1 (2H, m,CH_2), 3.4 (2H, s,CH_2), 3.76 (3H, s, OCH_3), 4.26 (2H, m,CH_2), 6.78-7.28 (13H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.84 (CH_3), 21.13 (CH_2), 23.12 (CH_2), 24.24 (CH_2), 36.72 (CH_2), 50.00 (CH_2), 53.82 (OCH_3), 63.15 (CH_2), 113.01-140.1 (ArC), 154.96 (ArC-OCH_2), 157.58 (ArC-OCH_3). HRMS(EI): Found 442.2746 (M^++H), $C_{30}H_{36}NO_2$ requires 442.2782.

4-Methoxy-2'-pivaloyloxybenzophenone (33)

2-Hydroxy-4'-methoxybenzophenone [40] (4 mmol) was stirred in acetone (20 mL) with potassium hydroxide (35.6 mmol) for 3 hours. Trimethylacetyl chloride (24.8 mmol) was added and the reaction was left stirring overnight. The reaction mixture was poured into water, basified and the product was extracted with dichloromethane. The dichloromethane was dried over sodium sulphate and removed under reduced pressure. The product was purified using flash chromatography (eluant: CH_2Cl_2:ethyl acetate 19:1) 40%, (R_f 0.4 hexane:diethyl ether 5:1) and was used in the next reaction without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3402 (OH), 2926 (CH), 1748 (C=O), 1653 (C=O). ^{1}H-NMR (400MHz, $CDCl_3$): 1.07 (9H, s, $OC(CH_3)_3$), 3.84 (3H, s, OCH_3), 6.90-7.32 (4H, m, ArH), 7.45-7.50 (2H, m, Ar-H), 7.79 (2H, m, Ar-H). ^{13}C-NMR (100MHz, $CDCl_3$): δ 26.45 ((CH_3)$_3$), 31.09 ($C(CH_3)_3$), 54.89 (OCH_3), 113.16 -131.83 (ArC), 147.9 (ArC-OCO), 163.35 (ArC-OCH_3), 175.7 ,192.7 (2xC=O).

4-Hydroxy-2'-pivaloyloxybenzophenone (34)

4-Methoxy-2'-pivaloyloxy-benzophenone **33** (4.67 mmol) was stirred in dichloromethane (8 mL) while boron trifluoride-dimethyl sulphide (18.7 mmol) was added dropwise over 30 min. Stirring was continued for a further 10 hours at room temperature. The solvent was then removed and the residue was dissolved in ethyl acetate (200 mL) and washed with saturated sodium bicarbonate solution, water and brine. The ethyl acetate was dried over Na_2SO_4 and then removed under reduced pressure. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 50:50) and afforded the title compound (65%) (R_f 0.3 hexane:diethyl ether 1:1) which was used in the following reaction without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2973 (OH), 1751 (C=O), 1659 (C=O), 1571. ^{1}H-NMR (400MHz, $CDCl_3$):6.89-6.90 (2H, m, ArH), 7.27-7.76 (6H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 26.56 ((CH_3)$_3$), 38.7 ($C(CH_3)_3$), 114.9 -139.17 (ArC), 150.42 (ArC-OCO), 160.92 (ArC-OH), 194.73 , 208.11 (2xC=O). HRMS(EI) Found 297.1117 (M^+-H), $C_{18}H_{17}O_4$ requires 297.1127.

2 - Benzyl - 1-(4-hydroxyphenyl)-1-(2-pivaloylphenyl)-but-1-ene (37)

Following the general method above for **7** using titanium tetrachloride (11.75 mmol), zinc dust (37.6 mmol), 4-hydroxy-2'-pivaloyloxybenzophenone **34** (4.7 mmol) and 1-phenyl-2-butanone (14 mmol) in dry THF(10 mL). The crude product was purified by flash chromatography (eluant: hexane:dichloromethane 40:60) to afford the title compound (85%) as a light yellow oil (R_f 0.34 dichloromethane:hexane 6:4). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3357 (OH), 3034, 2924 (CH), 1757 (C=O), 1607 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$):0.98

(3H, t, CH_3), 1.35-1.38 (9H, s, $OC(CH_3)_3$), 2.08 (2H, q, CH_2), 3.6 (2H, s,CH_2), 5.47 (1H, s, OH), 6.71-7.33 (13H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.75 (CH_3), 20.45 (CH_2), 26.65-26.7 (($\underline{C}H_3)_3$), 36.75 ($\underline{C}(CH_3)_3$), 43.08 (CH_2), 114.31-144.37 (ArC), 150.42 (ArC-OCO), 153.95 (ArC-OH), 175.00 (C=O). HRMS(EI) Found 414.2400 (M^+), $C_{28}H_{30}O_3$ requires 414.2194.

2 - Benzyl - 1 -(4-hydroxyphenyl)-1-(3-pivaloyloxyphenyl)-but-1-ene (38)

Following the general method above for **7** using titanium tetrachloride (11.75 mmol), zinc dust (37.6 mmol), 4-hydroxy-3'-pivaloyloxybenzophenone [24] **35** (4.7 mmol) and 1-phenyl-2-butanone (14.1 mmol) in dry THF(10 mL). The crude product was purified by flash chromatography (eluant: hexane:dichloromethane 40:60) to afford the title compound (90%) as a light yellow oil (R_f 0.15 dichloromethane:hexane 6:4) which was used in the following reaction without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3363 (OH), 3027, 2932 (CH), 1755 (C=O), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$):1.05 (3H, t, CH_3), 1.41 (9H, s, $OC(CH_3)_3$), 2.11 (2H, q, CH_2), 3.63 (2H, s, CH_2), 5.78 (1H, s, OH), 6.70-6.72 (2H, m, ArH) 6.98 (2H, dd, ArH), 7.11-7.13 (2H, m, ArH), 7.27-7.32 (7H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.4 (CH_3), 24.4 (CH_2), 26.8 (($\underline{C}H_3)_3$), 36.7 ($\underline{C}(CH_3)_3$), 39.6 (CH_2), 113.7 -143.07 (ArC), 157.6 (ArC-OCO), 158.55 (ArC-OH), 173.5 (C=O). LRMS (*m/z*): $C_{28}H_{30}O_3$; 414.2 (M^++H, 2%).

2 - Benzyl - 1 -(4-hydroxyphenyl)-1-(4-pivaloyloxyphenyl)-but-1-ene (39)

Following the general method above for **7** using titanium tetrachloride (11.75 mmol), zinc dust (37.6 mmol), 4-hydroxy-4'-pivaloyloxybenzophenone [23] **36** (4.7 mmol) and 1-phenyl-2-butanone (14.0 mmol) in dry THF(10 mL). The crude product was purified by flash chromatography (eluant: hexane:dichloromethane 40:60) to afford the title compound (49%) as a light yellow oil, (R_f 0.3 dichloromethane:hexane 6:4) which was used in following reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3356 (OH), 3033, 2928 (CHs, 1764 (C=O), 1610 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$):0.97 (3H, t, CH_3), 1.35 (9H, s, $OC(CH_3)_3$), 2.07 (2H, q, CH_2), 3.56-3.59 (2H, m, CH_2), 5.60 (H, s, OH), 6.72-6.74 (2H, dd, ArH), 7.01 (11H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.8 (CH_3), 24.1 (CH_2), 25.23 (($\underline{C}H_3)_3$), 35.7 ($\underline{C}(CH_3)_3$), 39.6 (CH_2), 113.2 -140.12 (ArC), 156.12 (ArC-OH), 173.5 (C=O).

2 - Benzyl - 1-(2-pivaloylphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (40)

Following the general method 1 above, using disopropyl-azodicarboxylate (3.5 mmol), **37** (1.3 mmol), triphenyl-phosphine (2.6 mmol) and 1-(2-hydroxyethyl)-pyrrolidine (3.9 mmol) in dichloromethane (10 mL), the product was obtained by flash column chromatography on silica gel (eluant: dichloromethane:methanol 80:20) as a light brown oil,(19%),(R_f 0.25 dichloromethane:methanol 80:20). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2916, 2953 (CH), 1742 (C=O), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (3H, t, CH_3), 1.28-1.36 (9H, s, $OC(CH_3)_3$), 1.82 (4H, s, CH_2), 2.07 (2H, q, CH_2), 2.65-2.66 (4H, s, CH_2CH_2), 2.89-2.94 (2H, m, CH_2), 3.56 (2H, s, CH_2), 4.08-4.14 (2H, m, CH_2), 6.83-7.32 (13H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.74-12.78 (CH_3), 23.03 (CH_2), 24.31 (C-3, CH_2), 26.65-26.7 ($C(\underline{C}H_3)_3$), 30.39 (CH_2), 36.75-36.87 ($\underline{C}(CH_3)_3$), 38.55-38.6 (CH_2), 54.17-54.61 (($CH_2)_2$), 66.41 (CH_2), 113.49-144.27 (C-1'), 150.45 (ArC-OCO), 156.96 (ArC-OCH_2), 172.30 (C=O). HRMS(EI) Found 512.3152, (M^++H) , $C_{34}H_{42}NO_3$ requires 512.3164.

2-Benzyl-1-(3-pivaloyloxyphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (41)

Following the general method 2 above using **38** (0.45 mmol), potassium carbonate (0.54 mmol), and 1-(2-chloroethyl)pyrrolidine.HCl (0.9 mmol) the product was obtained as a light brown oil (87%) (R_f 0.3 dichloromethane: methanol 80:20). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2931, 2966 (CH), 1752 (C=O), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (3H, t, CH_3), 1.37 (9H, s, $OC(CH_3)_3$), 1.88 (4H, s, CH_2), 2.06 (2H, m, CH_2), 2.76 (4H, s, CH_2), 2.97 (2H, m, CH_2), 3.57 (2H, s, CH_2), 4.1-4.18 (2H, m, CH_2), 6.82-6.85 (3H, m, ArH), 6.91-6.94 (2H, m, ArH), 7.06-7.08 (1H, d, ArH), 7.12-7.31 (8H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 11.9 (C-4,), 22.4 (CH_2), 23.54 (CH_2), 25.68 ($\underline{C}H_3)_3$), 31.29 (CH_2), 37.70 ($\underline{C}(CH_3)_3$), 39.60 (CH_2), 40.32 (CH_2), 61.24 (CH_2), 113.9 -143.07 (ArC), 156.12 (ArC-OCO), 157.55 (ArC-OCH_2), 173.5 (C=O). HRMS(EI) Found 512.3199 (M^++H), $C_{34}H_{42}NO_3$ requires 512.3165.

2-Benzyl-1-(4-pivaloyloxyphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (42)

Following the general method 2 above, and using **39** (0.45 mmol), potassium carbonate (0.54 mmol), and 1-(2-chloroethyl)pyrrolidine.HCl (0.9 mmol), the product was obtained using flash column chromatography (eluant: dichloromethane:methanol 80:20) as a light brown oil (40%) (R_f 0.35 dichloromethane:methanol 80:20). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2919, 2968 (CH), 1748 (C=O), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (3H, t, CH_3), 1.34 (9H, s, $OC(CH_3)_3$), 1.79 (4H, s, CH_2), 2.03-2.05 (2H, q, CH_2), 2.64 (4H, s, CH_2), 3.13 (2H, s, CH_2), 3.73 (2H, s, CH_2), 3.90-3.94 (2H, m, CH_2), 6.81-7.02 (4H, m, ArH), 7.17-7.2 (9H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.72 (CH_3), 21.2 ($CH_2)_2$, 23.1 (CH_2), 24.23 (($\underline{C}H_3)_3$) , 29.21 (CH_2), 36.7 ($\underline{C}(CH_3)_3$), 38.6 (CH_2), 49.8 (CH_2), 63.24 (CH_2), 113.7 - 139.12 (ArC), 155.12 (ArC-OCH_2), 173.5 (C=O). HRMS(EI) Found 512.3164 (M^++H), $C_{34}H_{42}NO_3$ requires 512.3165.

2 - Benzyl - 1 -(2-hydroxyphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (43)

A solution of **40** 6.64 mmol was treated with sodium hydroxide (33.2 mmol) in water:ethanol (1:4), (5 mL) at room temperature for 4 hours. The reaction mixture was acidified with HCl (10%), (10 mL) and the product extracted with dichloromethane (4x40mL). The organic layers were combined, dried over Na_2SO_4 and the solvent removed under reduced pressure. The residue was chromatographed over

silica gel (eluant: dichloromethane:methanol 19:1) to afford the product as a yellow oil (45%), (R_f 0.35 dichloromethane: methanol 19:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3300 (OH), 2926, (CH), 1627 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.95-0.97 (3H, t, CH_3), 1.86 (4H, s, CH_2), 2.07-2.01 (2H, q, CH_2), 2.77 (4H, s, CH_2), 3.09 (2H, s, CH_2), 3.53 (2H, s, CH_2), 4.19-4.22 (2H, m, CH_2), 6.69-7.42 (13H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.81 (CH_3), 22.81 (CH_2), 24.72 (CH_2CH_2), 36.58 (CH_2), 53.86 (CH_2CH_2), 54.14 (CH_2), 65.36 (CH_2), 113.01 -144.45 (ArC), 155.63 , 156.34 (ArC-OH, ArC-OCH_2). HRMS(EI) Found 428.2578 (M^++H), $C_{29}H_{34}NO_2$ requires 428.2590.

2 - Benzyl - 1-(3-hydroxyphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (44)

Boron trifluoride-dimethyl sulphide (1.33 mmol) was added dropwise over 30 min to a solution of **31** (6.67 mmol) in dichloromethane (8 mL). Stirring was continued for 24 hours at room temperature. The solvent was removed, the residue dissolved in ethyl acetate (200 mL) and washed with saturated sodium bicarbonate solution (2 x 50 mL), water (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield the title product as a yellow oil (30%) (R_f 0.2 dichloromethane: methanol 19:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3400 (OH), 2926, 2854 (CH), 1596 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.95-0.99 (3H, t, CH_3), 1.84 (4H, s, CH_2), 2.00-2.07 (2H, q, CH_2), 2.70 (4H, s, CH_2), 2.95-2.96 (2H, s, CH_2), 3.50 (2H, s, CH_2), 4.09-4.12 (2H, m, CH_2), 6.78-7.31 (13H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.89 (CH_3), 22.9 (CH_2), 28.8 (CH_2CH_2), 31.30 (CH_2), 53.32 (CH_2CH_2), 54.09 (CH_2), 65.89 (CH_2), 113.67 -142.70 (ArC), 156.60, 156.98 (ArC-OH, ArC-OCH_2). HRMS(EI) Found 428.2595 (M^++H), $C_{29}H_{34}NO_2$ requires 428.2590.

2 - Benzyl - 1-(4-hydroxyphenyl)-1-[4-(pyrrolidinylethoxy) phenyl]but-1-ene (16)

(Method 2) Boron trifluoride-dimethyl sulphide (1.33 mmol) was added dropwise over 30 min to a solution of **32** (6.7 mmol in dichloromethane (8 mL) following the procedure outlined above. The title product was isolated following chromatography over silica gel (eluant: dichloromethane:methanol 19:1) as a yellow oil (10%) (R_f 0.2 dichloromethane:methanol 19:1) and was identical to the sample prepared above HRMS(EI) Found 428.2563 (M^++H), $C_{29}H_{34}NO_2$ requires 428.2590.

4-[2-Benzyl-1-(4-fluorophenyl)-but-1-enyl]-phenol (47)

Following the general method for **7**, using titanium tetrachloride (11.75 mmol) zinc dust (37.6 mmol), 4-hydroxy-4'-fluorobenzophenone **45** (14.7 mmol) and 1-phenyl-2-butanone (14.1 mmol) in dry tetrahydrofuran (100 mL). The title product was obtained following flash column chromatography on silica gel (100%) as a brown oil, (eluant: dichloromethane:hexane 1:1)., R_f 0.85 (dichloromethane) IR: ν_{max} ($CHCl_3$) cm^{-1}: 3360 (OH), 2967 (CH), 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 1.02 (3H, t, CH_3), 2.43-2.47 (2H, m, CH_2), 3.65 (2H, s, CH_2), 6.85-6.87 (2H, m, CH_2), 7.00-7.06 (2H, m, ArH), 7.11-7.15 (2H, m, ArH), 7.22-7.28 (5H, m, ArH), 7.32-7.34 (3H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.85 (CH_3), 24.3 (CH_2), 36.78 (CH_2), 114.36-154.6 (ArC), 159.73 (ArC-OH), 162.17 (ArC-F). ^{19}F NMR ppm ($CDCl_3$) -117.0 (ArC-F). HRMS(EI) Found 332.1584 (M^+), $C_{23}H_{21}FO$ requires 332.1576.

4-[2-Benzyl-1-(4-bromophenyl)-but-1-enyl]-phenol (48)

Following the general method for 7 using zinc (12.0 mmol) powder, dry tetrahydrofuran (100 mL), titanium tetrachloride (6.0 mmol), 46 (1.40 mmol) and 1-phenyl-2-butanone (1.41 mmol), the product was purified by flash column chromatography over silica gel (eluant: hexane: diethyl ether 1:1). The title product was obtained as a brown oil (100%) (R_f 0.3 hexane). IR: ν_{max} ($CHCl_3$) cm^{-1} :3368 (OH), 2966 (CH), 1594 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.98-1.0 (3H, m, CH_3), 2.03-2.08 (2H, m, CH_2), 3.6 (2H, s, CH_2), 6.77-6.79 (2H, m, ArH), 7.08-7.10 (4H, m, ArH), 7.22 (3H, m, ArH), 7.30-7.33 (2H, d, ArH), 7.44-7.47 (2H, d, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 13.70 (CH_3), 24.28 (CH_2), 36.7 (CH_2), 114.7 -141.92 (ArC), 154.05 (ArC-OH). HRMS(EI) Found 392.0079 (M^+), $C_{23}H_{21}BrO$ requires 392.0076.

1-(2-{4-[2-Benzyl-1-(4-fluorophenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (49)

Following the general method 1, to a solution of 47 (4.626 mmol), triphenylphosphine (9.252 mmol) and 1-(2-hydroxyethyl)-pyrrolidine (13.8 mmol) in dichloromethane (10 mL) was added disopropylazodicarboxylate (11.6 mmol) slowly over 20 minutes at room temperature and left stirring for 12 hours. Flash column chromatography was used to purify the product (eluant: chloroform:methanol 4:1) which was recovered as a yellow oil (80%) (R_f 0.6 chloroform: methanol 4:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2853, 2930 (CH), 1604 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97-0.99 (3H, m, CH_3), 1.85 (4H, s, CH_2), 2-2.06 (2H, m, CH_2), 2.65 (4H, s, CH_2CH_2), 2.92-2.95 (2H, m, CH_2), 3.58 (2H, s, CH_2), 4.10-4.13 (2H, m, CH_2), 6.83-6.85 (2H, m, ArH), 6.98-7.02 (2H, m, ArH), 7.11-7.14 (2H, m, ArH), 7.14-7.22 (5H, m, ArH), 7.29-7.31 (2H, d, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ12.80 (CH_3), 23.00 (CH_2CH_2), 24.23 (CH_2), 36.7 (CH_2), 54.18 (CH_2CH_2), 54.60 (CH_2), 66.20 (CH_2), 113.75-140.07(ArC), 156.8 (ArC-OCH_2), 159.70 (ArC-F). ^{19}F NMR ppm ($CDCl_3$) -117.12 (ArF). HRMS(EI) Found 430.2546 (M^++H), $C_{29}H_{32}FNO$ requires 430.2541.

1-(3-{4-[2-Benzyl-1-(4-bromophenyl)-but-1-enyl]-phenyl}-propyl)-pyrrolidine (50)

Following the general method 2, a solution of 1-(2-benzyl-1-phenylbut-1-enyl)-4-bromobenzene 48 (0.4 mmol) in acetone:water 19:1 (10 mL) was heated under reflux with potassium carbonate (0.46 mmol), and 1-(2-chloroethyl) pyrrolidine.HCl, (1.9mmol). The product was obtained following purification by flash column chromatography (eluant: hexane:diethyl ether:methanol 1:1:1) as a light brown oil (95%) (R_f 0.1 hexane:diethyl ether:methanol 1:1:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2966 (CH), 1605 (C=C).

^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97-0.99 (3H, m, CH_3), 1.84 (4H, s, CH_2), 2.05-2.40 (2H, m, CH_2), 2.66-2.68 (4H, s, CH_2CH_2), 2.90-2.93 (2H, m, CH_2), 3.58 (3H, s, OCH_3), 4.08-4.11 (2H, m, CH_2), 6.81-6.82 (2H, m, ArH), 7.08-7.11 (4H, m, ArH), 7.18-7.23 (4H,m, ArH), 7.28-7.32 (5H, m, ArH), 7.43-7.44 (1H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.79 (CH_3), 22.98 (CH_2-CH_2), 24.24 (CH_2), 36.7(CH_2), 54.17 (CH_2-CH_2), 54.52 (CH_2), 66.27 (CH_2), 113.79-139.95 (ArC), 141.87 (ArC-Br). HRMS(EI) Found 490.1740 (M^++H), $C_{29}H_{32}BrNO$ requires 490.1745.

Biochemical Evaluation of Activity

All assays were performed in triplicate for the determination of mean values reported. Compounds were assayed as the free bases isolated from reaction.

Antiproliferation Studies

The human breast tumor cell line MCF-7 was cultured in Eagles minimum essential medium in a 95%O_2/5% CO_2 atmosphere with 10% fetal calf serum. The medium was supplemented with 1% non-essential amino acids. Cells were trypsinised and seeded at a density of 1.5 x 10^4 into a 96-well plate and incubated at 37°C, 95%O_2/5% CO_2 atmosphere for 24 h. After this time they were treated with 2 μL volumes of test compound which had been pre-prepared as stock solutions in ethanol to furnish the concentration range of study, 1 nM–100 μM, and re-incubated for a further 72 h. Control wells contained the equivalent volume of the vehicle ethanol (1% v/v). The culture medium was then removed and the cells washed with 100 μL phosphate buffered saline (PBS) and 50 μL MTT added, to reach a final concentration of 1 mg/mL MTT added. Cells were incubated for 2 h in darkness at 37°C. At this point solubilization was begun through the addition of 200 μL DMSO and the cells maintained at room temperature in darkness for 20 min to ensure thorough colour diffusion before reading the absorbance. The absorbance value of control cells (no added compound) was set to 100 % cell viability and from this graphs of absorbance versus cell density per well were prepared to assess cell viability and from these, graphs of percentage cell viability versus concentration of subject compound added were drawn.

Cytotoxicity Studies

Human MCF-7 breast cancer cells were plated at a density of 1.5 x 10^4 per well in a 96-well plate, then incubated at 37°C, 95%O_2/5% CO_2 atmosphere for 24 h. Cells were treated with the compound of choice at varying concentrations (1nM–100 μM), then incubated for a further 72 h. Following incubation 50 μL aliquots of medium were removed to a fresh 96-well plate. Cytotoxicity was determined using and LDH assay kit obtained from Promega, following the manufacturer's instructions for use. A 50 μL per well LDH substrate mixture was added and the plate left in darkness at room temperature for equilibration. Stop solution (50 μL) was added to all wells before reading the absorbance at 490 nm. A control of 100% lysis was determined for a set of untreated cells which were lysed through the addition of 20 μL lysis solution to the media 45 min prior to harvesting. Data was presented following calculation, as percentage cell lysis versus concentration of subject compound.

Receptor Binding Assay

ERα and ERβ fluorescence polarization based competitor assay kits were obtained from Panvera at Invitrogen Life Technologies. The recombinant ER(insect expressed, full length, untagged human ER obtained from recombinant baculovirus-infected insect cells) and the fluorescent estrogen ligand were removed from the -80 C freezer and thawed on ice for one hour prior to use. The fluorescent estrogen ligand(2nM) was added to the ER (30nM for ERα and 20nM for ERβ) and screening buffer (100mM potassium phosphate (pH 7.4), 100μg /mL BGG, 0.02%NaN_3 was added to make up to a final volume that was dependant on the number of tubes used (number of tubes (e.g.50) x volume of complex in each tube(50μL) = total volume (e.g.2500μL). Test compound (1μL , concentration range 1nM) to 100μM) was added to 49μL screening buffer in each borosilicate tube (6mm diameter). To this 50μL of the fluorescent estrogen / ER complex was added to make up a final volume of 100μL and final concentration range for the test compound of 0.01nM to 1μM. A vehicle control contained 1% (v/v) of ethanol and a negative control contained 50μLscreening buffer and 50μL fluorescent estrogen /ER complex. The negative control was used to determine the polarisation value when no competitor was present (theoretical maximum polarization). The tubes were incubated in the dark at room temperature for 2 hours and were mixed by shaking on a plate shaker. The polarization values were read on a Beacon single-tube fluorescent polarization instrument with 485nm excitation and 530nm emission interference filters. For ERα and ERβ, graphs of anisotropy (mA) versus competitor concentration were obtained for determination of IC_{50} values.

Fluorescence Activated Cell Sorting (FACS)

The following assay was completed to determine the apoptotic induction by compounds. The MCF-7 cells were seeded at a density of 8x10^5 cells /25cm^2 flask in 25mL of complete medium. After 24 hours, the cells were treated with either vehicle control (1% ethanol v/v) or apoptotic compounds (50μM). Following incubation for up to 72 hours, the cells were washed with three times with PBS before being trypsinised and centrifuged at 300g for 5 min. The pellet was then resuspended in 200μl PBS, made up to 2ml with ice-cold ethanol (70% v/v) and left to sit on ice for at least 1h to fix them. Approximately 1h prior to use they were centrifuged at 300g for 3min and the supernatant carefully pipetted off. The pellet was resuspended in 800μ PBS. RNAase (100μL; 1mg/mL) and 100μL of the fluorescent dye propidium iodide (PI; 400μg/mL) which binds DNA were added. The tubes were vortexed and incubated in the dark at 37° for 30 min. Flow cytometry was performed with a FACS caliber flow cytometer from Becton Dickinson. FACS data was analysed using the programme Cell Quest. The presence of a subG1 peak is indicative of apoptotic cells.

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^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97-0.99 (3H, m, CH_3), 1.84 (4H, s, CH_2), 2.05-2.40 (2H, m, CH_2), 2.66-2.68 (4H, s, CH_2CH_2), 2.90-2.93 (2H, m, CH_2), 3.58 (3H, s, OCH_3), 4.08-4.11 (2H, m, CH_2), 6.81-6.82 (2H, m, ArH), 7.08-7.11 (4H, m, ArH), 7.18-7.23 (4H,m, ArH), 7.28-7.32 (5H, m, ArH), 7.43-7.44 (1H, m, ArH). ^{13}C-NMR (100MHz, $CDCl_3$): δ 12.79 (CH_3), 22.98 (CH_2-CH_2), 24.24 (CH_2), 36.7(CH_2), 54.17 (CH_2-CH_2), 54.52 (CH_2), 66.27 (CH_2), 113.79-139.95 (ArC), 141.87 (ArC-Br). HRMS(EI) Found 490.1740 (M^++H), $C_{29}H_{32}BrNO$ requires 490.1745.

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The human breast tumor cell line MCF-7 was cultured in Eagles minimum essential medium in a 95%O_2/5% CO_2 atmosphere with 10% fetal calf serum. The medium was supplemented with 1% non-essential amino acids. Cells were trypsinised and seeded at a density of 1.5 x 10^4 into a 96-well plate and incubated at 37°C, 95%O_2/5% CO_2 atmosphere for 24 h. After this time they were treated with 2 μL volumes of test compound which had been pre-prepared as stock solutions in ethanol to furnish the concentration range of study, 1 nM–100 μM, and re-incubated for a further 72 h. Control wells contained the equivalent volume of the vehicle ethanol (1% v/v). The culture medium was then removed and the cells washed with 100 μL phosphate buffered saline (PBS) and 50 μL MTT added, to reach a final concentration of 1 mg/mL MTT added. Cells were incubated for 2 h in darkness at 37°C. At this point solubilization was begun through the addition of 200 μL DMSO and the cells maintained at room temperature in darkness for 20 min to ensure thorough colour diffusion before reading the absorbance. The absorbance value of control cells (no added compound) was set to 100 % cell viability and from this graphs of absorbance versus cell density per well were prepared to assess cell viability and from these, graphs of percentage cell viability versus concentration of subject compound added were drawn.

Cytotoxicity Studies

Human MCF-7 breast cancer cells were plated at a density of 1.5 x 10^4 per well in a 96-well plate, then incubated at 37°C, 95%O_2/5% CO_2 atmosphere for 24 h. Cells were treated with the compound of choice at varying concentrations (1nM–100 μM), then incubated for a further 72 h. Following incubation 50 μL aliquots of medium were removed to a fresh 96-well plate. Cytotoxicity was determined using and LDH assay kit obtained from Promega, following the manufacturer's instructions for use. A 50 μL per well LDH substrate mixture was added and the plate left in darkness at room temperature for equilibration. Stop solution (50 μL) was added to all wells before reading the absorbance at 490 nm. A control of 100% lysis was determined for a set of untreated cells which were lysed through the addition of 20 μL lysis solution to the media 45 min prior to harvesting. Data was presented following calculation, as percentage cell lysis versus concentration of subject compound.

Receptor Binding Assay

ERα and ERβ fluorescence polarization based competitor assay kits were obtained from Panvera at Invitrogen Life Technologies. The recombinant ER(insect expressed, full length, untagged human ER obtained from recombinant baculovirus-infected insect cells) and the fluorescent estrogen ligand were removed from the -80 C freezer and thawed on ice for one hour prior to use. The fluorescent estrogen ligand(2nM) was added to the ER (30nM for ERα and 20nM for ERβ) and screening buffer (100mM potassium phosphate (pH 7.4), 100μg /mL BGG, 0.02%NaN_3 was added to make up to a final volume that was dependant on the number of tubes used (number of tubes (e.g.50) x volume of complex in each tube(50μL) = total volume (e.g.2500μL). Test compound (1 μL , concentration range 1nM) to 100μM) was added to 49μL screening buffer in each borosilicate tube (6mm diameter). To this 50μL of the fluorescent estrogen / ER complex was added to make up a final volume of 100μL and final concentration range for the test compound of 0.01nM to 1μM. A vehicle control contained 1% (v/v) of ethanol and a negative control contained 50μLscreening buffer and 50μL fluorescent estrogen /ER complex. The negative control was used to determine the polarisation value when no competitor was present (theoretical maximum polarization). The tubes were incubated in the dark at room temperature for 2 hours and were mixed by shaking on a plate shaker. The polarization values were read on a Beacon single-tube fluorescent polarization instrument with 485nm excitation and 530nm emission interference filters. For ERα and ERβ, graphs of anisotropy (mA) versus competitor concentration were obtained for determination of IC_{50} values.

Fluorescence Activated Cell Sorting (FACS)

The following assay was completed to determine the apoptotic induction by compounds. The MCF-7 cells were seeded at a density of 8x10^5 cells /25cm^2 flask in 25mL of complete medium. After 24 hours, the cells were treated with either vehicle control (1% ethanol v/v) or apoptotic compounds (50μM). Following incubation for up to 72 hours, the cells were washed with three times with PBS before being trypsinised and centrifuged at 300g for 5 min. The pellet was then resuspended in 200μl PBS, made up to 2ml with ice-cold ethanol (70% v/v) and left to sit on ice for at least 1h to fix them. Approximately 1h prior to use they were centrifuged at 300g for 3min and the supernatant carefully pipetted off. The pellet was resuspended in 800μ PBS. RNAase (100μL; 1mg/mL) and 100μL of the fluorescent dye propidium iodide (PI; 400μg/mL) which binds DNA were added. The tubes were vortexed and incubated in the dark at 37° for 30 min. Flow cytometry was performed with a FACS caliber flow cytometer from Becton Dickinson. FACS data was analysed using the programme Cell Quest. The presence of a subG1 peak is indicative of apoptotic cells.

Estrogenic Activity: Alkaline Phosphatase Assay

Following the procedure of Littlefield *et al.* [36], human Ishikawa cells are maintained in Eagle's Minimum Essential Medium (MEM containing 10% vol/vol fetal bovine serum (FBS) and supplemented with 100 U/mL penicillin and 10μg/mLl streptomycin, 2mM glutamine and 1mM sodium pyruvate. Cells were plated at 1.5 x 10^6 cells/75cm^2 surface area and were cycled twice weekly. Twenty four hours before the start of the experiment, near confluent cells were changed to an estrogen-free medium (EFBM), A 1:1 mixture of phenol-free Ham's F-12 and Dulbescco's Modified Eagles Medium, together with the supplements listed above, and 5% calf serum, stripped of endogenous estrogens with dextran coated charcoal. On the day of the experiment, cells were harvested with 0.25% trypsin and plated in 96-well flat bottomed microtitre plates in EFBM at a density of 2.5x10^4 cells/well. Test compounds were dissolved in ethanol at 10^{-3}M, diluted with EFBM (final concentration of ethanol 0.1%)and filter sterilised. After addition of the test compounds, (plated in 50μL, added estradiol in 50μL, and blank medium to give a final volume 150μL) the cells were incubated at 37C in a humidified atmosphere containing 95%O_2/5% CO_2 for 72 hours. All experimental values were obtained in triplicate. The microtitre plates were then inverted and the growth medium removed. The plates were then rinsed by gentle immersion and swirling in 2 L of PBS (0.15M NaCl, 10mM sodium phosphate, pH 7.4). The plates were removed from the container, the residual saline in the plate was not removed, and the wash was repeated. The buffered saline was then shaken out, and the plate blotted on paper towel. The covers were replaced and the plates are held at -80 C for at least 15min. then thawed at room temperature for 5-10min. The plates were then placed on ice and 50μL ice cold solution containing 50mM *p*-nitrophenyl phosphate, 0.24mM $MgCl_2$ and 1M diethanolamine(pH 9.8) was added. The plates were warmed to room temperature (time zero), and the yellow colour from the production of *p*-nitrophenol was allowed to develop. The plates were monitored at 405nm until maximum stimulation of the cells shows absorbance of approximately 1.2.

COMPUTATIONAL METHODS

***Docking*:** PDB entry 3ERT [39] was downloaded from the RCSB website and utilised for all modelling studies. The file was manually edited to extract the OHT ligand. Hydrogens were added to the apo protein using the Biopolymer Module in InsightII from Accelrys, assuming a pH of 7.0 with charged residue capping, and random hydrogen orientation for water molecules. The protonated apo structure was written as a PDB file and used without further modification. A FlexX [44] input file was created in Sybyl 6.9 from Tripos by describing an active site of radius 10 Angstroms around the residue ensemble of Arg394, His524, Asp351 and Glu353. The receptor data file was modified so that Asp and Glu residues were treated as deprotonated acids, while Arg and His were protonated in the simulation. No explicit water was included in the active site treatment. Formal charge calculation was specified, and inversion of N3 centres was permitted. The DrugScore scoring function was selected for ranking of predicted poses. A total of 30 solutions was requested. On completion of the simulation all 30 solutions were manually inspected, with the highest ranking solution used for further analysis.

Ligand Protein Contacts: PDB entry 1ERR [47] was downloaded from the RCSB website and used with the 3ERT structure and the docked solution for **16** in the calculation of ligand protein contacts for the system [45,46].

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