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Design, Synthesis and Pharmacological Evaluation of Novel Heterocyclic Guanidine Derivatives as α_2-Adrenoceptor Antagonists for the Treatment of Depression

A thesis presented to Trinity College Dublin for the degree of Doctor of Philosophy

by

Aoife Flood B. A. (Mod.)

Under the supervision of Prof. Isabel Rozas

March 2012

Trinity College Dublin

Declaration

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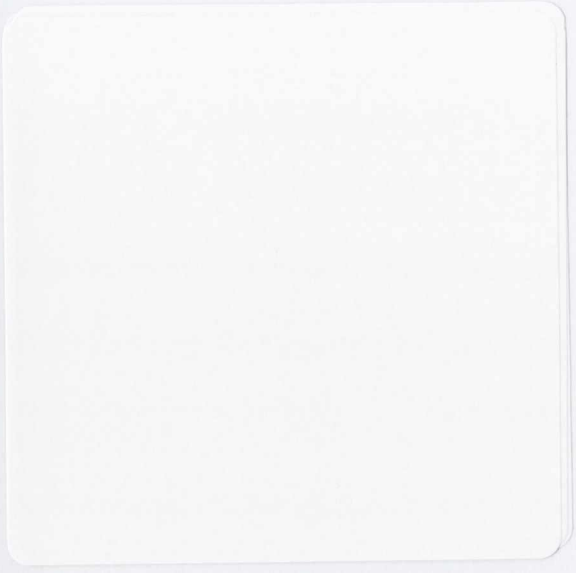

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Abbreviations

5-HT	5-hyroxytryptamine (serotonin)
5-HTP	5-hydroxytryptophan
5-HTTLPR	serotonin transporter linked polymorphic region
α_2-AR	α2-adrenoceptor
AcOAc	acetic anhydride
AcOH	acetic acid
ACTH	adrenocorticotropic hormone
app.	apparent
Arg	arginine
Asn	asparagine
Asp	aspartate
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
BINAP	2,2'-*bis*(diphenylphosphino)-1,1'-binapthyl
Boc	*tert*-butoxy-carbonyl
BOP	benzotriazole-1-yloxy-tris-(dimethylamino)phosphonium hexafluorophosphate
bp	base pair
brs	broad singlet
C	cytosine
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
COMT	catechol-*O*-methyltransferase
CREB	cAMP response element binding protein
CRF	corticotropin-releasing factors
CV	column volume
Cys	cysteine
d	doublet
D_2	dopamine 2 receptor
DA	dopamine
DCHPB	2-(dicyclohexylphosphino)biphenyl
DCM	dichloromethane

DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DG	denate gyrus
DiBocGua	di-*tert*-butoxycarbonyl guanidine
DIPEA	*N*,*N*-diisopropylethylamine
DMSO	dimethyl sulfoxide
EAS	electrophillic aromatic substitution
Et	ethyl
EtOH	ethanol
EtOAc	ethyl acetate
eq.	equivalent
ESI	electron spray ionisation
ES TOF	electron spray time of flight
Fmoc	9-fluromethyloxy carbonyl
FST	forced swimming test
G	guanine
GABA	gamma aminobutyric acid
Gβγ	the β and γ subunits of the G protein
GDP	guanosine diphosphate
Gly	glycine
GPCR	G-protein coupled receptor
GRK2/3	G protein-coupled receptor kinases 2 and 3
GTP	guanosine triphosphate
Gua	guanidine
Gua HCl	guanidine hydrochloride
$HgCl_2$	mercury (II) chloride
HgS	mercury sulfide
HMBC	heteronuclear multiple-bond correlation spectroscopy
HPA	hypothalamic-pituitary-adrenal
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
Hz	hertz
IR	infrared

L-Dopa	L-3,4-dihyroxyphenylalanine
Leu	leucine
Lit.	literature
LRMS	low resolution mass spectrometry
Lys	lysine
m	multiplet
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
mbar	millibar
MCH	melanin-concentrating hormone
Me	methyl
MeOH	methanol
µL	microlitre
mL	millilitre
mmHg	millimetres mercury
Mol	mole
MS	mass spectrometry
NA	noradrenaline
Na_2S	sodium sulfide
NaO^tBu	sodium *tert*-butoxide
NAc	nucleus accumbens
NAS	nucleophillic aromatic substitution
NAT	noradrenaline transporter
NCS	*N*-chlorosuccinimide
nM	nanomolar
NMR	nuclear magnetic resonance
PET	positron emission tomography
PFC	prefrontal cortex
Phe	phenylalanine
q	quartet
qC	quaternary carbon
quint	quintet
R_f	retention factor

r.t.	room temperature
RX821002	2-methoxy idazoxan
s	singlet
SAR	structural activity relationship
Ser	serine
SERT	serotonin transporter
SNAP	synaptosomal-associated protein
SNP	single nucleotide polymorphism
SNRI	serotonin and noradrenaline reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
t	triplet
^{t}Bu	*tert*-butyl
TCA	tricyclic antidepressant
TEA	triethylamine
TFA	trifluoroacetic acid
TST	tail suspension test
TLC	thin layer chromatography
THF	tetrahydrofuran
TOCSY	total correlation spectroscopy
TPH	tryptophan hydroxylase
TrkB	tyrosine kinase receptor B
UV	ultraviolet
VAMP	vesicle-associated membrane protein
VEGF	vascular endothelial growth factor
VTA	ventral tegmental area

Table of Contents

1 Introduction

1.1 Neurotransmission

Messages travel through the brain and nervous system in two ways, the first is in the form of an electrical impulse, called an action potential, which travels along a cell called a neuron. The second is via a chemical messenger known as neurotransmitter, which travels through the gap between two neurons. The process by which the neurotransmitter travels through the intracellular gap or synapse is known as *neurotransmission* (Figure 1.1). Neurotransmitters are taken up into storage vesicles at the end of the presynaptic neuron or axon terminus. There are two pools of storage vesicle in the neuron: the first pool or proximal pool are vesicles which are close to the cell membrane that can fuse with the membrane after an action potential and are thus ready for immediate release.[1] The second pool consists of reserve vesicles which are further away from the membrane, which can be recruited into the proximal pool after the release of neurotransmitters from the proximal pools. When an action potential reaches the presynaptic terminus, it alters the polarity of the cell membrane causing calcium ion channels on the cell membranes to open, which results in an influx of Ca^{2+} into the neuron. This in turn causes storage vesicles to fuse with the cell membrane, releasing the neurotransmitter they contain into the synapse. From there they diffuse across the synapse and can bind to receptors on the post synaptic neuron. If the number of binding events which occur exceed a threshold amount, a new action potential is fired in the post synaptic neuron propagating the signal. These postsynaptic receptors can either be ion channels which allow a specific ion or ions to enter the cell on receptor binding and thus affect the electrical potential of the cell directly, or they can be another protein which is altered by the receptor binding and causes a cascade of further binding events to occur. These types of receptor are respectively known as ionotropic and metabotropic receptors.

The neurotransmitter molecules can also interact with receptors on the presynaptic neuron, which can result in a reduction in the release of the neurotransmitter into the synapse. Such binding events inhibit further release of the neurotransmitter into the synapse, and thus allow for regulation of neurotransmitter release by a process known as feedback inhibition. When the receptor binds the same type of neurotransmitter that is released by that neuron these receptors are called autotropic receptors, whereas if they recognise a different neurotransmitter than that released by the synapse they are known as heteroceptors.

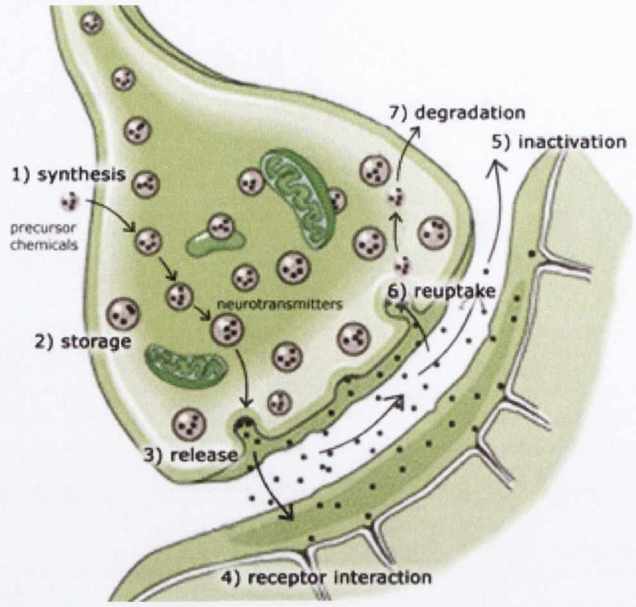


Figure 1.1 - Neurotransmission at a synapse[2]

After the neurotransmitters bind to the receptors they can be taken back up into the presynaptic neuron by reuptake proteins or can be metabolised in the synapse. In Figure 1.2, the structure of an adrenergic synapse is shown including a depiction of (i) noradrenaline (NA) anabolism in the synapse and within the vesicle, synthesis can be inhibited by α-methylparatyrosine (metyrosine) or Reserpine (ii) monoamine transporter proteins, which is inhibited by cocaine and tricyclic antidepressants and (iii) autoreceptors e.g the α_2-AR. Other features are the vesicle-associated membrane proteins (VAMPs) and synaptosomal-associated proteins (SNAPs) which are involved in the fusion of the vesicle to the cell membrane of the synapse, both of which can be inhibited by bretylium and guanethidine. The structures of the inhibitors mentioned are depicted in Figure 1.3.

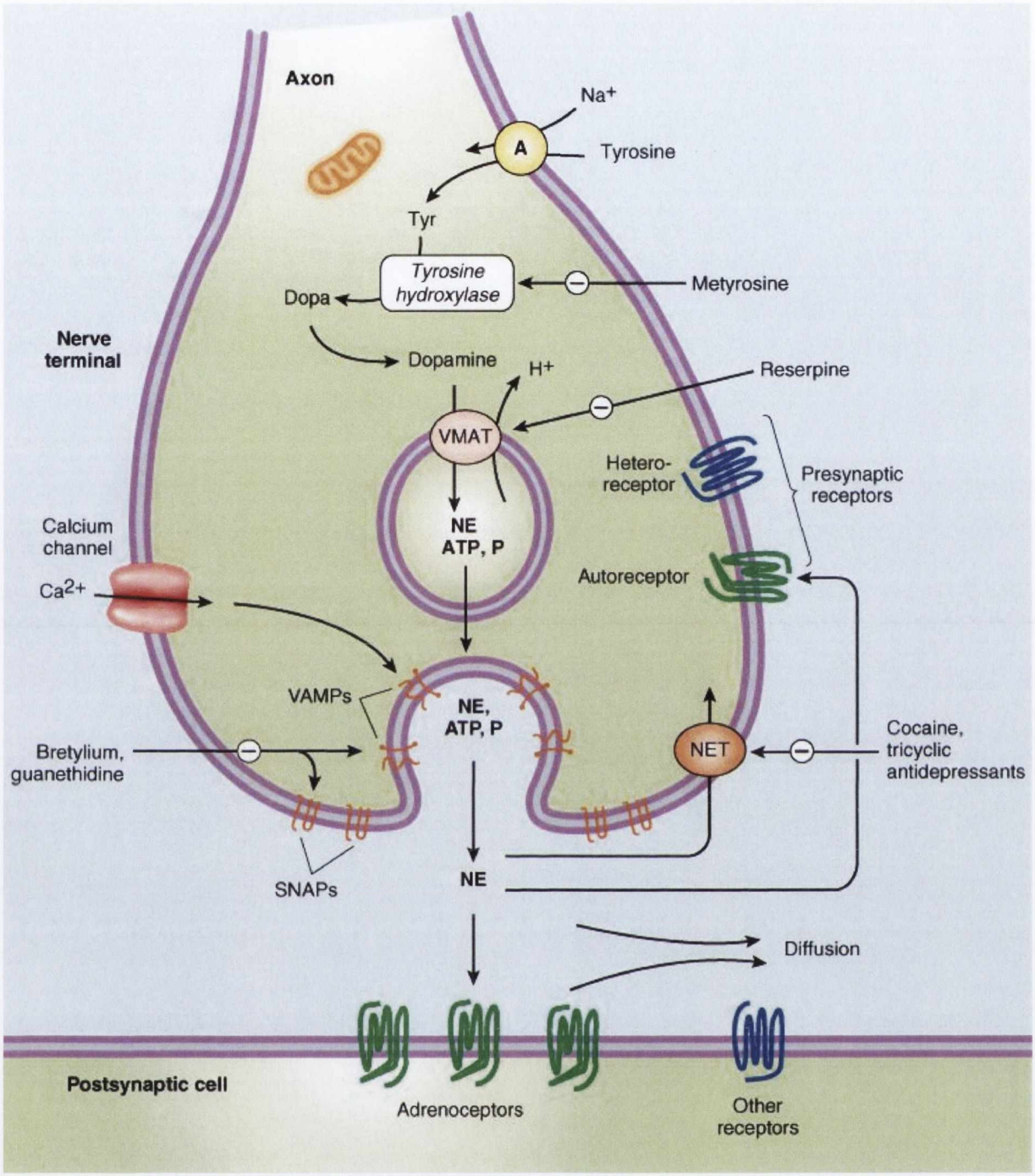


Figure 1.2 - The adrenergic synapse, depicting (a) the biosynthesis of NA (NE in image) in the synapse (top centre) and (b) vesicle (centre), (c) adrenoreceptors present on the postsynaptic cell (below), (d) autoreceptors e.g. the α_2-AR (right) (e) the NA transporter protein involved in reuptake (bottom right)[3]

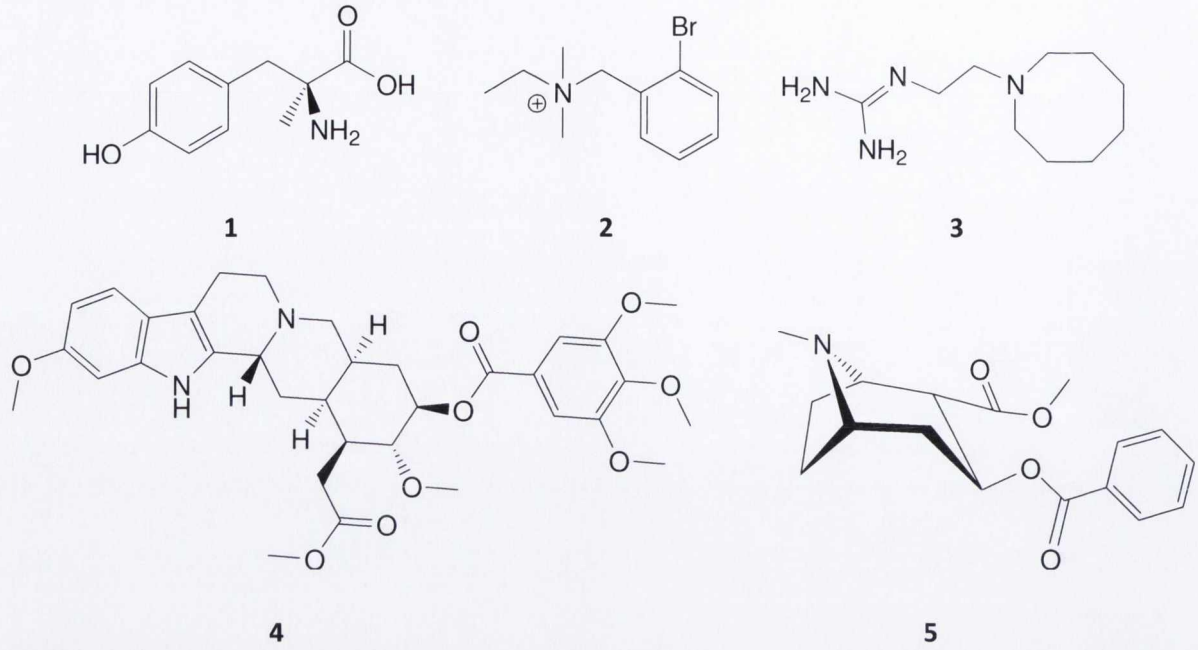


Figure 1.3 – Structures of α-methylparatyrosine (**1**), bretylium (**2**), guanethidine (**3**), reserpine (**4**) and cocaine (**5**)

1.2 The α_2-Adrenoceptor

1.2.1 Structure

The α_2-AR is a G-Protein Coupled Receptor (GPCR), a type of cell membrane protein, arranged into seven transmembrane helices, which span the cell membrane, with intracellular and extracellular loop regions which allow for interactions both inside and outside the cell.[4,5] The lengths of the three intracellular and four extracellular loops are fairly similar except for the intracellular loop connecting transmembrane helices V and VI, which varies depending on the receptor.[4] The α_2-AR differs from the α_1- and β- adrenoceptors by having relatively short amino and carboxyl termini and a very long third intracellular loop.[5]

GPCRs are coupled to a G protein which consists of three subunits, α, β and γ. The α subunit has a binding pocket which can bind guanyl nucleotides, specifically guanosine diphosphate (GDP) and guanosine triphosphate (GTP) which is vital for the protein's function. There are a number of different types of G protein, including G_s, G_i/G_o and $G_{q/11}$, with the α_2-AR being a G_i coupled protein. The main differences between the G protein is the activity of the α subunit. In particular, α_s stimulates adenylate cyclase, α_i inhibits that enzyme and may also activate K^+ channels, α_o inhibits Ca^{2+} ion channels and $\alpha_{q/11}$ activates an enzyme called phospholipase C.

Mutation of cysteine^{351} (Cys) in the G_i protein residue that binds to the α_2-AR, shows that reduced hydrophobicity at this amino acid leads to reduced agonist binding.[6] Studies of chimeric receptors have shown that substituting helix VII of the α_2-AR conferred weak yohimbine selectivity to the β_2-adrenoceptor, a feature specific to the α_2-AR, while substitution of helices I-IV was needed to confer full selectivity.[5] This suggests that helix VII is important for substrate binding to the receptor. Similarly if phenylalanine-412 (Phe) of helix VII is mutated to asparagine (Asn), the affinity for several α_2-AR antagonists is reduced by several orders of magnitude.[5] Mutation of aspartate-113 (Asp) to Asn eliminates the specific binding of Yohimbine.[5] Mutation studies show an interaction between serine-204 (Ser) with the *para* hydroxyl group of catecholamines such as NA, but no interaction between Ser-200 with the catecholamines *meta* hydroxyl group,[5] indicating that the *para* hydroxyl group may be more important in catechol binding. Homology modelling of the α_{2c}-AR based on bacteriorhodopsin as a template suggests that residues leucine-128 (Leu), Asp-131, Ser-183, Phe-184, Leu-211, Cys-214 and Phe-422 are important in agonist binding.[7] In particular Asp-131 on helix III forms a salt bridge with the protonated nitrogen of the substrates modelled.

1.2.2 Function

The α_2-AR is an autoreceptor inhibiting the release of NA, therefore antagonists of the receptor stop this negative feedback and increase NA concentrations in the synapse, resulting in antidepressant activity.[8] Blockade of the α_2-AR also has a role in the increase of levels of dopamine (DA),[9] acetylcholine[10] and 5-hyroxytryptamine or serotonin (5-HT)[11] in rat and human.

Upon binding of NA in a deep pocket between the transmembrane helices, the receptor and G_i protein bind changing conformation and allowing for the exchange of bound GDP for GTP (Figure 1.4). This is brought about by the G-protein coupled receptor kinases GRK2/3, which can recognise and phosphorylate the agonist bound receptor.[12] Site mutagenesis and recombinant expression studies have shown that the site of phosphorylation is Ser-360 in the carboxy-terminal region of the third intracellular loop of the α_{2A}-AR.[13] Upon binding GTP, the α subunit of the G protein splits from the others ($G\beta\gamma$). This α subunit is an inhibitor of adenylate cyclase, an enzyme which converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). This process is shown in Figure 1.4. This product is a secondary messenger involved in a signalling cascade which causes a decrease in Ca^{2+} influx following an action potential and results in a decrease in the release of NA into the synapse. Blocking the α_2-AR therefore prevents this decrease from occurring.

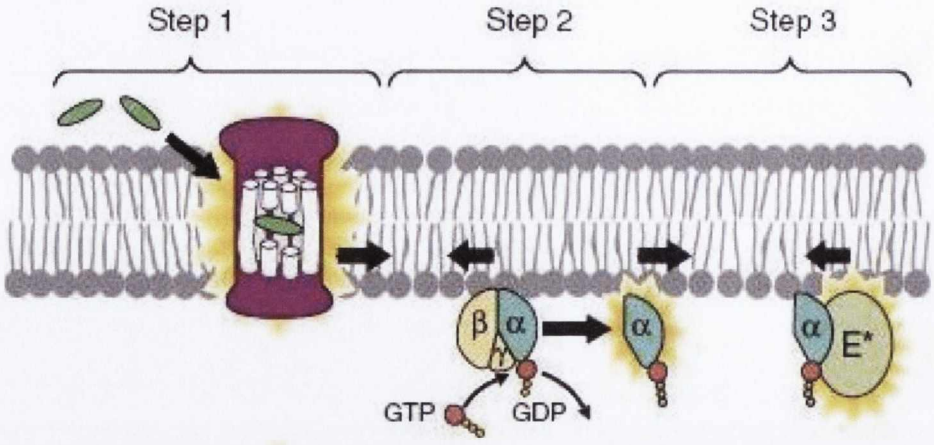


Figure 1.4 – Mode of Action of a GPCR[14]

As well as this, there are at least three conformations of the α_2-AR,[15] one of which has very high affinity for agonists but not for antagonists.[16] The high affinity conformation itself is induced by agonists, and when the α subunit dissociates from the G protein, the receptor then adopts the lower affinity conformation.

The Gβγ complex also plays a role by allowing the movement of a specific kinase for receptor regulation into the cell membrane.[17] Increased Gβγ availability also allows for increased phosphorylation of the agonist-bound receptor as it binds and activates GRK2/3.[17]

There are 4 subtypes of the receptor: α_{2A}-, α_{2B}-, α_{2C}- and α_{2D}-AR. The α_{2A}-AR is present throughout the human body, with the α_{2B}-AR largely in peripheral tissues.[18] Most prejunctional α_2-ARs in the rabbit cortex are of the α_{2A}- subtype, while the α_{2D}- subtype, a species homologue of α_{2A}-, is most common in the rat.[19] This indicates that the α_{2A}- subtype could be the most important in the treatment of depression, although other radioligand studies have shown a mixed population of α_2-AR subtypes in rat cortex, including the α_{2C}- subtype,[20] which is present almost exclusively in the central nervous system (CNS).[18] These findings give credence to the theoretical viability of using α_2-AR antagonists, and in particular antagonists of the α_{2A}- or α_{2c}- subtypes, in the treatment of depression. Further findings suggest that the α_{2A}- subtype is involved in controlling the release of NA and DA, whereas the α_{2C}- subtype is involved in regulating 5-HT release.[21,22]

A number of studies have implicated the role of the α_2-AR in depression. For example, depression has been associated with a selective increase in the high-affinity conformation of the α_{2A}-AR in the

brain.[15,16,23,24] A study by Callado *et al.* showed that the α_2-AR densities in suicide brain and in control groups were the same, whereas the density of α_2-ARs labelled by agonists were significantly higher than that for antagonists. Other studies have shown high densities of α_{2A}-AR in the prefrontal cortex (PFC) in depressed suicide victims upon post-mortem examination.[16] Chronic variable stress, a condition which is associated with depression, has been shown to induce supersensitivity of the α_2-AR in the rat.[24] Also, reserpine was found to increase the density of the high affinity state of the α_2-AR in the rat attributed to an increased turnover of the state.[15]

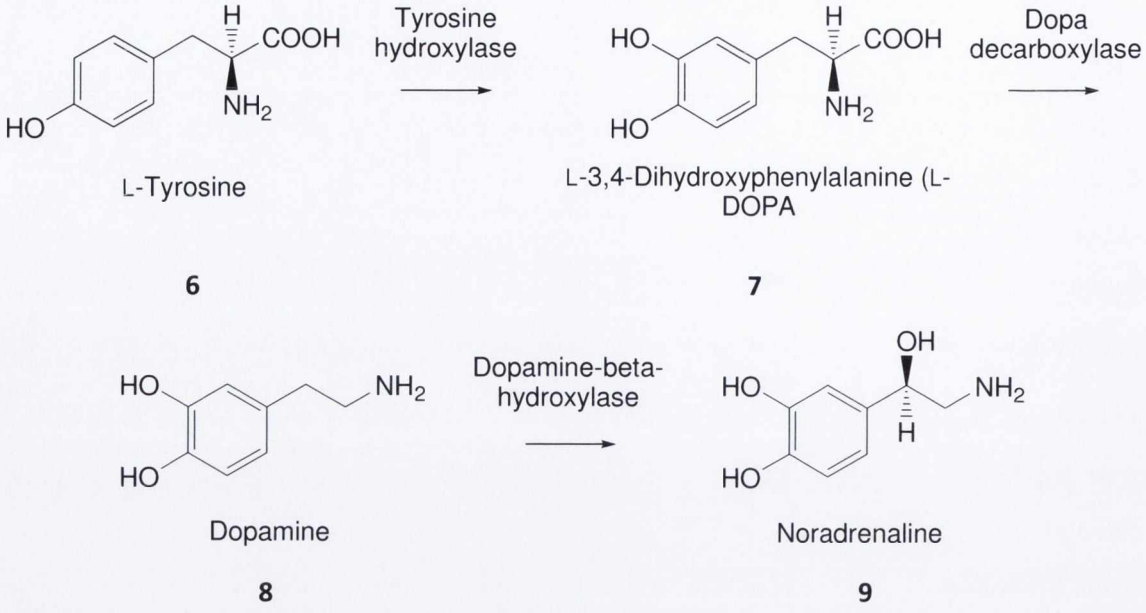
Additional experimental support for this theory comes from the following findings among others: Chronic antidepressant treatment has been found to cause in vivo desensitisation of the α_2-ARs involved in NA regulation.[21,25] In particular, endocrine responses to the α_2-AR agonist clonidine are reduced.[26] Antagonists of the α_2-AR have been shown to increase the release of NA in the prefrontal cortex,[27,28] and can also augment NA increase in conjunction with tricyclic antidepressant (TCA) drug desipramine.[29] Therefore, α_2-AR antagonists have potential application as both stand alone antidepressants and as combined treatments with other drug classes.

Analysis of the onset of action of mirtazapine *versus* the selective serotonin reuptake inhibitor (SSRI) antidepressants fluoxetine, paroxetine and citalopram has shown that more patients respond to treatment and reach remission earlier than with the SSRIs.[30] As well as this fact, patients taking α_2-AR antagonists, such as mirtazapine and yohimbine, to augment other antidepressants, including fluoxetine, show statistically significant remission rates.[31,32]

Few α_2-AR antagonists have been reported thus far in comparison to agonists, thus making the synthesis of novel α_2-AR antagonists and the elucidation of factors which imbue antagonistic properties and subtype specificity a foremost area of research.

1.3 Monoamines: Biosynthesis and Metabolism

The biosynthesis of NA begins with the synthesis of L-3,4-dihyroxyphenylalanine (L-Dopa) from tyrosine in the adrenergic synapse by the enzyme tyrosine hydroxylase (Scheme 1.1). Decarboxylation of L-Dopa by Dopa decarboxylase yields DA, which is then taken up into the storage vesicles within the synapse by a vesicular monoamine transporter protein. Finally, dopamine is converted to NA by dopamine-β-hydroxylase within the vesicle (Scheme 1.1)



L-Tyrosine

6

L-3,4-Dihydroxyphenylalanine (L-DOPA

7

Dopamine

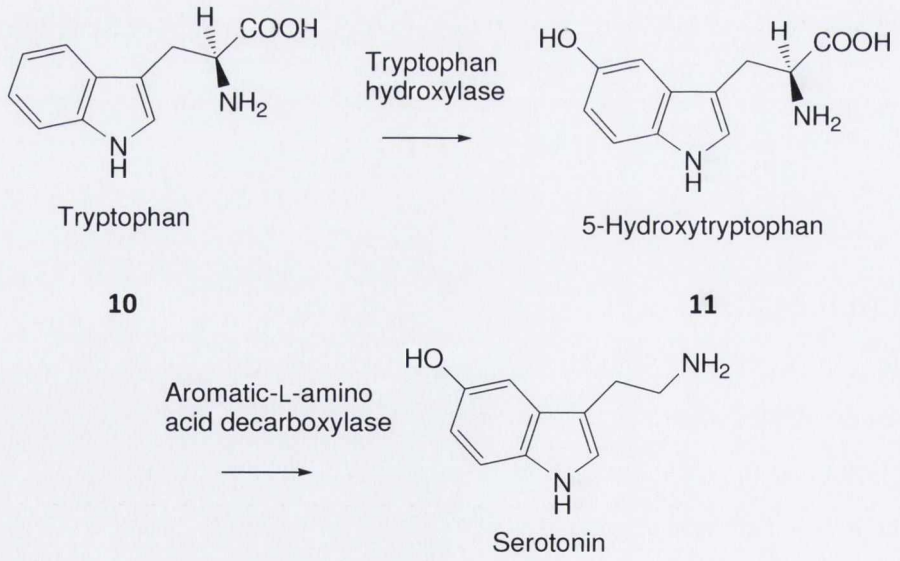
8

Noradrenaline

9

Scheme 1.1

The biosynthesis of 5-HT begins with the *meta* hydroxylation of the amino acid tryptophan to 5-hydroxytryptophan (5-HTP) through the action of the rate limiting enzyme tryptophan hydroxylase (TPH). Aromatic-L-amino acid decarboxylase then converts 5-HTP to 5-HT. This is depicted in Scheme 1.2.



Tryptophan

10

5-Hydroxytryptophan

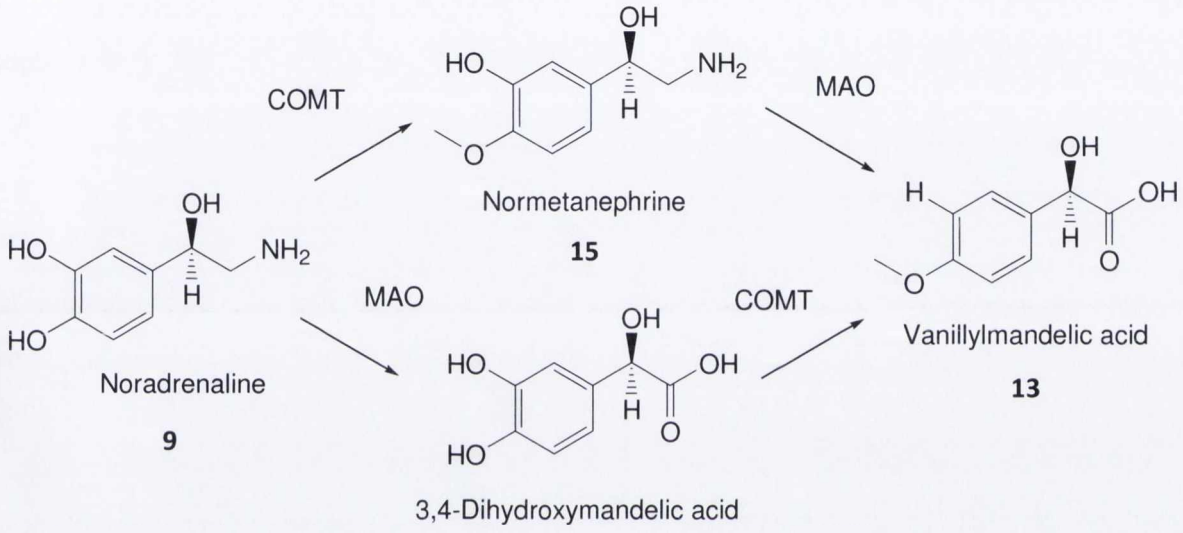
11

Serotonin

12

Scheme 1.2

NA catabolism involves two main enzymes, COMT and MAO, the latter of which has two subtypes - MAO-A and MAO-B – with MAO-A being more NA selective than MAO-B. Although breakdown of NA can begin with either MAO-A or COMT to give normethanephrine or 3,4-dihydroxymandelic acid respectively (Scheme 1.3), little or no COMT is found in monoaminergic neurons, thus making MAO-A the most important enzyme in the breakdown of NA in the synapse.[1] Action of the final enzyme results in Vanillylmandelic acid (Scheme 1.3).



9 **15** **13** **14**

Scheme 1.3

MAO-A also preferentially catabolises 5-HT to produce 5-hydroxyindolacetic acid as shown in Scheme 1.4.

12 **16**

Scheme 1.4

1.4 Depression

1.4.1 History and Symptoms

Depression is a common mental disorder that is characterised by a number of symptoms including sadness, decreased interest in pleasurable activities, weight change, insomnia or hypersomnia, fatigue, diminished ability to think or concentrate and recurrent thoughts of death or suicide.[33] It is more than just a feeling of sadness and can last from weeks to years. Depression cannot be clinically tested; therefore, a diagnosis of depression is made based on achieving a certain score on a number of symptomatic criteria for example on the diagnostic and statistical manual, the Hamilton rating scale for depression, the Montgomery-Åsberg depression rating scale or on the clinical global impression scale. High scoring individuals are deemed to have major depression, whereas those scoring in the intermediate range are termed dysthymia patients.

Sometimes there is a clear cause for depression such as bereavement, divorce, illness or redundancy, when this is the case it is known as reactive depression. Other types of depression include psychological depression where traumatic events cause a feeling of hopelessness about the future, and chemical depression, where there is no known external cause, as well as bipolar disorder where the patient experiences bouts of depression and elation.

In antiquity, Hippocrates first used the term melancholia (which means black bile) around 400 B.C. and recognised most of the major symptoms observed today in depression, including links to anxiety and excessive alcohol consumption.[34] It was not until the 19th century that a link was perceived between depression and the brain.

1.4.2 Statistics

Depression affects about 121 million people worldwide at any given time and it is estimated that about 15% of people will be affected at some point in their lives.[35] Prevalence is mainly in those between 15 and 30 years old, with more women affected than men,[36] but with men being more likely to commit suicide. According to Suicide Prevention approximately 400 people take their lives in Ireland every year, and it is estimated that the majority of these lives could be saved with antidepressant treatment and intervention.[37] A 2006 Central Statistics Office national survey reported that an estimated 17-20% of people in Ireland have a disability and that 9.5% of these people cite depression as their disability.[38] This means that almost 2% of people in Ireland were disabled due to depression.

According to the World Health Organization, it is one of the leading causes of disability and by 2020 it is expected to be the second largest health burden following heart diseases.[39] This means that the economic cost of depression will be greater than that of cancer by this date. The economic costs are considerable, with the International Labour Organization placing them at 3-4% of the gross national product in EU member states.[40] The cost of treating depression is only 2-11% of the total direct costs,[41] when indirect costs such as morbidity and mortality are taken into account, antidepressant treatment is only a small fraction of the total cost. This means that treating depression is cheaper than not treating it.

1.4.3 Causes

In multivariance familial, adoption and twin studies, it was shown that depression was 31-42% based on genetics and 58-67% on individual-specific environmental effects, and was therefore caused by both.[42] The non genetic causes are poorly defined, but could possibly be caused by childhood trauma or emotional stress,[43] physical illness or viral infections[44] such as Borna virus.[34]

Despite the prevalence of the condition, the exact physiological cause of depression remains unknown. The most widely accepted theory to date is the monoamine theory.[1,45] Aternative, non-monoaminergic theories exist; however, difficulties in finding reliable animal models to test these theories and the lack of alternative antidepressants reaching the market are notable problems with these theories.[44]

What all theories for depression have in common is that depression causes an imbalance in the process of neurotransmission at some point in the brain, with the frontal cortex and the hippocampus being the areas where this imbalance most likely occurs.[44] Indeed, hippocampal activity is seen to increase in the cornu ammonis and decrease in the denate gyrus (DG) using an animal model of depression.[46] Disturbances in these areas could result in the cognitive abnormalaties which are symptomatic of depression. Other areas where a disturbance could occur include the amygdala, which controls the brain's reward stimuli or the ventral tegmental area (VTA) and nucleus accumbens (NAc),[47] disruption of which can cause lack of motivation.

Given that depression is characterised by a set of symptoms rather than any one distinct pathophysiology, it is possible that depression consists of a number of separate illnesses with individual aetiologies.[44] For this reason, the investigation of novel types of antidepressants is of

considerable importance, and the understanding on how these antidepressants act upon neurotransmission is essential.

1.5 Theories for Depression

1.5.1 The Monoamine Theory

As mentioned earlier the most widely accepted theory is the monoaminergic hypothesis for depression, which states that depression occurs due to a depletion of the monoamine neurotransmitters NA, 5-HT and DA in the brain (see structures in Scheme 1.1 and Scheme 1.2).[1]

This depletion could occur due to a variety of different causes. Insufficient monoamine biosynthesis can occur due to a lack of dietary amino acids tyrosine or tryptophan or due to underexpression of any of the enzymes involved in the anabolism of the monoamines. Over expression of autotropic or heterotropic receptors such as the α_2-AR or the 5-HT_{1A}[26] or under expression of postsynaptic receptors could also result in depression. A number of cases in the literature support this theory and in particular implicate the importance of catecholamines such as NA in depression. Reserpine (**4**), a drug which was once used as an antihypertensive agent, inhibits the uptake of catecholamines into neuronal storage vesicles and causes release of NA from the reservoir storage vesicles (Figure 1.2). However, its use was discontinued as many patients experienced serious depression as a side effect.[45,4] This is due to the fact that too much of the neurotransmitter is initially released from the synapse and is quickly metabolised by MAO, resulting in a lower amount of reserve neurotransmitter left on the arrival of the next action potential, which in turn causes the depressive symptoms.

Similarly, α-methyl-*para*-tyrosine (**1**), used in the treatment of pheochromocytoma (catecholamine secreting tumours), caused a relapse of depression in patients being treated with NA reuptake inhibitors related to imipramine.[45] This is attributed to the drugs action as a competitive inhibitor of tyrosine hydroxylase, the rate limiting enzyme in catecholamine biosynthesis (Scheme 1.1). Amphetamine enhances the release of NA in the brain and its actions are enhanced by both MAOIs and NA reuptake inhibitors.[26] All of these cases demonstrate a link between reduced levels of NA in the brain and depression. Thus, treatment of depression involves increasing the concentration of either or both NA and 5-HT in the brain.

1.5.2 The Neurotrophic Theory of Depression

The neurotrophic theory of depression states that changes in the concentration of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), could be partially responsible for the reduced neurogenesis and structural damage seen in the brain in depression.[44,48] Therefore, the hippocampal pyramidal neurons which are innervated by monoaminergic, glutaminergic as well as other types of neurons, are abnormal in depression (Figure 1.5). Severe stress causes changes in these neurons, including reduced dendritic arborisation or birth of new neurons. BDNF has been described as producing antidepressant effects in behavioural models in the rodent.[49]

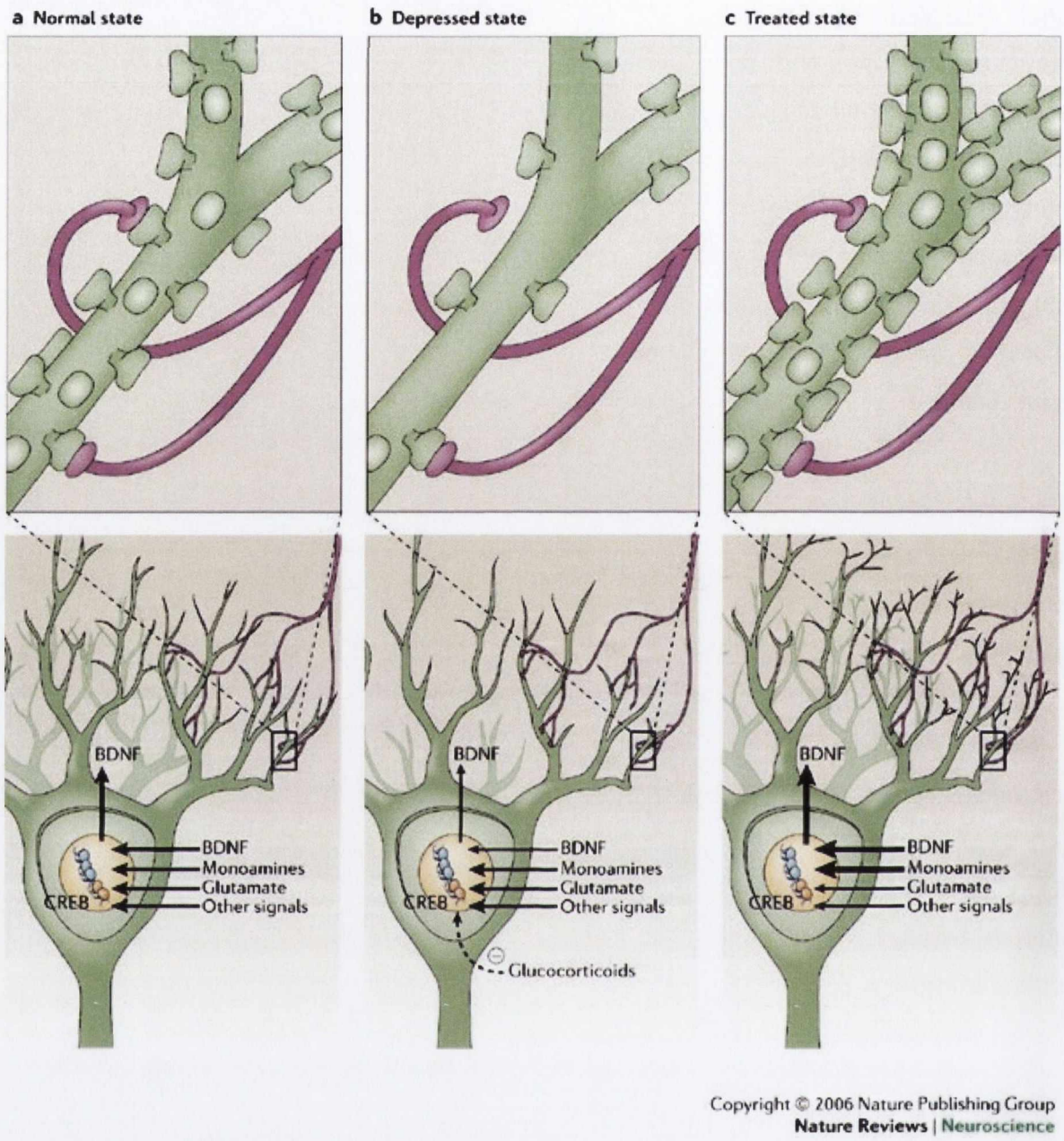


Figure 1.5 - Neurotropic mechanisms of depression and antidepressant action[44]

Neurotrophic factors were first discovered to regulate neural growth during development, but it has also now been shown that they play an important role in the plasticity and survival of adult neurons. BDNF itself is a protein of 14kDa, which binds to tyrosine kinase receptor B (TrkB) as a dimer, so it is difficult to develop small molecule agonists of TrkB.

BDNF regulates a complex cascade of post-receptor pathways involving the extracellular signal regulated kinase and phosphatidylinositol 3-kinase.[44] It may, therefore, be possible to target any of

the proteins downstream of BDNF in the signalling cascade but there are a number of problems. These include a lack of small molecule agonists available, lack of knowledge of which proteins are best to target and toxicity issues due to the presence of these proteins in many areas of the brain.

Antidepressants, such as fluoxetine, tranylcypromine and reboxetine, have been shown to increase neurogenesis and neurogenesis precursors such as BDNF, VEGF and trkB in the hippocampal DG.[46,50-53] In addition to this, blockade of the α_2-AR can accelerate the neurotrophic and behavioural effects of chronic antidepressant treatments.[54] In a 2010 paper, Jhaveri *et al.* reported that noradrenaline activates adult hippocampal neurogenesis via β_3 adrenergic receptors (β_3-AR).[55] Their conclusions were based on a minor drop in the levels of neurospheres formed using a β_3-AR antagonist, while they failed to note that a large increase in neurosphere formation compared to controls when an α_2-AR antagonist was used could indicate a role for that receptor in neurogenesis since it is an autoreceptor.

The role of neurogenesis in depression is summarised by Sahay *et al.* in saying "that adult hippocampal neurogenesis is more likely a substrate for the behavioural effects of antidepressants than a pivotal contributer to the etiology."[56]

1.5.3 Alternative Theories for Depression

Disturbances in the hypothalamic-pituitary-adrenal (HPA) gland, such as a hypersecretion of cortisol, have also been implicated in depression.[57] In particular, failure to suppress plasma cortisol concentrations after administration of dexamethasone, an agent which reduces the secretion of adrenocorticotropic hormone (ACTH) required for cortisol release, has been observed in untreated depressed patients,[58] and has been cited as a possible marker for depression.[59] Failure to suppress cortisol secretion has been most commonly found in patients with psychotic or mixed state depression.[60]

Corticotropin-releasing factors (CRF) are involved in regulating the HPA axis (Figure 1.6). This occurs when CRF is secreted from the paraventricular nuclei of the hypothalamus into the hypophyseal portal system and is then transported to the anterior pituitary causing the release of ACTH,[57] which in turn regulates the HPA axis. It is thought that if this theory of depression proves valid, CRF antagonists, vasopressin receptor antagonists and glucocorticoid ligands could prove to be effective antidepressants.[44] Interestingly, CRF also regulates catecholamine synthesis and release from the adrenal gland, influencing adrenaline and noradrenaline secretion.[61]

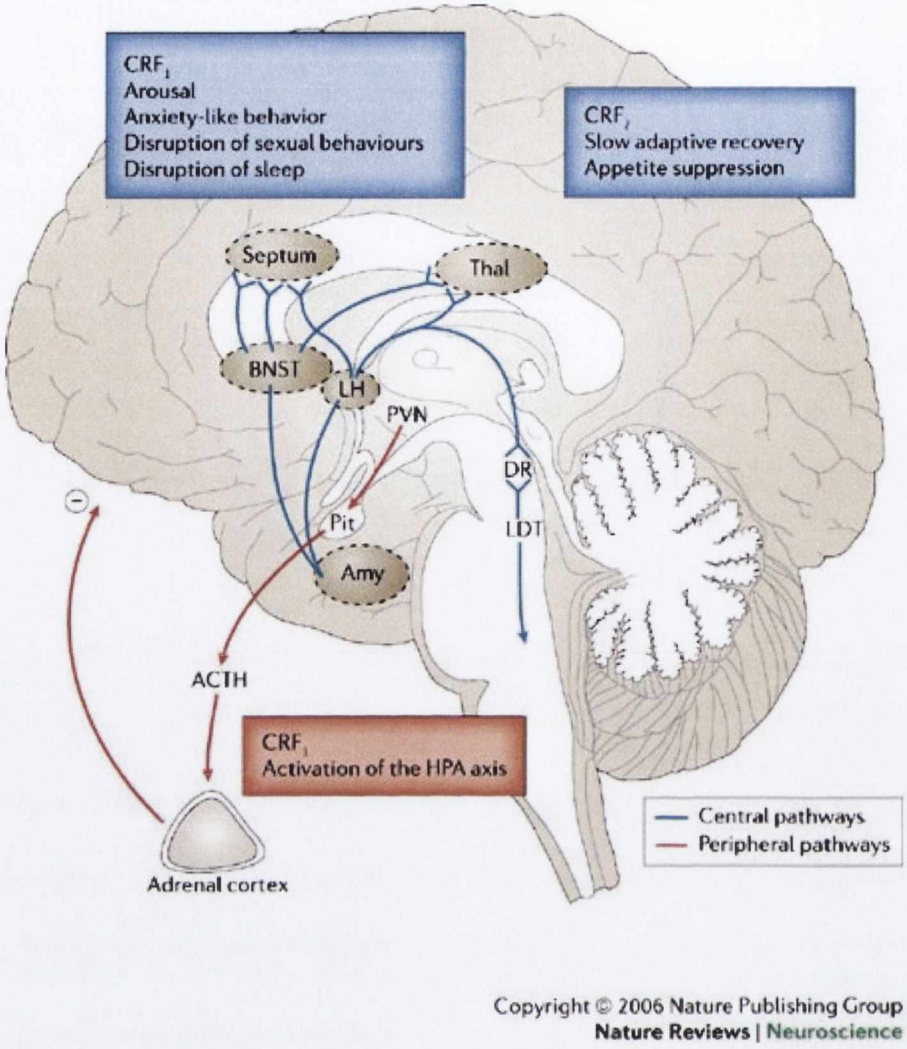


Figure 1.6 – The corticotrophin-releasing factor system in depression[44]

Other than the frontal cortex and the hippocampus, an area of the brain which is also implicated in depression is the NAc, which links to the VTA. This area of the brain is also important in drug abuse and is responsible for natural rewards such as food, sex and social interactions. Interestingly, it is reported that stress can cause many of the changes in this area which are seen after chronic drug abuse and that stress has been linked to depression.[47] Neurotransmission imbalances along this axis of DA and gamma aminobutyric acid (GABA), as well as protein imbalances of dynorphin and hypothalamic feeding peptides have all been implicated in depression.[44,47] Transcription factor Clock and circadian genes have also been associated with depression theories in this region of the brain. The key interactions involved in this area are depicted in Figure 1.7.

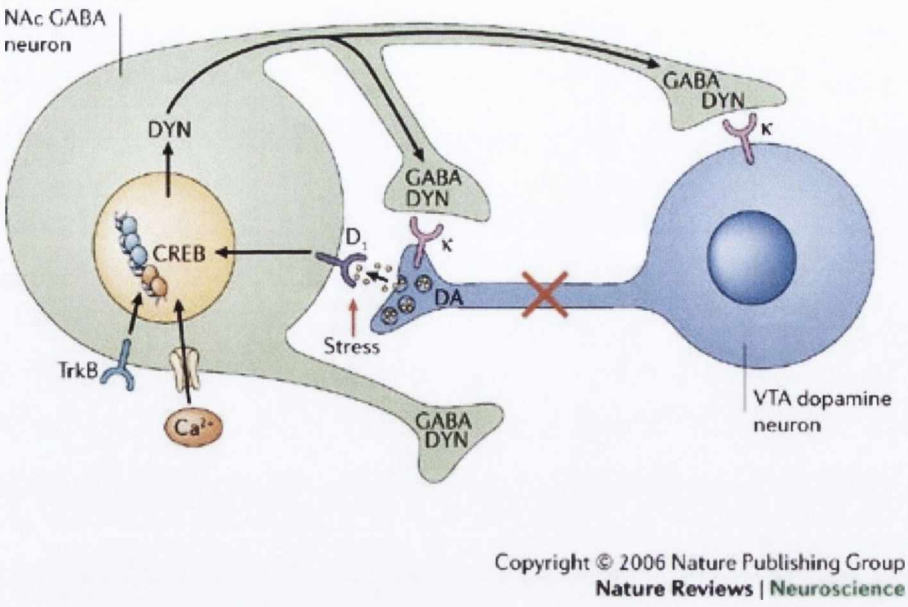


Figure 1.7 – CREB and dynorphin in the nucleus accumbens in depression[44]

Reportedly, the transcription factor cAMP response element binding protein (CREB) has a profound effect on emotional responsiveness and its levels are controlled by GABAergic and dopaminergic pathways. Depression has been characterised by both increased and decreased levels of CREB in different areas of the brain, making its use as a possible antidepressant unfeasible. However, κ opoid receptor antagonists have been reported to regulate CREB activity in the NAc and could be potential antidepressants. BDNF also has mixed effects in different regions of the brain. Melanin-concentrating hormone (MCH) is an important pro-appetite protein and antagonists of the MCH_1 receptor have shown antidepressant activity.

1.6 Studies on the Genetics of Depression

Considerable attention has been given to the 5-HT receptors as possible genes involved in depression. It is suggested that a cytosine (C) to guanine (G) conversion at position 1019 on the gene for the 5-HT_{1A} receptor can cause depression and an increased tendency towards suicide with twice as many depressed patients having the homozygous G allele than controls.[62] This difference was four fold in suicide victims. The mechanism of action is believed to be based on the fact that the G allele does not bind to a repressor, called nuclear deformed epidermal autoregulatory factor -1-related protein, as well as the C allele, so more of the 5-HT_{1A} autoreceptor is produced. An association between a single

nucleotide polymorphism (SNP) in the TPH gene, TPH_2, and major depression had also been found.[63] Another study found no association between the rs10748185 SNP located in the promoter region of TPH_2 and suicidal behaviour.[64] A further study purports a connection between a polymorphism of TPH_1 and depression, while also implicating the 5-HT_{2A} receptor as a factor in depression.[65]

One of the studies carried out on the 5-HT transporter (SERT) suggests that a 44 base pair (bp) deletion/insertion polymorphism in the transporter promoter gene, called the serotonin transporter linked polymorphic region (5-HTTLPR), is not involved in depression or suicide.[66] Another study suggests a link between 5-HTTLPR and depression in women only.[65] The 5-HT_{1D}, dopamine D_2 and cannabinoid receptor 2 have been also linked with an increased susceptibility to depression in association with 5-HTTLPR.[67]

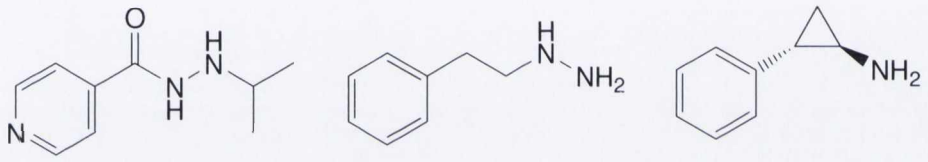
A number of studies have been done to examine the effect of the α_2-AR on depression. For instance, a study on the behaviour of α_{2C}-AR knockout and overexpressed mice showed that the α_{2C}-AR is associated with the control of emotion in the CNS, the development of behavioural despair and response to stress.[18,68] In humans a rare C to G conversion at neucleotide 753 of the α_2-AR, leading to a Asn to lysine (Lys) mutation at amino acid 251 (N251K), has been shown not to be involved in depression.[69] Using the dexmethasone/corticotrophin-releasing hormone test, it was revealed that a polymorphism of the α_{2A}-AR gene which converts C to G at nucleotide 1291 plays a role in imbalance of the HPA and depression in men but not women, whereas women with depression tend to have a polymorphism of the β_2-AR which involves a change at amino acid 16 from arginine (Arg) to glycine (Gly).[70] A further study implicated a role for the G protein β_3 subunit in depression, with a polymorphism at C825T causing genetic susceptibility.[71]

Genetic association studies broadly support a link between CRF and CRF receptors in depression but whole-genome association studies do not.[72] A study on the neuropeptide Y gene indicated that the rs16147-399C allele is associated with a slower response and failure to reach remission with antidepressant treatment.[73]

In conclusion, a few association studies have been carried out in order to determine if a relationship exists between various receptors, transporters, peptides and enzymes and depression. Unfortunately, no consensus has been reached as to which genes are conclusively involved in depression with many conflicting results having been published, therefore further studies need to be done in order to determine which genes are most closely linked with depression.

1.7 Classical Antidepressants

Traditionally, antidepressants have targeted two processes involved in removing neurotransmitters from the synapse after their release.[4] Iproniazid (Figure 1.8) a drug initially intended for use in the treatment of tuberculosis in the 1950s was observed to cause "a subtle general stimulation... [and] renewed vigor [which] occasionally served to introduce disciplinary problems"[74] in patients treated with the drug. It was thus the first antidepressant discovered and was found to act by inhibiting the breakdown of monoamine neurotransmitters by MAO, at or near the synapse as shown in Figure 1.2. Therefore, this class of drug was termed the monoamine oxidase inhibitors (MAOIs). Because enzymatic degradation of monoamines is slowed by MAOIs, a greater number of post-synaptic binding events can then occur. This in turn increases the propagation of the signal at the post-synaptic neuron, alleviating the symptoms of depression. Other drugs in this class include phenylzine and tranylcypromine.



17 **18** **19**

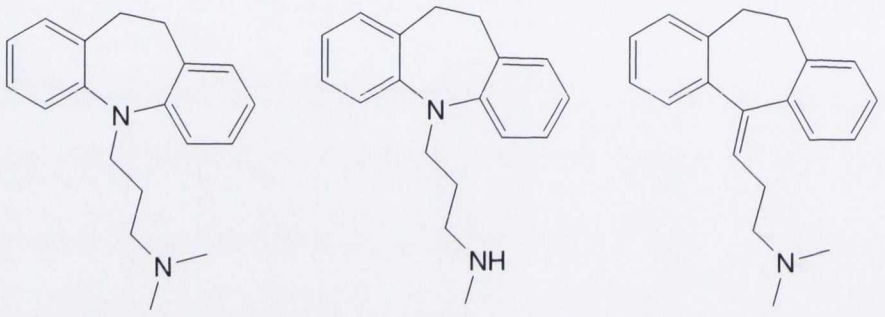
Figure 1.8 - MAOI drugs: Iproniazid (**17**), phenylzine (**18**) and tranylcypromine (**19**)

MAOIs possess a reactive group such as a hydrazine or cyclopropane in the case of tranylcypromine. This reactive group can bind covalently with the active site of MAO resulting in their antidepressant activity. MAOIs are thus irreversible inhibitors.[26]

Though there is no clear evidence to show abnormal MAO expression in depression, disruption of the gene for MAO-A in mice causes increased brain accumulation of 5-HT and to a lesser extent, NA.[26] A notable problem with MAOIs is that since they are non-selective, they can interact with other amines present in food. For example ripe cheese contains tryptamine, which is normally metabolised by MAO in the gut wall and liver, and so does not enter the bloodstream. However MAOIs can prevent this reaction, allowing the circulation of tryptamine in the body which can interact with the adrenergic system and cause severe headaches and hypertension.[4] Other side effects of MAOIs include

hypotension, tremors, insomnia, weight gain, dry mouth and blurred vision and convulsions in overdose.[26] Moreover, MAOIs do not distinguish between MAO-A and MAO-B.

The second process targeted by traditional antidepressants is the inhibition of neurotransmitter reuptake into the presynaptic neuron by neurotransmitter transporters such as the NA (NAT/NET, see Figure 1.2) or SERT transporters. This process occurs shortly after postsynaptic neurotransmitter binding, and results in the removal of up to 95% of the neurotransmitters from the synapse. Drugs which target both NAT and SERT are known as TCAs and examples are imipramine, desipramine and amitriptyline shown in Figure 1.9.



20 **21** **22**

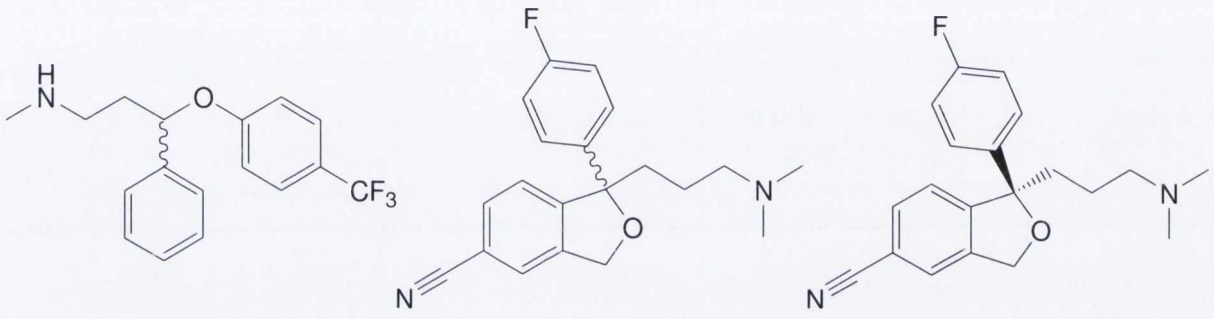
Figure 1.9 – Tricyclic drugs: Imipramine (**20**) desipramine (**21**) and amitriptyline (**22**)

This class of drugs was also discovered serendipitously during research on antihistimines. These drugs are structurally related to the antipsychotic phenothiazines, and indeed the first TCA drug, imipramine, did not begin life as an antidepressant, but instead was tried as an antipsychotic.[26] TCAs have an extra atom in the central ring than phenothiazines, which twists the structure and removes the planarity of the parent drug class.[26] It is thought that they exhibit their antidepressant effect since they are partly super imposable on NA and thus can block the transporter proteins.[4] Tertiary amine TCAs are rapidly demethylated *in vivo* to secondary amines or are hydroxylated; it has been found that both metabolites can act as antidepressants in their own right.[26] Some TCAs are also thought to increase neurotransmitter release indirectly by antagonising presynaptic α_2-ARs.[26] The action of desipramine is NA specific, however due to its structure it is classed as a TCA.[26]

The main problems with the TCA class of antidepressants include side effects such as sedation, confusion and poor motor coordination. These are due to the action of TCAs at the muscarinic

acetylcholine, histamine and 5-HT receptors. They also have a high risk of overdose on their own causing confusion, mania and cardiac dysrythmia and when combined with CNS depressants.[26]

There are also drugs which selectively inhibit the reuptake of 5-HT or NA alone, which sometimes result in lower side effects. Drugs that only inhibit 5-HT reuptake are classed as SSRIs, the first of which to be available commercially was racemic fluoxetine (Figure 1.10) in the late 1980s. Since then SSRIs with different structures have been discovered such as racemic citalopram (Figure 1.10) or its (+) enantiomer escitalopram.

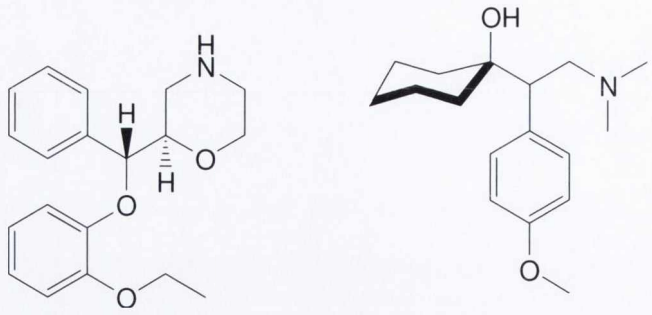


23 **24** **25**

Figure 1.10 – SSRIs: Fluoxetine (**23**) and citalopram (**24**) and escitalopram (**25**)

SSRIs have fewer side effects than TCAs, are less likely to cause anticholinergic side effects and less dangerous in overdose. However, SSRIs are thought to be less effective in treating severe depression and also show side effects such as nausea, anorexia, insomnia and loss of libido.[26]

More recently a number of 5-HT and NA reuptake inhibitors (SNRIs) have been developed including reboxetine and venlafaxine whose structures are shown in Figure 1.11.[26,75] Venlafaxine has been reported to act more rapidly than other antidepressants and works better in the treatment of resistant depression.[26]



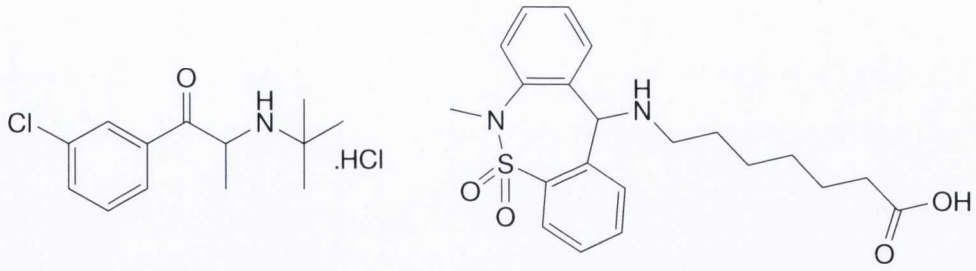
26 **27**

Figure 1.11 – Structures of SNRIs reboxetine (**26**) and venlafaxine (**27**)

Lithium salts have also been used in the treatment of depression, and of manic depression in particular. This cation is a known inhibitor of phosphatidylinositol phosphatases, adenylyl cyclases, G proteins and glycogen synthase kinase 3β, but the target which is responsible for its antidepressant activity is as yet unknown.[44] Lithium is administered as lithium carbonate, lithium citrate, lithium sulphate or lithium aspartate.

A lack of the monoamine DA can also play a role in depression, with the DA reuptake inhibitor, bupropion (Figure 1.12), proving to be an effective antidepressant in some patients. [26,44] Side effects include drowsiness, vomiting, weightloss,[76] anxiety and seizures.[26] The drug has a number of contraindications, for example it cannot be administered to patients with epilepsy or patients taking MAOIs,[76] however it can be effectively co-administered with SSRIs.

Tianeptine (Figure 1.12) is an activator of monoamine reuptake,[44] which was first developed in 1981 by Antoine Deslandes and Michael Spedding and was marketed as an antidepressant shortly afterwards.[77] Tianeptine is also believed to act by inhibiting and even reversing stress induced neuronal damage.[77]



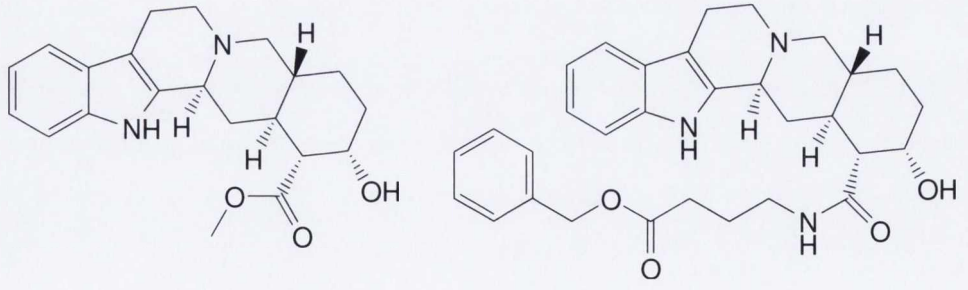
28 **29**

Figure 1.12 - Bupropion (**28**) and tianeptine (**29**)

Not all patients respond to these treatments, however, and a 4-6 week delay occurs before therapeutic efficacy is achieved,[30,78] clearly indicating a need for research and development of novel antidepressant agents. This makes the α_2-AR a promising new target for the treatment of depression.

1.8 α_2-Adrenoceptor Antagonists

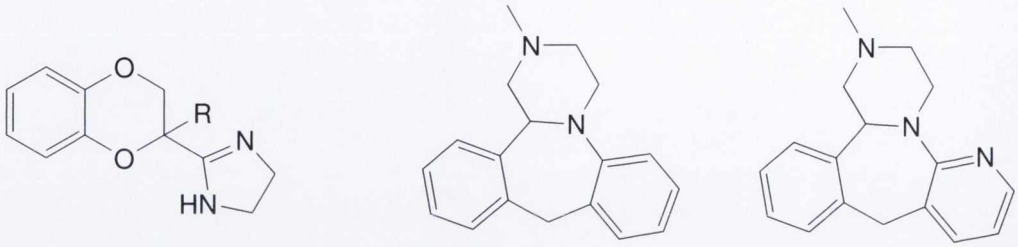
Yohimbine (Figure 1.13) is a naturally occurring alkaloid and selective α_2-AR antagonist. It is mainly used as a drug for improving sexual dysfunction, but can also be used in the treatment of post traumatic stress disorder. In order to provide ligands for use in receptor function studies, Mustafa *et al.* developed a number of yohimbine derivatives with high binding affinities and good α_{2C}-AR selectivities.[79] The most promising compound is shown in Figure 1.13, having a better binding affinity for the α_{2C}-AR than yohimbine while also being more selective for the α_{2C}-AR subtype.



30 **31**

Figure 1.13 – Yohimbine (**30**) and Mustafa's most potent and selective α_{2C}-AR antagonist (**31**)

Idazoxan, shown in Figure 1.14, also shows affinity for the α_2-AR.[26] It is not used clinically but is used experimentally to analyse α_2-AR subtypes. Compound 2-methoxy idazoxan (RX821002), shown in Figure 1.14, is also used experimentally.



R = H (idazoxan), R = OMe (RX821002)

32 **33** **34** **35**

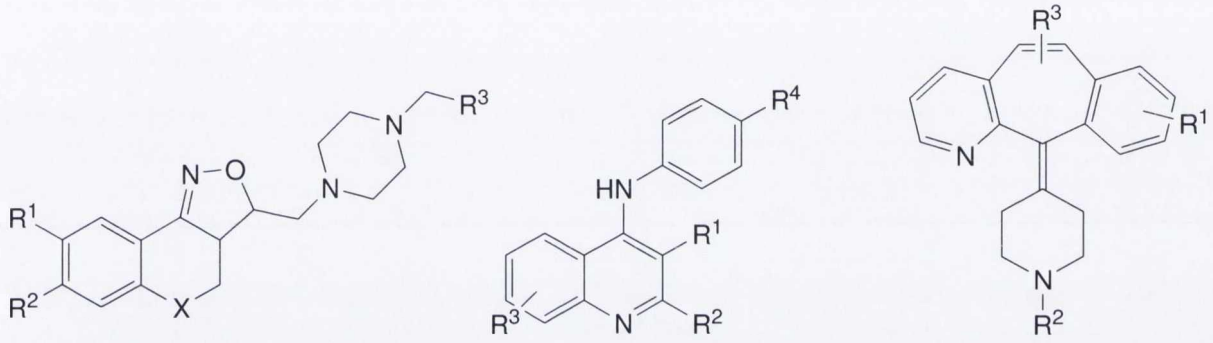
Figure 1.14 - Idazoxan and RX821002 (**32** and **33**), mianserin (**34**) and mirtazapine (**35**)

Mianserin and mirtazapine (Figure 1.14) are the first commercially available α_2-AR antagonists for use against depression. Neither affect the reuptake of NA or 5-HT, however mianserin does inhibit both histamine-1 receptors and α_1-AR resulting in sedation and postural hypotension.[80,81] Mirtazapine possesses less unwanted side effects, with sedation and weight gain being the most common. Mirtazapine acts through inhibition of α_2-ARs as well as 5-HT autoreceptors,[82] whereas mianserin has a relatively weak action at 5-HT2 and 5-HT3 receptors and therefore owes its efficacy principally to its action at the α_2-AR, while its activity at the 5-HT1 receptors could partially inhibit noradrenergic neurotransmission.[83] Mianserin is mainly used to augment the therapeutic effects of other drugs in the treatment of resistant patients.[84]

From 2003 to 2007, Andrés *et al.* from Janssen Pharmaceutica have developed a number of tricyclic isoxazolines containing a cinnamyl group (at the R^3 position) as dual α_2-AR antagonists and 5-HT reuptake inhibitors, the general structure of which is shown in Figure 1.15.[85-89] In particular, some compounds showed nanomolar binding affinities for α_{2A} and α_{2C}-AR subtypes as well as good results in the inhibition of zylazine or metetomididine induced loss of righting test. This is a reliable test for α_2-AR blocking activity when occurring without behavioural stimulant effects, with some of the Janssen compounds having an ED_{50} < 1.0 mg/kg. Some of the alterations made to the core structure included the substitution of R^1 and R^2 with different ether and ester functional groups, the use of X =

C, O or N and the modification of the cinnamyl fragment including halogen substitution and heterocyclic moieties. When enantiomers were separated and tested *in vivo*, the (+) enantiomers gave better binding affinities.

Höglund *et al.* developed a number of quinoline derivatives as potent and selective α_{2C}-AR antagonists in 2006.[90] The compounds had *para*-amino substituted anilines and it was found that the quinoline core was necessary for α_2-AR binding affinity, with a substituent in the 3-position of the quinoline system giving improved results. The general structure is shown in Figure 1.15.

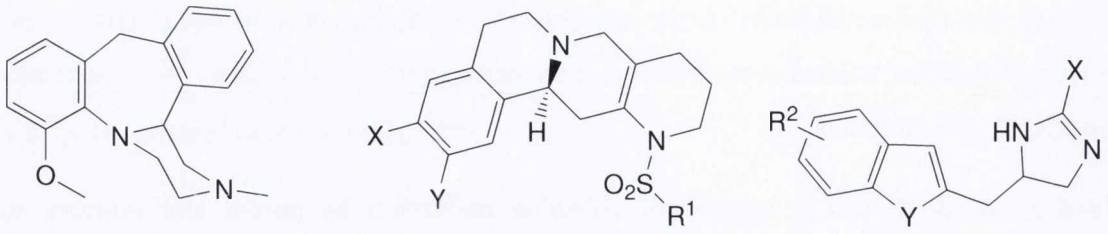


36 **37** **38**

Figure 1.15 – General structure of the derivatives of Janssen's (**36**) Höglund's quinoline derivatives (**37**) and van der Berg's multicyclic derivatives (**38**)

In 2006, van der Berg submitted a patent for aromatic, multicyclic derivatives as antidepressants related to Tolvon and Remeron, known α_2-AR antagonists.[91] The derivatives contain both a pyridine and benzene ring fused to a heptacyclic ring which is also bonded to an *N*-alkyl piperidine group as shown in Figure 1.15.

Wikström *et al.* have synthesised and tested the mianserin derivative, 1,2,3,4,10,14b-hexahydro-6-methoxy-2-methyldibenzo[*c,f*]pyrazine[1,2-*a*]azepin (Figure 1.16), and its individual enantiomers as α_2-AR antagonists as well as testing its affinity at a number of other receptors including the 5-HT_{2A} and 5-HT_{2C} receptors at which the parent compound had very high binding affinities.[92]



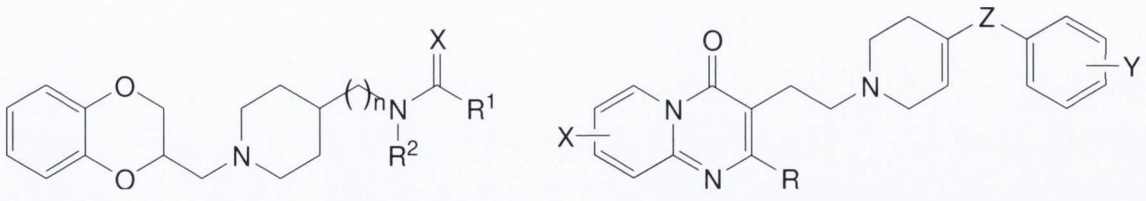
39 **40** **41**

Figure 1.16 – Structures of Wikström's derivative (**39**), general structure of Szántay's azaberbene derivatives (**40**) and general structure of some of Cordi's napamezole derivatives (**41**)

In 2002, Szántay *et al.* developed a number of azaberbene derivatives as α_{2A}-AR antagonists.[93] Their general structure is depicted in Figure 1.16. Competition experiments versus xylazine, an α_2-AR agonist, showed that the compounds were competitive antagonists.

A series of napamezole derivatives with dual α_2-AR antagonist and monoamine reuptake inhibitor properties was developed in 2001 by Cordi *et al.*[94] The most potent derivative was found to be an isomer of napamezole having the alternative connectivity of the 4,5-dihydro imidazole ring. The structure of the most potent derivative and related compounds is shown in Figure 1.16. Other derivatives included compounds with 4,5-dihydro oxazole and imidazole substituents instead of 4,5-dihydro imidazole.

In 2000 a number of novel substituted 1-(2,3-dihydrobenzo[1,4] dioxin-2-ylmethyl)piperidin-4-yl derivatives were developed by Mayer *et al.*[95] These structures were based on R47243, a previous α_2-AR antagonist, which was developed as an anti-Parkinsonian agent and their general structure is shown in Figure 1.17. The compounds were also tested at the α_1-AR and the dopamine 2 (D_2) receptors to determine selectivity, with some compounds showing modest selectivity, although none proved to be as selective as either yohimbine or idazoxan.

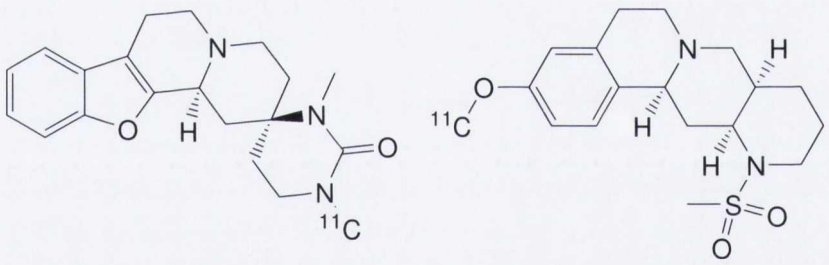


42 **43**

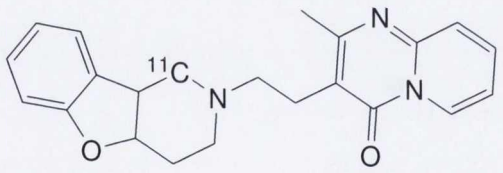
Figure 1.17 – General structures of Mayer's derivatives (**42**) and Kennis' derivatives (**43**)

Also in 2000, Kennis *et al.* developed a number of 2-substituted 1,2,3,4-tetrahydrobenzofuro[3,2-*c*]pyridines, whose structure is depicted in Figure 1.17.[96] Unfortunately, these compounds proved to have poor α-AR subtype selectivity, also blocking the α_1-ARs.

A number of radiolabelled α_2-AR antagonists have been developed previously such as [^{11}C]MK-912 (Figure 1.18),[97] [*N*-methyl-^{11}C]mianserin,[98] [*O*-methyl-^{11}C]RS-15385-197 (Figure 1.18),[99] RX821002 based radioligands,[100] [*N*-methyl-^{11}C]mirtazapine,[101] and [^{11}C]R107474[102] (Figure 1.18) but to date no radioligand has been selected for use in positron emission tomography (PET) in humans.



44 **45**

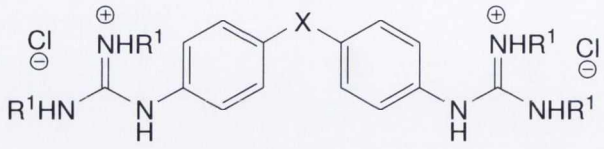


46

Figure 1.18 - MK-912 (**44**), RS-15385-197 (**45**) and R107474 (**46**)

1.9 Previous Research by Rozas and Co-workers

Previous investigations carried out by our group on the α_2-AR binding properties of over 60 guanidinium-like containing compounds including symmetric *bis*-cationic derivatives of the general structures **47** and *mono*-cationic derivatives such as **48** to **50** as depicted in Figure 1.19, and have resulted in the discovery of five antagonists with strong binding affinities.[103-105]



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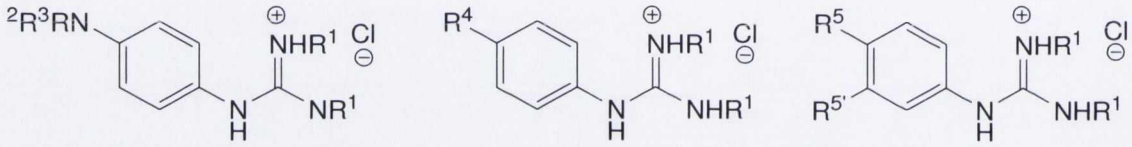


48 **49** **50**

Figure 1.19 - General structures of most previous compounds evaluated at α_2-AR by our group where R^1 = H, $-CH_2CH_2-$; X = CH_2, CH_2CH_2, S, O, NH, CO; R^2, R^3 = H, Me, Et; R^4 = H, Me, Et, CH_2Ph; R^5, $R^{5'}$ = Me, $-(CH_2)_3-$, $-(CH_2)_4-$, OMe, $-OCH_2O-$, $O(CH_2)_2O-$.

The α_2-AR binding affinities of all compounds were measured by competition with selective radioligand [^{3}H]RX821002 in human prefrontal cortex (PFC). Compounds with binding affinities (pK_i) larger than 7, were tested further to assess their activity.

Activities were determined *in vitro* using functional [^{35}S]GTPγS binding assays in human PFC. The [^{35}S]GTPγS binding assay constitutes a functional measure of the interaction of the receptor and the G–protein and is a useful tool to distinguish between agonists (which increase the nucleotide binding), inverse agonists (which decrease the nucleotide binding), and neutral antagonists (which do not affect the nucleotide binding) of GPCRs.[106] Agonists stimulated the binding of [^{35}S]GTPγS, showing a typical agonist dose-response plot. Their EC_{50} values and percentage efficacy relative to the well-known α_2-AR agonist UK14304 was measured.

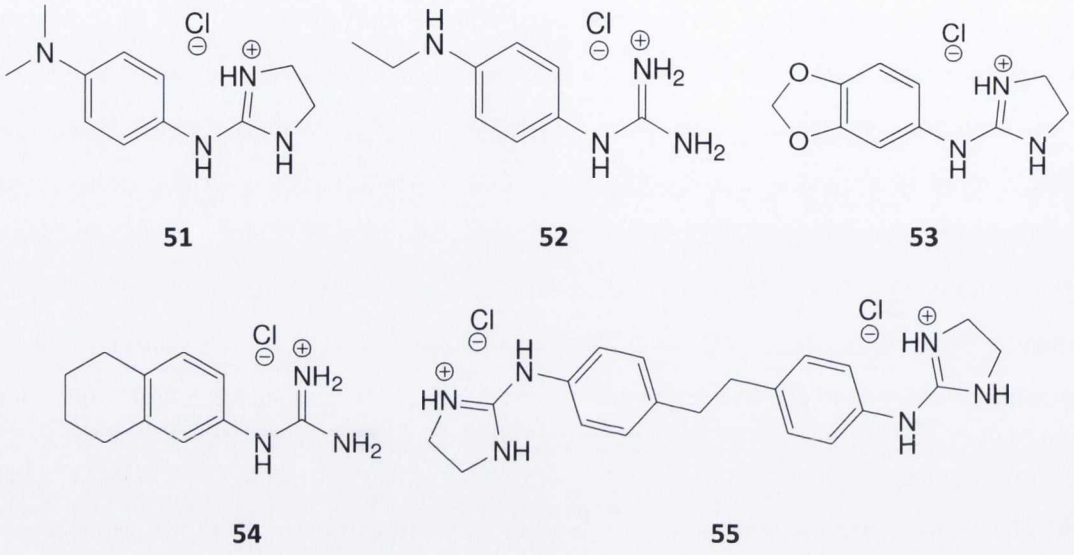
Those compounds which did not stimulate binding of [^{35}S]GTPγS on their own, were subjected to new [35S]GTPγS binding experiments and tested against UK14304. If a rightwards shifting of the concentration-response curve for UK14304 was observed in assays in which the tested compounds were included, this would confirm the antagonist effect of these derivatives against the α_2-ARs. Considering the antagonistic effect and relatively good affinities over the α_2-ARs obtained for compounds **51** to **55** (Figure 1.20), it was decided to test them *in vivo* to evaluate their potential effect on noradrenergic transmission. As presented in previous sections, α_2-AR antagonists are expected to increase the extracellular NA concentrations in the brain, and for that reason these five compounds are expected to produce such an effect. The microdialysis technique is designed to collect virtually any substance from the brains of freely moving animals with limited tissue trauma. Thus, microdialysis experiments allow the measurement of local neurotransmitter release.[107] In control rats, local administration of artificial cerebrospinal fluid did not change extracellular NA basal levels.

On the contrary, local administration of these compounds increased extracellular NA in the PFC, in a concentration dependent manner, hence confirming their antagonistic activity. Local administration of Idazoxan or RX821002, two well-known α_2-AR antagonists, increased extracellular NA, when were compared to control. At this point, the effect of these compounds administered systemically was also assessed and thus, intraperitoneal administration of **51** to **55**, increased extracellular NA on the PFC demonstrating the compounds ability to cross the blood brain barrier.[105]

Notable symmetric compounds include agonist **47** where R^1 = -CH_2CH_2- (*i.e. bis*-2-aminoimidazolinium derivatives) and X = CH_2, which, with pK_i = 8.80, has the highest binding affinity of any compound synthesised by our group so far. Symmetric compounds with heteroatomic linkers where R^1 = -CH_2CH_2- and X = S, O and NH (*bis*-2-aminoimidazolinium derivatives) also had strong binding affinities. Also of note is compound **55** (Figure 1.20) which differs from compound **47** described above, by only one CH_2 in the linker X but this difference is enough to confer it with antagonist activity. Of the *mono*-guanidinium-like compounds **52**, **53** and **54** (Figure 1.20) showed antagonistic activity. In addition to this, compounds **52** and **54** have been tested in two animal models of stress such as the tail suspension tests (TST) on mice and the forced swimming tests (FST) on rats. These tests are considered as animal models for depression. Compound **52** is of particular interest, having demonstrated an antidepressant efficacy greater than fluoxetine in the TST and in the FST.

However, determining what exact properties result in antagonistic activity has so far proved elusive, with compounds with little structural variation from the antagonists described, showing agonistic activity. The determination of structural activity relationships (SARs) of 2-amino-2-oxazolines,[7]

imidazolylmethylthiophenes[108] and pyridazinone derivatives[109] at the α_2-AR has also proven difficult, indicating that more work is necessary in order to understand this phenomenon.



51 **52** **53**

54 **55**

Figure 1.20 - Structures of the five antagonistic compounds developed by Rozas' group

1.10 The use of Bioisosterism

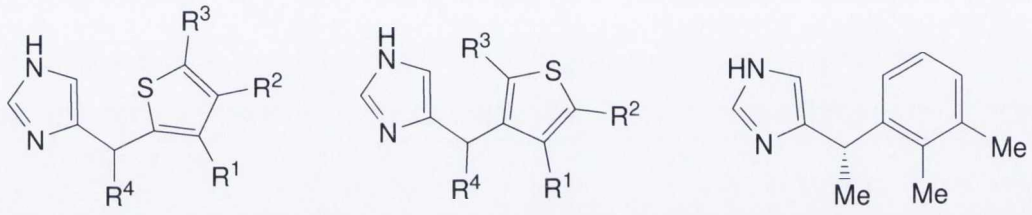
In drug design there are certain chemical groups that are interchangeable yielding compounds with similar biological response. This interchange process is known as bioisosterism

1.10.1 Thiophene

The target compounds intended to be synthesised and tested within the scope of this project are *bis*- and *mono*-guanidinium-like thiophene analogues of the lead compounds described in Section 1.9 and other related compounds. The use of thiophene as an ring equivalent of benzene in medicinal chemistry has often been reported,[110] Thiophene, having only atoms with similar electronegativities as carbon in its ring, has greater aromaticity than analogous five-membered rings with other heteroatoms; this strong aromaticity lends itself to the effective mimicking of benzene. Due to thiophene having only 5 atoms in the ring, there is increased anular electron density resulting in an altered three dimensional shape, which is also affected by distortions induced by the large sulfur atom. This results in thiophene being both strongly aromatic and at the same time having electronic

and geometric differences from benzene significant enough to warrant the synthesis of thiophene analogues in medicinal chemistry.

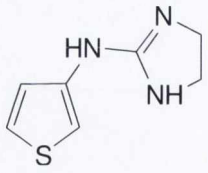
The most closely related thiophene based compounds tested at the α_2-AR are imidazomethyl-2- and imidazomethyl-3-thiophene based compounds, such as those shown in Figure 1.21, and have exhibited agonistic activity at α_2-AR at nanomolar (nM) concentrations, with analogues of dexmedetomidine (pK_i = 9.4) having comparable or improved binding affinities.[108] All these results strongly support the investigation of thiophene analogues of our lead compounds.



56 **57** **58**

Figure 1.21 - General structure of imidazomethyl-2- (**56**) and imidazomethyl-3-thiophenes (**57**) and dexmedetomidine (**58**)

Syntheses of simple 2-iminoimidazolidine and guanidine thiophenes are extremely rare in the literature, with, to the best of our knowledge, (4,5-dihydro-1*H*-imidazol-2-yl)thiophen-3-ylamine (Figure 1.22) being the only example of this class of compound reported to date.[111] The synthesis of aromatic 2-iminoimidazolidine and guanidine derivatives currently employed by our group,[112] involves the use of aromatic amine intermediates. Since many thiophene amines are known to be unstable, this could explain the lack of thiophene guanidine-like compounds reported thus far. As such all target compounds synthesised will be novel, and it may be necessary to develop new synthetic methodologies for the preparation of thienylguanidines. Thus the synthetic component of this project represents a significant advancement in the area of thienylamidines.



59

Figure 1.22 - (4,5-Dihydro-1*H*-imidazol-2-yl)thiophen-3-yl-amine (**59**)

1.10.2 Thiazole

Replacement of a phenyl ring with a thiazole is also a classical bioisosteric strategy. Thiazole having nitrogen in its ring, which is a more electronegative atom than the corresponding carbon atom in thiophene, is less aromatic than the latter species, due to the fact that a greater portion of electron density is present around this N atom, disrupting the equal distribution of electrons common in aromatic species. 2-Aminothiazoles are readily available commercially, and a number of thiazole guanidines have already been prepared by other methods than our guanidylation process. These methods, which will be discussed in Chapter 6, make the use of this bioisostere on molecules of the type developed in our group more attainable.

In the literature, bioisosterism is often used in medicinal applications for example the substitution of an oxazole moiety for a thiazole in a recent paper by Kaspady *et al.*[113] or in the classical antibacterial sulfonamides the change of a benzene by a thiazole ring to produce sulfathiazoles.

2 Objectives

2.1. Guanidine-like Thiophenes

The primary objective of this project is to design, synthesise and test a number of twin and single thiophene amidine-like hydrochloride salts as potential α_2-AR antagonists. The target molecules envisaged to date are analogues of the lead compounds developed by Rozas and co-workers and described in section 1.9, with general structures as shown in Figure 2.1.

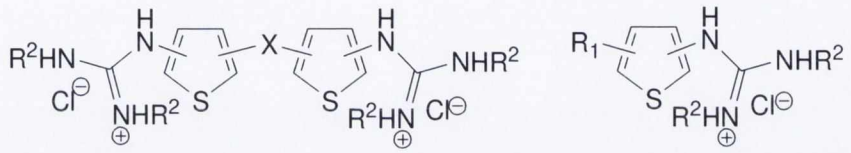


Figure 2.1 - General structures of target compounds with R^2 = H, $-CH_2CH_2-$

In order to carry out this synthesis, the applicability of the standard method used in Rozas' group, the Kim and Qian[112] method (Section 3.2; Scheme 3.3), must be tested. This will be done through the use of an easily accessible aminothiophene under the standard conditions involving thiophilic mercury (II) chloride ($HgCl_2$). Should this method prove ineffective, other methods such as the use of Mukaiyama's reagent with relevent thiophene amines will be explored.

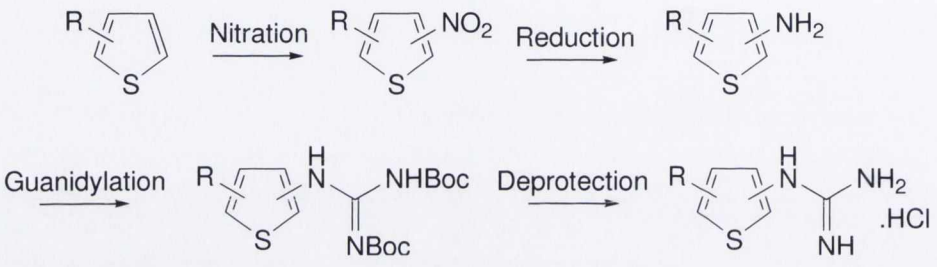
The deprotection to generate the hydrochloride salts of the thienylguanidines will be similarly examined. There are a variety of reagents available for the removal of *tert*-butoxycarbonyl (Boc) groups from substituted guanidines, so a number of these including the use of 1:1 trifluoroacetic acid (TFA): dichloromethane (DCM), or HCl solutions in different organic solvents will be examined.

After the suitability of the methods mentioned above has been established, the next step will consist of the synthesis of a variety of *bis*-thienyl diamine and aminothiophene intermediates which will then be coupled to the guanidine precursor (guanidylated) using the current methodologies.

Where possible, synthetic strategies which are either divergent, or can be readily applied to the synthesis of similar molecules will be favoured, as this will allow a greater number of final compounds to be synthesised.

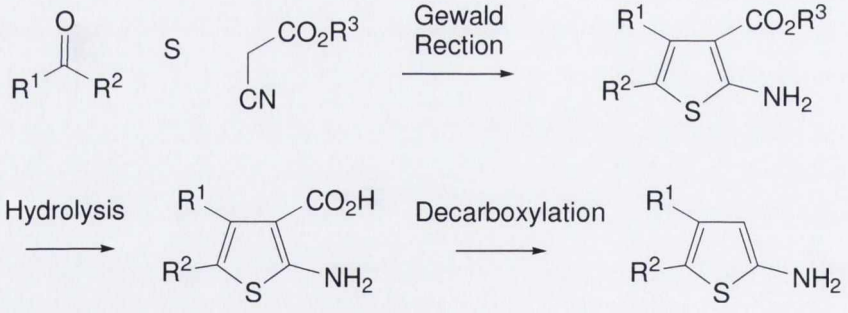
The use of synthetic strategies involving both standard electrophillic (EAS) and nucleophillic aromatic substitutions (NAS) can be envisaged at this stage as both are reported in relation to the thiophene nucleus.

In particular, in terms of EAS based strategies, the nitration, followed by reduction, and subsequent guanidylation and deprotection of substituted thiophenes, as depicted in Scheme 2.1, will be examined.



Scheme 2.1

The synthesis of aminothiophene from acyclic starting materials will be also be examined such as the Gewald synthesis of alkyl-2-aminothiophene-3-carboxylates.[114] It is envisaged that the ester group could be removed via hydrolysis and decarboxylation as shown in Scheme 2.2.



Scheme 2.2

If the guanidylating methods mentioned above prove successful, the Dardonville and Rozas[115] approach will be applied to the aminothiophene intermediates, in order to make the 2-iminoimidazolidino derivatives as well.

The synthesis of guanidine thiophenes using starting materials other than aminothiophenes may prove necessary for some molecules due to the inherent instability of molecules of this type. It may, therefore, be necessary to come up with novel synthetic strategies which bypass this class of intermediate. In particular the use of metal mediated coupling will be examined.

2.2. Guanidine-like Thiazoles

In a similiar fashion, the applicability of the Kim and Qian and Dardonville and Rozas methods must be examined for the thiazole series. Such a study will be carried out on 2-aminothiazoles and 2-aminobenzothiazoles, of which some are commercially available. This should give rise to molecules of the structures shown in Figure 2.2.

Figure 2.2 - General structures of target thiazole compounds.

Similarly, the deprotection step must also be validated.

Alternative methods for the synthesis of thiazol-2-ylguanidines such as that of Beyer and Hantschel[116] will also be examined, especially for the synthesis of *N*-(5,6-dihydro-4*H*-cyclopenta-[1,3]-thiazol-2-yl)guanidine hydrochloride (**60**, Figure 2.3) and *N*-(4,5,6,7-tetrahydro-4*H*-benzo-[1,3]-thiazol-2-yl)guanidine hydrochloride (**61**, Figure 2.3, which are analogues of compound **54** (Figure 1.20).

60 **61**

Figure 2.3 - Structures of target thiazole compounds **60** and **61**

Likewise, for making benzothiazol-2-ylguanidines the method documented by Dolzhenko and Chui[117] will be assessed.

2.3. Pharmacological Evaluation

The final salts will be pharmacologically evaluated for their affinity towards α_2-AR on post-mortem human frontal cortex tissue using competition binding studies against [^{3}H]RX821002, an α_2-AR radioligand.

Compounds showing binding affinities in the region of the known α_2-AR ligand Idazoxan (pK_i >7) will be subjected to [^{35}S]GTPγS assays to determine their activities: whether they act as agonists or antagonists.

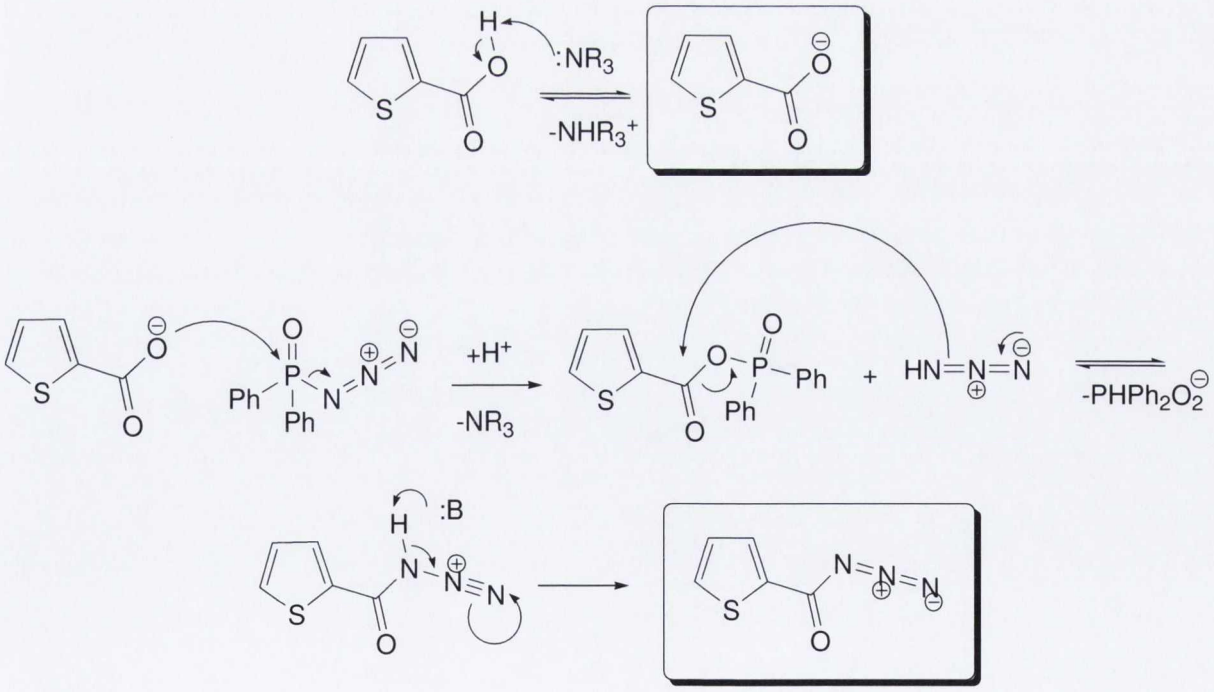
The results of the initial experiments will be analysed, and structures with high affinity or which in general lead to antagonistic activity will be taken and used as new lead compounds on which future target molecules could be based.

3 Guanidine-like Thiophenes: From Nitrothiophene Derived Amines

3.1 Introduction: Aminothiophenes

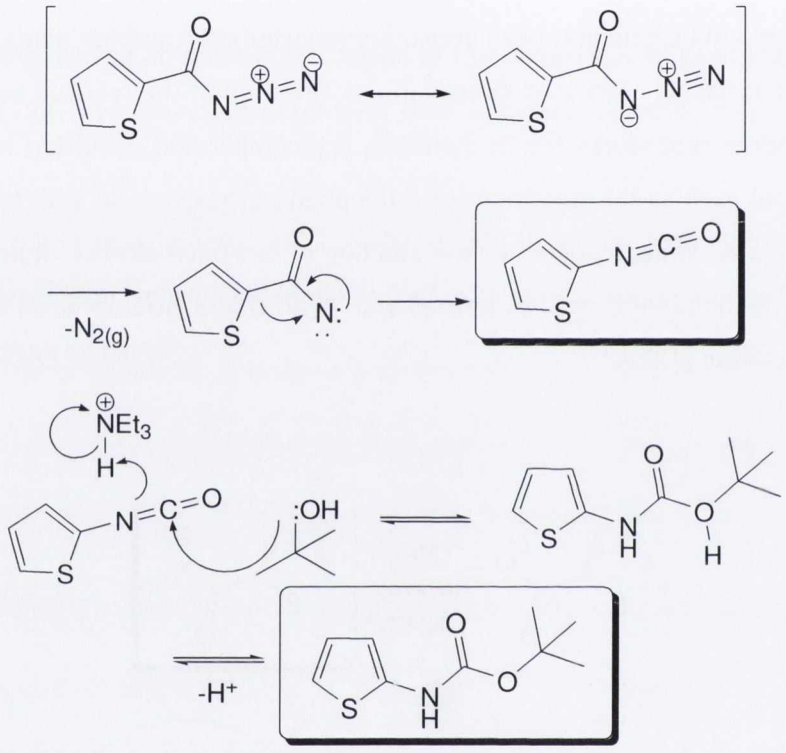
There is a significant lack of simple commercially available aminothiophenes from prominent companies such as Sigmaaldrich[118] and Acros Organics,[119] and there are relatively few published syntheses of basic aminothiophenes, such as the starting materials required for the synthesis of the target molecules, which are the objective of this thesis. This therefore complicates the synthesis of guanidine like thiophenes.

Preparation of 2-aminothiophene has been previously reported via a number of methods, though it is generally reported as having been used directly in the next step of the reaction sequence due to its instability.[120,121] Some procedures for its synthesis are complicated, involving many steps (often without purification) such as the treatment of 2-thiophenecarboxylic acid with triethylamine (TEA) and diphenylphosphoryl azide, followed by the addition of *tert*-butyl alcohol, deprotection of the 2-*tert*-butoxycarbonylaminothiophene thus formed with hydrochloric acid, removal of the water using azeotroping and addition of TEA.[120]



Scheme 3.1

A mechanism for the transformation is proposed in Scheme 3.1. Firstly, the acid is deprotonated by TEA, which allows the oxyanion to attack at the phosphorous atom resulting in the expultion of an azide anion. The azide ion then attacks the carbonyl group resulting in the expulsion of a phosphorous-containing anion. (Scheme 3.1). A Curtius rearrangement then takes place (Scheme 3.2) resulting in a isocyanate. Nucleophilic attack by *tert*-butyl alcohol, at the carbon of the isocyanate, followed by proton transfer results in the Boc-protected amine (Scheme 3.2).



Scheme 3.2

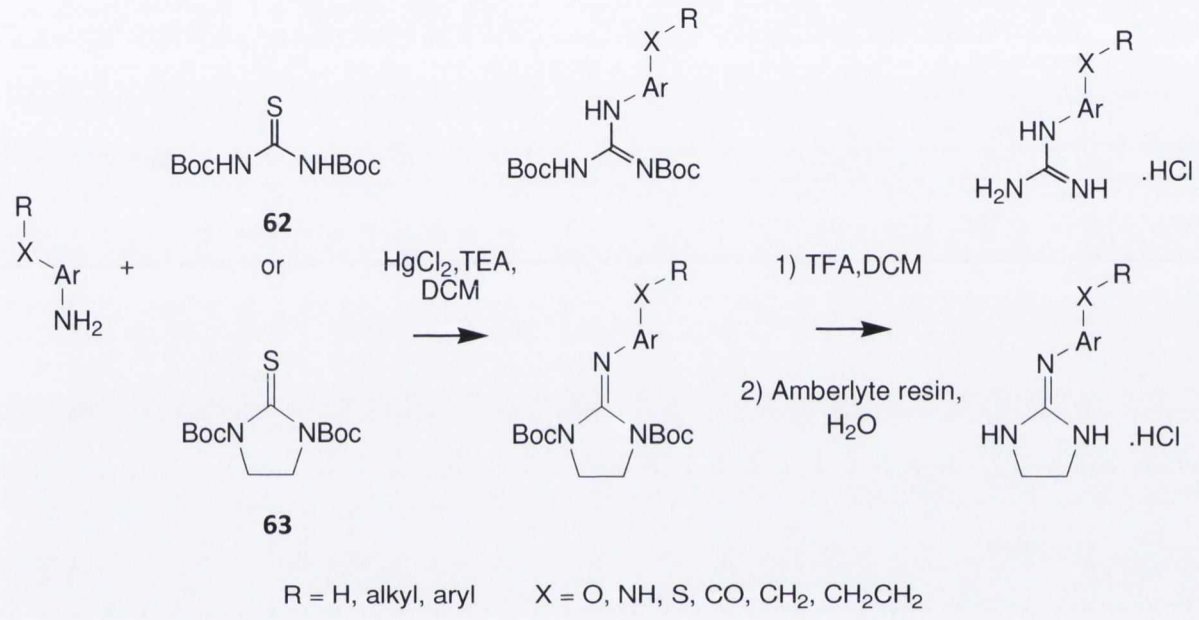
Others such as the reduction of 2-nitrothiophene using palladium(II) acetate, aqueous potassium fluoride and polymethylhydrosiloxane involve the use of hazardous reagents.[122] The reduction of 2-nitrothiophene by Schnute *et al.*[121] using Sn in HCl was therefore viewed as the most favourable strategy, and the preparation of aminothiophenes based on the reduction of nitrothiophenes was attempted.

The most common synthesis of 3-aminothiophene is via the decarboxylation of 3-aminothiophene-2-carboxylic acid. Syntheses related to this procedure will be discussed in Chapter 4.

3.2 The Kim & Qian and Dardonville & Rozas Methods

3.2.1 Introduction

Previous aromatic amidine derivatives synthesised in Rozas' group have employed the method of Kim and Qian,[112] involving the use of *N,N'*-di(*tert*-butoxycarbonyl)thiourea (**62**) or the method of Dardonville and Rozas[115] using *N,N'*-di(*tert*-butoxycarbonyl)imidazoline-2-thione (**63**) as guanidine and 2-iminoimidazolidine precursors respectively, with $HgCl_2$ acting as a coupling agent as shown in Scheme 3.3. An activating agent is needed as aromatic amines are not nucleophilic enough by themselves to couple to the guanidine precursors.



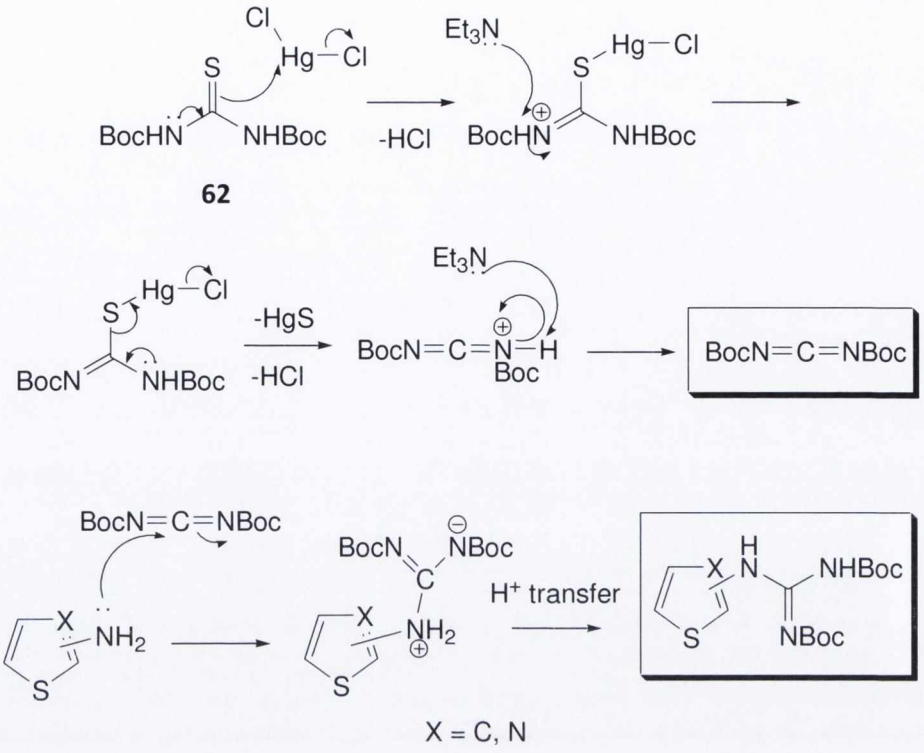
Scheme 3.3

The deprotection of the di-(*tert*-butoxycarbonyl)guanidines produced is via the method of Poss *et al.*[123] and the conversion of the trifluoroacetate salts to hydrochloride salts is also via the method of Dardonville and Rozas.[115] The method begins with the deprotection of the Boc groups in 50% v/v TFA in DCM over 4 hours (h), followed by the removal of the solvents. The residue is then dissolved in Millipore water, to which approximately 1-2 g of Amberlite per 100 mg of the Boc-protected guanidine is added. After stirring for 24 hours, the Amberlite is removed via filtration, and the solvent removed from the filtrate to yield the hydrochloride salt. If the presence of the trifluoroacetate salt is detected by ^{19}F NMR spectroscopy, the treatment with Amberlite must be repeated.

The coupling step takes advantage of the thiophilicity of mercury chloride; therefore, the first stage in the project was to determine whether coupling in the presence of thiophene was possible using the standard conditions. This was necessary to ensure that the thiophene ring itself was not destroyed using these reaction conditions because of the thiophilicity of the metal.

3.2.1.1 Mechanism of the Kim & Qian and Dardonville & Rozas Methods

The mechanism for the Kim and Qian method is reported to proceed via a carbodiimide intermediate.[112] A proposed mechanism is shown in Scheme 3.4. The lone pairs on the nitrogen atoms adjacent to the thiocarbonyl carbon would be involved in a resonance structure which would place a formal negative charge on the sulfur atom, increasing its nucleophilicity and aiding the first step of the mechanism. It is envisaged that the sulfur atom of **62** attacks the mercury atom of mercury chloride, with chloride acting as a leaving group. The non nucleophilic base TEA would quickly deprotonate the positively charged nitrogen on the intermediate due to its higher basicity than this nitrogen (pKa 10.65[124] vs. 7.14[125] for *N*-phenyl-*S*-methylisothiourea, a compound with similar functionality). These two steps are then repeated, as there is another lone pair on the second nitrogen atom which can emulate the first. This results in the elimination of mercury sulfide (HgS) and produces the requisite carbodiimide.



Scheme 3.4

The carbodiimide then reacts quickly with the only free nucleophile available: the aromatic amine. Due to the absence of any pure, isolated intermediate thus far, a short lived, highly reactive species such as *N,N'-bis-*(*tert*-butoxycarbonyl)carbodiimide is suspected. A species possibly related to the carbodiimide was observed by Kim and Qian, with mass spectrometry confirming $[M - H]^-$ for what appears to be the carbodiimide minus one Boc group at 141 (Calc. 141.0664).[112] Mercury metal is reported to precipitate out of a DCM solution of the *mono*-Boc-protected carbodiimide species; indicating that it may indeed be a mercury complex of the same. A $[M - H]^-$ peak of 242 was also observed which is reported to be the carbodiimide itself, however this is not consistent with $[M - H]^-$ for that molecule but is more consistent with the mercury complex shown in Figure 3.1 ($[M - 2H]^{2-}$ *m/z* calc. for 242.0523). This indeed provides evidence for the presence of a carbodiimide species.

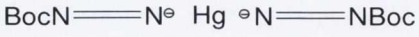
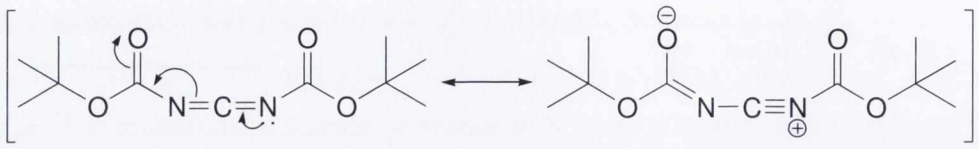


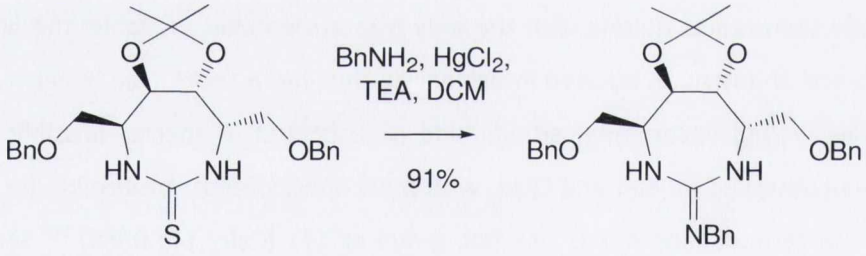
Figure 3.1 – Possible structure of mercury complex found by Kim and Qian.

It is thought that N substitution such that in compound **62** is necessary for the completion of the reaction. It is postulated that the carbamate groups help to stabilise the carbodiimide by resonance, as shown in Scheme 3.5, allowing the molecule to exist long enough to be attacked by the desired nucleophile.



Scheme 3.5

Other nitrogen substituents have been reported, such as in the synthesis of imino-diazepane (**64**) from diazepan-2-thione (**65**) reported by Le Merrer *et. al,*[126] shown in Scheme 3.6 in which the N atoms of the precursor are substituted with alkyl groups.

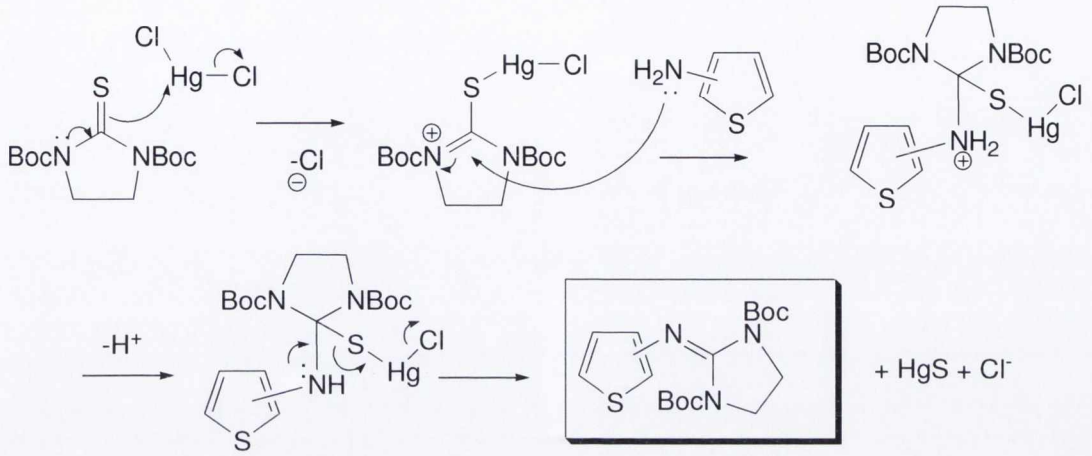


64 **65**

Scheme 3.6

It is difficult to conceive that the Dardonville and Rozas method proceeds via the above described mechanism, due to the implication that the carbodiimide would be highly strained within a five membered ring system. Although unusual bond configurations have been observed, such as the "banana bonds" of three membered ring systems, it is likely that the method proceeds via an alternative mechanism. Additionally, there are no free protons on the N atoms which can be deprotonated in order to form the carbodiimide. Therefore the mechanism probably proceeds via the mechanism depicted in Scheme 3.7. Differences from the carbodiimide mechanism are attack by the

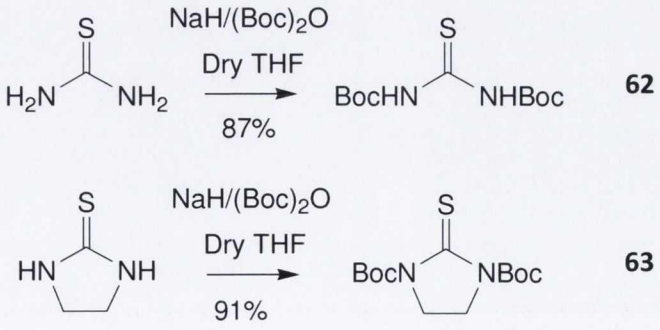
amine at the carbon of the positively charged intermediate in the second step and a second attack by the same amino group leading to the elimination of mercury sulfide and a chloride ion.



Scheme 3.7

3.2.2 Preparation of Boc-protected Amidine Precursors

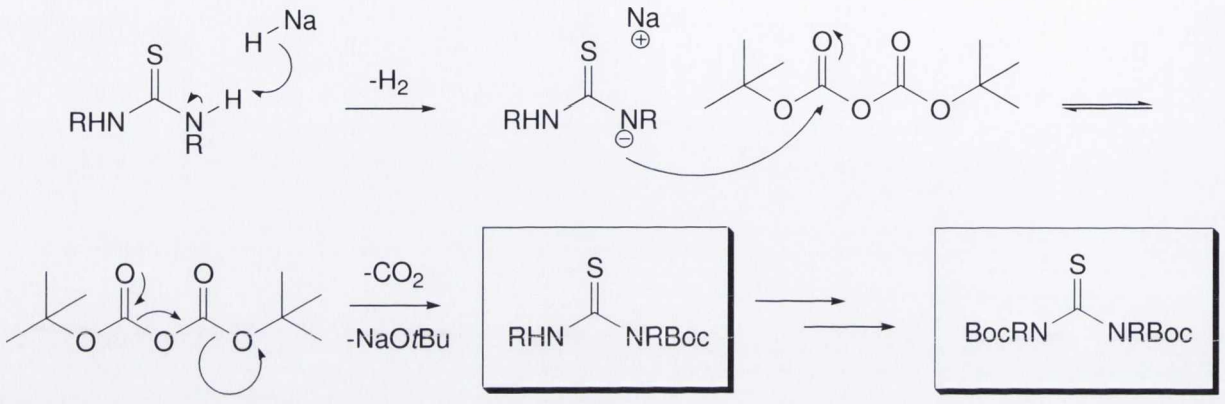
The precursors **62** and **63** were synthesised from thiourea and imidazoline-2-thione using 4.5 equivalents (eq.) of NaH and 2.2 eq. of di-*tert*-butyldicarbonate in THF as previously described by the Rozas group (Scheme 3.8).[115] The obtained yields were good compared with those in the literature, with a total yield of 87% for **62** lower than the reported yield of 95%,[123] but with a yield of 91% for **63**, which is higher than the literature value of 72%.[115] Purity was also high with melting point data for **63** in good agreement with the literature value (117 °C versus 117 – 119 °C).



Scheme 3.8

3.2.2.1 Mechanism of Boc Protection of Amidine Precursors

A very strong base, such as NaH, is needed for the reaction to occur. NaH is an extremely powerful base, therefore it will fully deprotonate thiourea and the mechanism will proceed as follows (Scheme 3.9):



Scheme 3.9

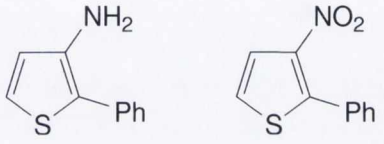
Firstly, thiourea is deprotonated irreversibly by NaH resulting in the liberation of H_2 gas. The highly basic and reactive thiourea salt attacks di-*tert*-butyldicarbonate to yield the relevant *mono-tert*-butoxycarbonyl-protected thione, CO_2, and sodium *tert*-butoxide. As there is a further thioamide group on the intermediate, the two steps previously mentioned are repeated, resulting in the formation of **62** or **63**.

3.3 Investigation of Coupling Reagents for the Amidylation Reactions

3.3.1 Preparation of 3-Nitro-2-phenylthiophene (67)

3.3.1.1 Introduction

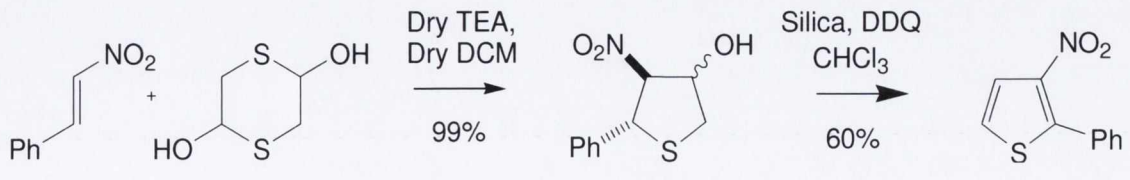
Few aminothiophenes are commercially available, and of those, many are prohibitively expensive. It was therefore decided to test the coupling using 3-amino-2-phenylthiophene (**66**, Figure 3.2), a precursor of which, 3-nitro-2-phenylthiophene (**67**, Figure 3.2), had previously been prepared in good yield in the Southern group at Trinity College Dublin.[127]



66 **67**

Figure 3.2 – Structures of 3-amino-2-phenylthiophene (left) and 3-nitro-2-phenylthiophene (right)

The synthesis of **67** follows a procedure devised by Southern *et al.* to make 2-substituted-3-aminothiophenes, where the 2 substituent can be alkyl or aryl. The initial reaction involves a tandem Michael-intermolecular Henry reaction to form a tetrahydrothiophene from 1,4-dithiane-2,5-diol and a nitroalkene. It was reported that in both diastereomers of the tetrahydrothiophene, the nitro and R groups were in an *anti* configuration, whereas the hydroxyl group could be either *syn* or *anti* to the nitro group, with the *anti* configured diastereomer predominating. The steps are as follows: the formation of 4-nitro-5-phenyltetrahydrothiophen-3-ol (**68**, Scheme 3.10) and its one pot dehydration and oxidation using silica and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to form **67** as depicted in Scheme 3.10.[127]

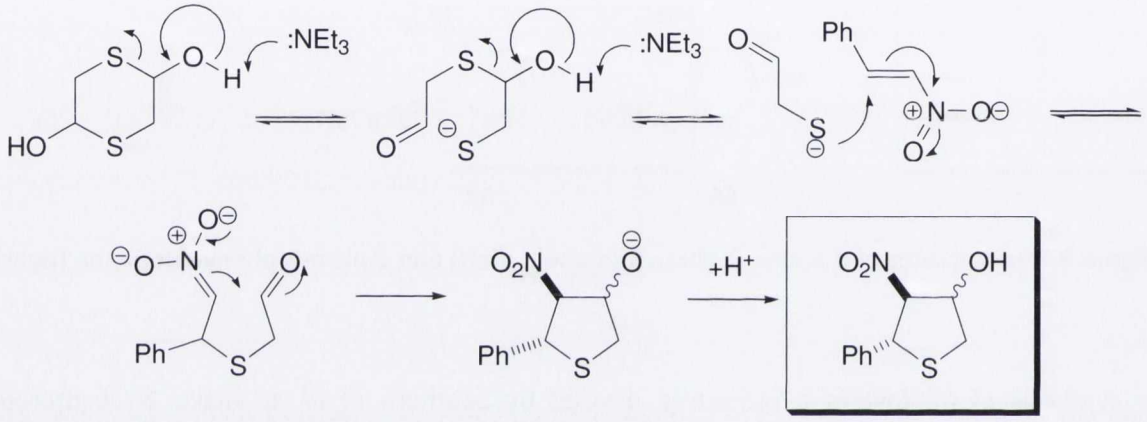


68 **67**

Scheme 3.10

3.3.1.2 Mechanism of the Formation of 4-Nitro-5-phenyl-tetrahydrothiophen-3-ol (68)

The mechanism of the formation of **68** is depicted in Scheme 3.11. Double deprotonation of 2,5-dihydroxy-1,4-dithiane by TEA produces 2 eq. of a thiolate anion. The thiolate anion then attacks the double bond of *trans*-β-nitrostyrene, via a Michael addition, forming a thioether ketone, which has a negative charge stabilised on the nitro group. This molecule is prearranged to undergo an intramolecular cyclisation via the Henry reaction to form the oxalate anion of **68**, which in turn is protonated by the conjugate acid of TEA to produce **68**. The ring closing step being 5-exo-trig is allowed by Baldwin's rules.[128]

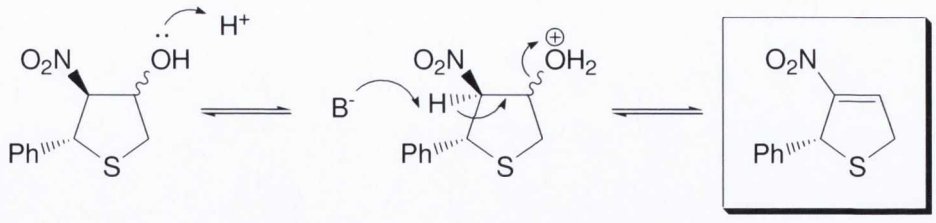


68

Scheme 3.11

3.3.1.3 Mechanisms of the Dehydration and Oxidation of 4-Nitro-5-phenyl-tetrahydrothiophen-3-ol (68)

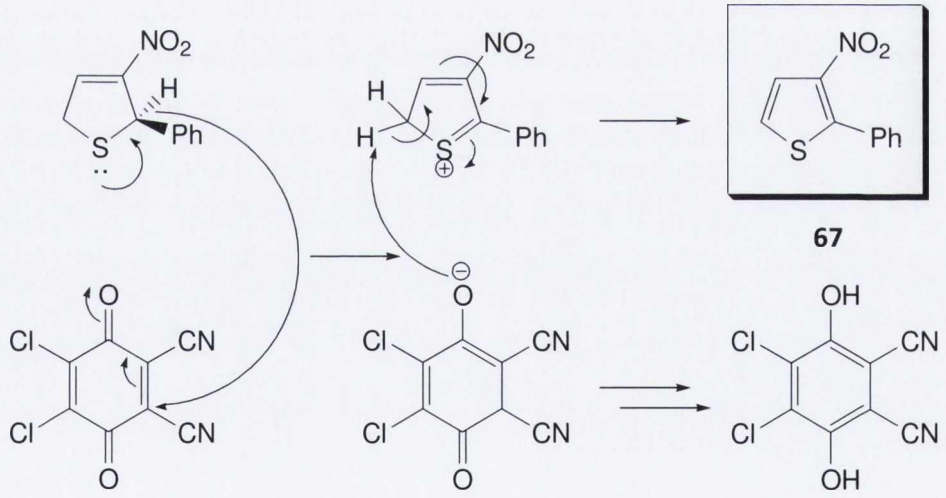
Dehydration of **68** is thought to proceed via a standard dehydration pathway such as that depicted in Scheme 3.12. The oxygen lone pair of the alcohol group picks up a proton from the alumina, forming an oxycation. The hydrogen atom geminal to the nitro group is relatively acidic due to the electron withdrawing inductive effect of the aforementioned group. Therefore, this oxycation can be easily deprotonated geminal to the nitro group, which occurs concomitantly with the formation of a new double bond and the expulsion of water to generate a dihydrothiophene intermediate.



68

Scheme 3.12

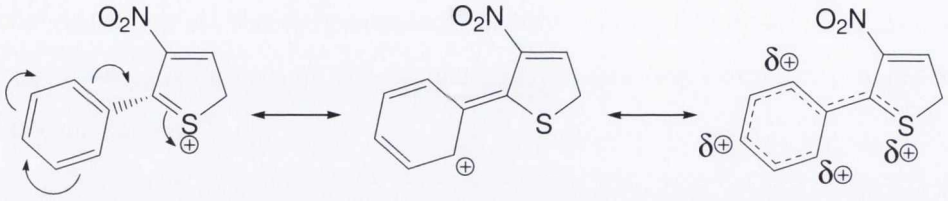
The dihydrothiophene intermediate generated in the dehydration step is then oxidised by DDQ in the same pot. The mechanism is depicted in Scheme 3.13. When any two molecules of the dihydrothiophene and DDQ come close enough, the lone pair on the sulfur atom of the dihydrothiophene can assist the delivery of a hydride ion geminal to a chlorine atom on DDQ, with the simultaneous rearrangement of the double bonds in DDQ. This results in the generation of an ion pair of a sulfonium and an oxyanion. The oxyanion is then capable of removing a proton on the carbon adjacent to the sulfonium ion, which occurs with the rearrangement of the double bonds in the thiophene intermediate, neutralising the positive charge and forming the thiophene **68**. DDQ is capable of reducing two molecules of the dihydrothiophene and forms a diphenol.



67

Scheme 3.13

It is envisaged that the hydrogen in the 2 position would be the one eliminated as a hydride as this proton is benzylic and thus the electron density of the phenyl ring can stabilise the sulfonium ion by resonance as is shown in Scheme 3.14.



Scheme 3.14

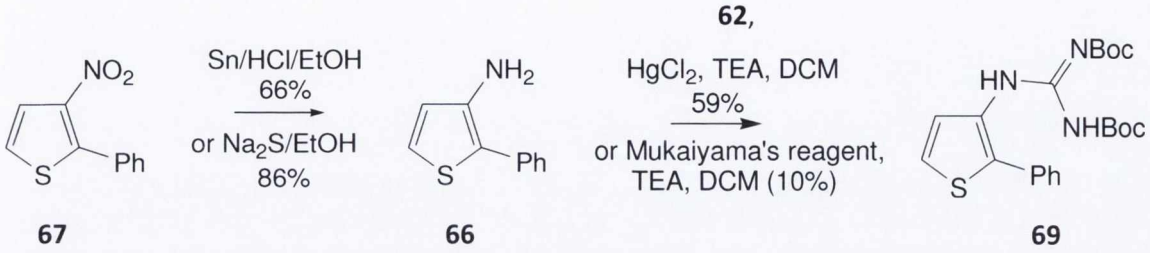
3.3.1.4 Synthesis of 3-Nitro-2-phenylthiophene (67)

The first step proceeded smoothly using *trans*-β-nitrostyrene (1 eq.) and 2,5-dihydroxy-1,4-dithiane (0.75 eq.) using TEA (0.2 eq.) as catalyst and resulted in a yield of 99% of **68** as a mixture of diastereomers. It is of note that the literature yield of 90%[127] was exceeded with a quantitative yield obtained. The diastereomers were not purified further as chirality is removed in the next step, with both isomers giving the same product. Proton nuclear magnetic resonance (^{1}H NMR) data was consistent with both reported isomers and the retention factor (R_f) values of 0.25, 0.16 were in good agreement with the literature [(Lit.)[127] 0.24, 0.16].

The high-yielding synthesis of **67** described in the literature was carried out in a microwave, and was not applicable to easy scale-up, therefore the reaction was performed using conventional equipment using 8 eq. w/w silica and 4 eq. DDQ. Purification by column chromatography and recrystallisation from water/ethanol (EtOH) 1:1 resulted in a golden solid with a melting point of 100 - 101 °C, in good agreement with literature values (101.5 – 102.5 °C).[129] Considering the lower yields generally obtained from larger scale reactions, and the fact that a microwave was not used and so the temperature of 125 °C could not be reached, the obtained yield of 60% is reasonable. It is also of note that Southern *et al.* report a similar large scale method for this two-pot procedure, using trifluoroacetic anhydride as dehydrating agent resulting in the similar yield of 59%. However, it was reported that extensive column chromatography was needed via this approach, so the method chosen was a mixture of the two methods previously reported.[127,129]

3.3.2 Reduction of 3-Nitro-2-phenylthiophene (67)

Preparation of 3-amino-2-phenyl thiophene (**66**) was then attempted by reducing the nitro group. This also allowed for the testing of nitro reduction conditions, which could be used in the synthesis of subsequent aminothiophenes.



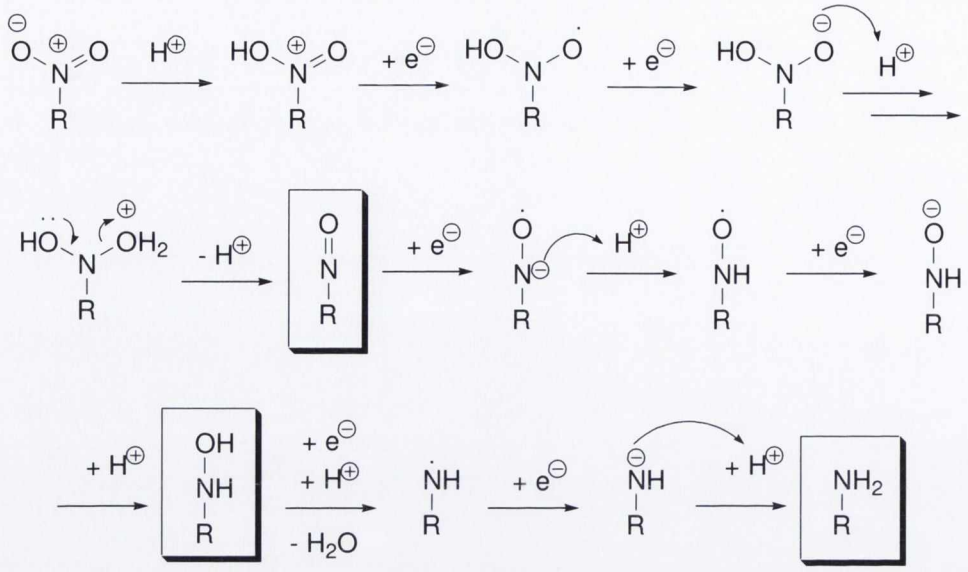
67 **66** **69**

Scheme 3.15

The first conditions tested for the reduction of **67** were reduction using tin with concentrated (conc.) HCl. The reaction is reported to proceed via Equation 3.1.[130,131]

Equation 3.1: $3Sn + ArNO_2 + 6HCl \rightarrow 3SnCl_2 + ArNH_2 + 2H_2O$

A mechanism for this reduction based on a dissolving metal reduction, consistent with Equation 3.1 is depicted in Scheme 3.16. As the metal dissolves in the acid, it donates electrons to the nitro group of the nitroarene. A series of radicals and ions are generated by the sequential capture of the aforementioned electrons and protons from the solution. There are two intermediates in the reaction, the nitroso compound and the hydroxylamine derived from the nitroarene. Two molecules of water are also eliminated during the course of the reaction, and due to the acidic conditions involved, the free amine produced is protonated to form an ammonium salt (not shown) which is subsequently hydrolysed by base.



Scheme 3.16

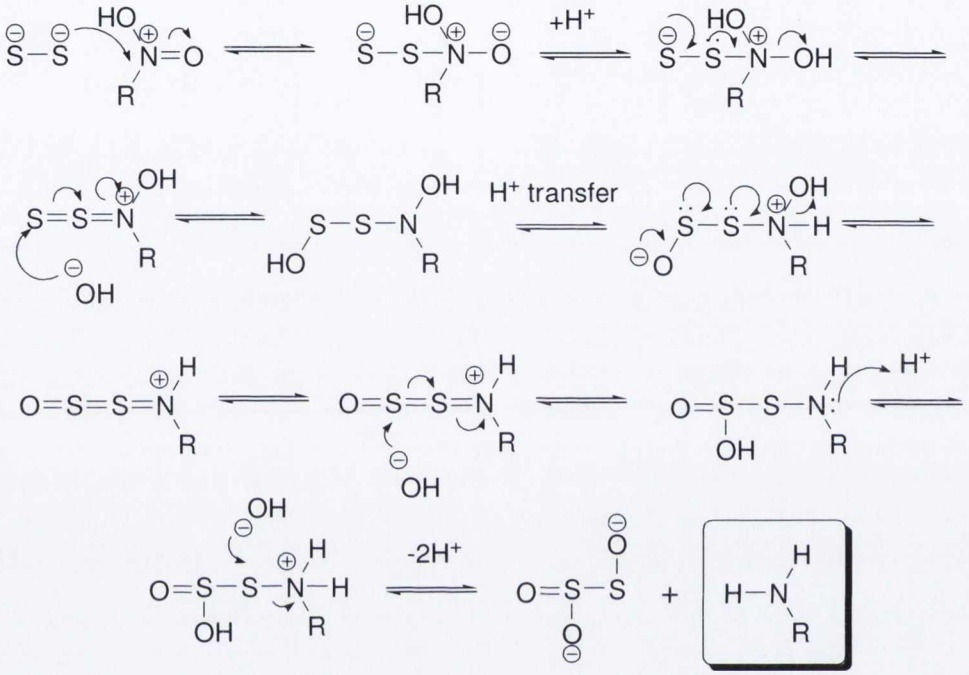
Overnight reduction using 3 eq. tin in HCl was attempted; however, **67** proved poorly soluble and no product was formed using the procedure of Kenning and Mitchell.[132] Since **67** can be recrystallised from aqueous EtOH,[133] EtOH added to aid solubility (Scheme 3.15). This enhanced the reaction significantly, with thin layer chromatography (TLC) showing partial conversion to a product, so a further 2.5 eq. tin was added which resulted in the formation of the desired product in 66% yield after column chromatography. ^{1}H NMR values (δ 7.55, 7.44, 7.29, 7.15, 6.70, 3.48) are consistent with those reported by Yoshikawa *et al.*[134] (7.52, 7.42, 7.22-7.29, 7.12, 6.66, 3.82).

It was thought that a higher yield could be obtained by using sodium sulfide (Na_2S) which had worked well in the Rozas group previously. The reduction is reported to be mainly due to the action of di- and polysulfide ions, with sulfide, hydrosulfide and hydrodisulfide ions playing a minor role.[135] It is expected to proceed according to Equation 3.2.

Equation 3.2: $4\ ArNO_2 + 3S^{2-} + 7H_2O \rightarrow 4ArNH_2 + 3S_2O_3^{2-} + 6\ OH^-$

A possible mechanism for the transformation is depicted in Scheme 3.17. Deprotonation of ethanol by the nitro group creates an intermediate with a positively charged nitrogen. It is presumed that the disulfide ion attacks the nitro group at the positively charged nitrogen forming a new charged

intermediate. A second deprotonation is followed by the attack of sulfur by sulfide, which causes the subsequent oxidation of sulfur and reduction of nitrogen via the elimination of hydroxide ion. Addition of a further hydroxide ion causes the oxidation of that ion, and the reduction of the nitrogen atom. Proton transfer occurs again allowing further oxidations and reductions to take place.



Scheme 3.17

Reduction using Na_2S (4.8 eq.) in refluxing EtOH over 6 h[135,136] was also attempted and proved successful giving a higher yield (86%), purer product, and was easier to carry out. It was decided to further purify **66** by acidic extraction; however this resulted in the complete loss of product upon basification, indicating acid instability. Thus, amine **66** was used without purification to test the guanidylation reaction, exploring the possible coupling conditions.

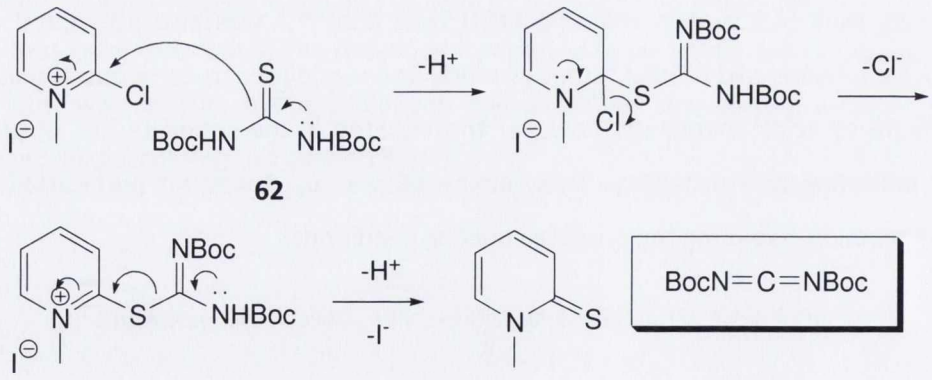
3.3.3 Investigations on Coupling Reagents for the Amidylation Process

The coupling reaction of **62** (1.2 eq.) and **66** was then carried out using 1.1 eq. $HgCl_2$ with 3.5 eq. TEA in dry DCM,[115] resulting in a moderate yield (42%) of Di-Boc-protected thiophenylguanidine (**69**, Scheme 3.15) after column chromatography. The ^1H NMR spectrum of the product shows that the

NH_2 signal at 3.48 ppm in thiophene **66** has been replaced with two less broad NH signals at 11.61 and 10.42 ppm. Also, the Boc signal now appears as two distinct peaks at 1.53 and 1.50 ppm instead of just one at 1.52 ppm, confirming that the coupling has indeed taken place. Both pieces of data support the assignment of the product as molecule **69**. A subsequent reaction using 1.5 eq. $HgCl_2$ afforded a slightly improved yield (59%) after purification.

In an attempt to improve yields, the coupling was carried out using Mukaiyama's reagent, which had been described in the literature[137] as a substitute for mercury chloride in this type of reaction and it has been used in the group previously.

It is proposed that the method occurs via generation of a carbodiimide as depicted in Scheme 3.18, which can then react with an amine in an analogous manner to the mechanism proposed for the Kim and Qian reaction. Firstly the lone pair of a nitrogen from **62** feeds into the C-N bond forming a pi bond, while breaking the pi bond between the carbon and sulfur. The electrons in this bond attack Mukaiyama's reagent at the electron deficient 2 position, breaking aromaticity and quenching the positive charge on nitrogen. Aromaticity is reformed when the lone pair on the pyridinium nitrogen feeds into the C-N bond which in turn causes Cl^- to leave. Repeat of the initial attack by the second nitrogen of the thiourea-based moiety causes the formation of the carbodiimide by breaking the C-S bond. A double bond is also formed between sulfur and the carbon in the 2 position of what was Mukaiyama's reagent, and this in turn quenches the positively charged nitrogen, breaking aromaticity and forming the side product 1-methyl-1*H*-pyridine-2-thione.



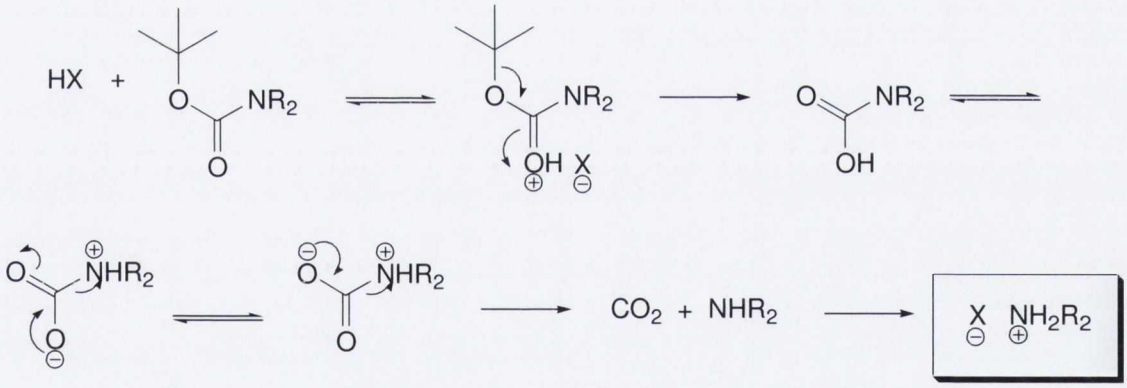
Scheme 3.18

The coupling was carried out using **62** (1.2 eq.), TEA (2.2 eq.) and Mukaiyama's reagent (1.2 eq.).[137] Six spots with very similar TLC R_f values to **69**, made isolation of the pure product difficult with only a 10% yield obtained. Due to the purification difficulties using Mukaiyama's reagent, and the lower yield and purity obtained than when using $HgCl_2$, the latter was selected as coupling reagent in subsequent reactions.

3.3.4 Deprotection of [2,3-Di(*tert*-butoxycarbonyl)]-1-(2-phenylthiophen-3-yl)guanidine (69)

3.3.4.1 Mechanism of Acid-catalysed Boc Deprotection

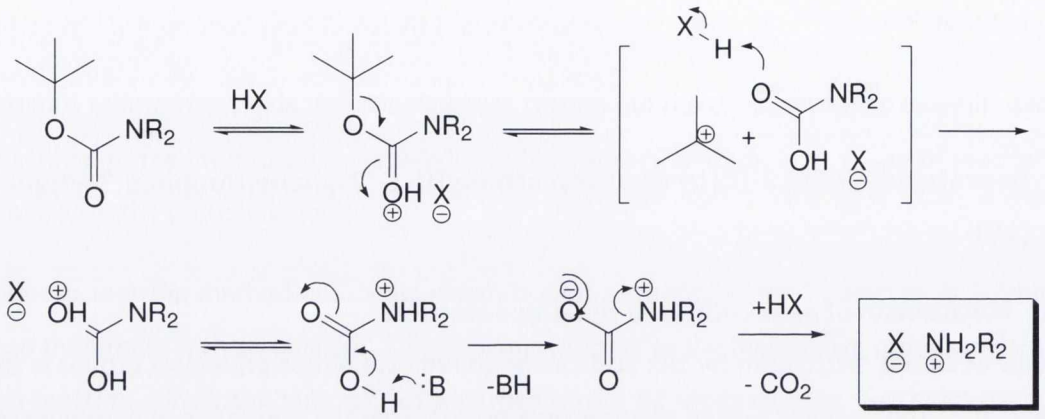
The widely accepted mechanism for the acid-catalysed removal of Boc protecting groups is shown in Scheme 3.19.[138] The carbonyl oxygen acquires up a proton from the solution to generate an oxonium ion which can then undergo loss of a *tert*-butyl cation to give a carbamic acid. Proton exchange, from the oxygen of the acid moiety to the nitrogen, allows the decarboxylation of the carbamic acid to occur resulting in the free amine, which is rapidly protonated to generate a salt.



Scheme 3.19

However, recent kinetic studies by Ashworth *et al.* have shown that the reaction is in fact second order in relation to acid concentration,[139] not first order as would be expected if the above mechanism were correct. A number of possible mechanisms are given by the group which account for the observed kinetics of which that shown in Scheme 3.20 is most consistent with the empirical data. Notable differences in the new mechanisms are the presence of a molecule-ion pair after the

elimination of *tert*-butyl cation with is followed by a slow dissociation step, and that the species which is decarboxylated is a monocation as opposed to a zwitterion in the standard mechanism.

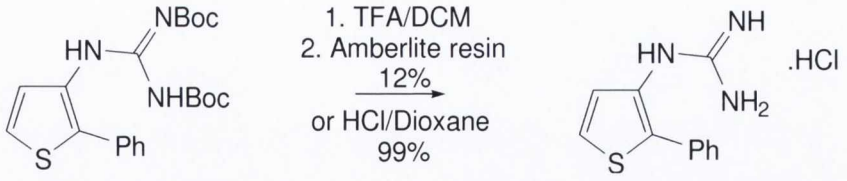


Scheme 3.20

3.3.4.2 Synthesis of *N*-(2-Phenylthien-3-yl)guanidine Hydrochloride (70)

Due to the low quantity of **69** prepared initially, its deprotection was not carried out to begin with, since there would not be enough material for pharmacological testing. In addition, the resulting guanidine hydrochloride salt (**70**, Scheme 3.21) was not initially a target molecule. However, after the difficulties which later arose in relation to synthesising the target molecules, it was decided to synthesise **70** for pharmacological testing.

Acidic deprotection of the Boc groups in **69** using TFA/DCM 1:1 generated a trifluoroacetate salt, which was not characterised, but was converted directly to the hydrochloride salt **70** by treatment with Amberlite 400-IRA in the chloride form.[115] Unfortunately, the yield was very low at 12%. It was hypothesised that the low yield could be due to steric interference caused by the proximity of the phenyl group to the guanidine moiety of **69**. However, later deprotection via the 4M HCl/dioxane method (discussed in 4.2.8.2) resulted in a yield of 99%. The product was not soluble in $CDCl_3$, and so D_2O was used as an NMR solvent. The ^1H NMR spectrum showed loss of both Boc groups, with no aliphatic signals present. The ^{13}C NMR spectrum showed loss of all carbon peaks from both Boc groups, while all other carbon atoms were accounted for. Therefore the product is consistent with the desired structure.



69 **70**

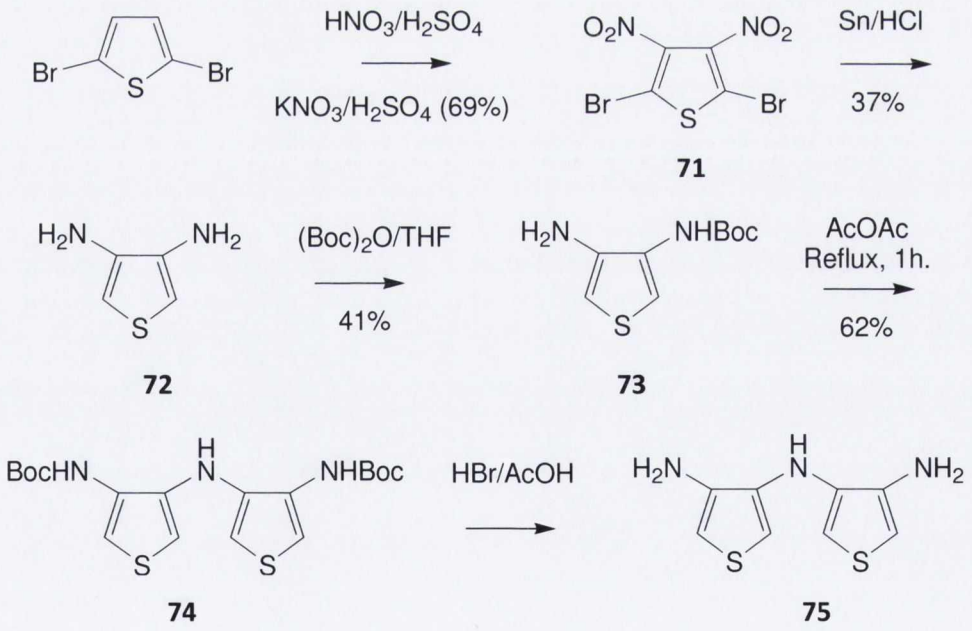
Scheme 3.21

3.4 Attempted Synthesis of Derivatives of *Bis*-(4-aminothien-3-yl)amine (75)

3.4.1 Attempted Preparation of *Bis*-(4-aminothien-3-yl)amine (75)

3.4.1.1 Introduction

Bis-(4-aminothien-3-yl)amine was selected as precursor for the first *bis*-thienyl guanidine because its preparation as 5-step synthesis had previously been described across two papers by both Kenning *et al.*[132] and Brugier *et al.*[140] (Scheme 3.22).



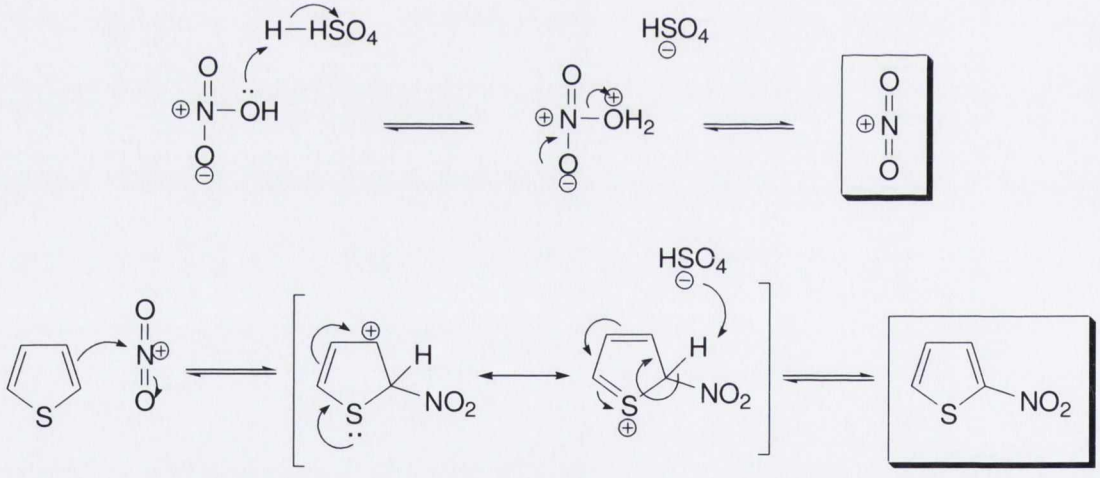
72 **73** **74** **75**

Scheme 3.22

The synthesis proceeded as follows: 2,5-dibromothiophene was nitrated using a mixture of nitric and sulfuric acid resulting in the dinitrated product 2,5-dibromo-3,4-dinitrothiophene (**71,** Scheme 3.22). The reduction of both nitro moieties to amino groups was carried out simultaneously with the radical debromination of **71** to produce 3,4-diaminothiophene (**72**, Scheme 3.22). *mono*-Boc-protected aminothiophene (**73**, Scheme 3.22) was then prepared from **72** using di-*tert*-butyldicarbonate. **73** was converted to Boc-protected aminothiophene dimer (**74**), using acetic acid (AcOH) as catalyst, and finally the acid catalysed deprotection of **74** afforded the free aminothiophene dimer **75 (**Scheme 3.22).

3.4.1.2 Nitration of 2,5-Dibromothiophene

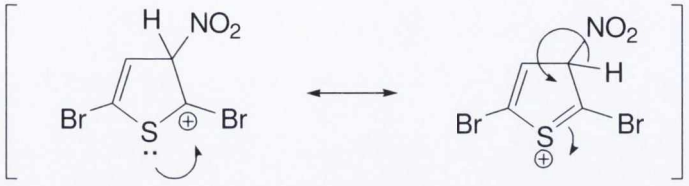
The mechanism of the nitration of 2,5-dibromothiophene follows the standard mechanism of EAS, which is illustrated in Scheme 3.23 for the case in which the nitronium ion is the electrophile and 2,5-dibromothiophene is the nucleophile.



Scheme 3.23

Nitric acid is protonated by the stronger acid, sulfuric acid, resulting in a species which formally has two adjacent positive charges, which due to its own instability, eliminates water to form a nitronium ion. The thiophene nucleus then attacks the ion, temporarily losing its aromaticity, to form a resonance stabilised cationic intermediate. This intermediate is then deprotonated geminal to the added nitro group to reform aromaticity and generate a nitrothiophene. For unsubstituted

thiophene, the nitro group will most often add in the 2 or 5 position, as shown in Scheme 3.23 as the positive charge is stabilised over 4 atoms. However in the case of 2,5-dibromothiophene, since no substitution in the 2 or 5 positions can take place, substitution occurs at the 3 position, forming an intermediate in which the cation is only stabilised over only 2 atoms as shown in Scheme 3.24.



Scheme 3.24

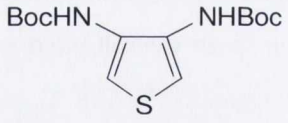
Nitration of 0.44 mmol 2,5-dibromothiophene using fuming HNO_3 in H_2SO_4 as described in the literature[132] (Scheme 3.22) produced **71** in a poor yield (8%) after recrystallisation from methanol (MeOH) compared to the literature (47%). This discrepancy is mainly accredited to the difficulty in recrystallising on a µmol scale without microscale apparatus. A second reaction at the same scale was carried out using the milder procedure of KNO_3 in H_2SO_4.[141] The reaction conducted with the KNO_3 mixture was found to give a higher yield (36%) than that with HNO_3, and since the stoichiometry of the reaction can be controlled and less acid waste is produced, the KNO_3 procedure was used preferentially in subsequent nitrations. The mother liquor obtained from the recrystallisation of all reactions to make **70** was kept, and following solvent removal, the residue could be recrystallised further or purified by column resulting in an overall yield of 69%. Characterisation data via both methods were consistent with literature values (136-138 °C compared with 135-137 °C).[132]

The reaction itself did go to completion; however it resulted in the formation of 3 other by-products, two of which have one proton, while the other two do not. It was postulated that one of the proton-containing by-products was 3-nitro-2,5-dibromothiophene, while the other two products involved substitution at the bromine atoms. However further investigation of the side products would be necessary to confirm this.

3.4.1.3 Preparation of *tert*-Butyl-(4-aminothien-3-yl)carbamate (73)

Then, diaminothiophene **72** was prepared using Sn in HCl and according to the literature it is isolated as a mixed salt involving hydrochloride and tin(IV)chloride.[132] The isolation of the salt of **72** proved difficult due to the large quantities of tin salts which precipitate out of solution on basic work up. It was found that by cooling the solution in a freezer overnight and by carefully controlling the solvent volume, the collection of the salt precipitate by filtration was possible. Subsequent basic extraction of the salt and removal of solvent without heating, afforded **72** in 37% yield (Lit.[132] 61%). ^{1}H and ^{13}C NMR data were found to be consistent with literature values, however the melting point was lower than expected (88-96 °C compared to 95.5-96.9 °C) which indicates that the compound was not isolated completely pure, probably containing small quantities of residual solvents or impurities, in quantities not visible via ^{1}H NMR, which should not interfere materially with the subsequent reaction . Due to the instability of diamine **72**, it was found that it could only be stored as the salt as was noted in the literature.

The next step in the synthetic route involves the preparation of **73** using 1 eq. of di-*tert*-butyl dicarbonate and **72** in THF (Scheme 3.22). Compound **73** was isolated in 41% yield (lit.[140] 55%). It was found that use of a tetrahydrofuran (THF)/light petroleum mix as solvent as reported in the literature gave a poorer yield (18%) than when THF was used alone. A by-product from the reaction, *di*-Boc-protected aminothiophene (**76**) was also isolated in a yield of 39%, accounting for most of the rest of the material from the reaction.



76

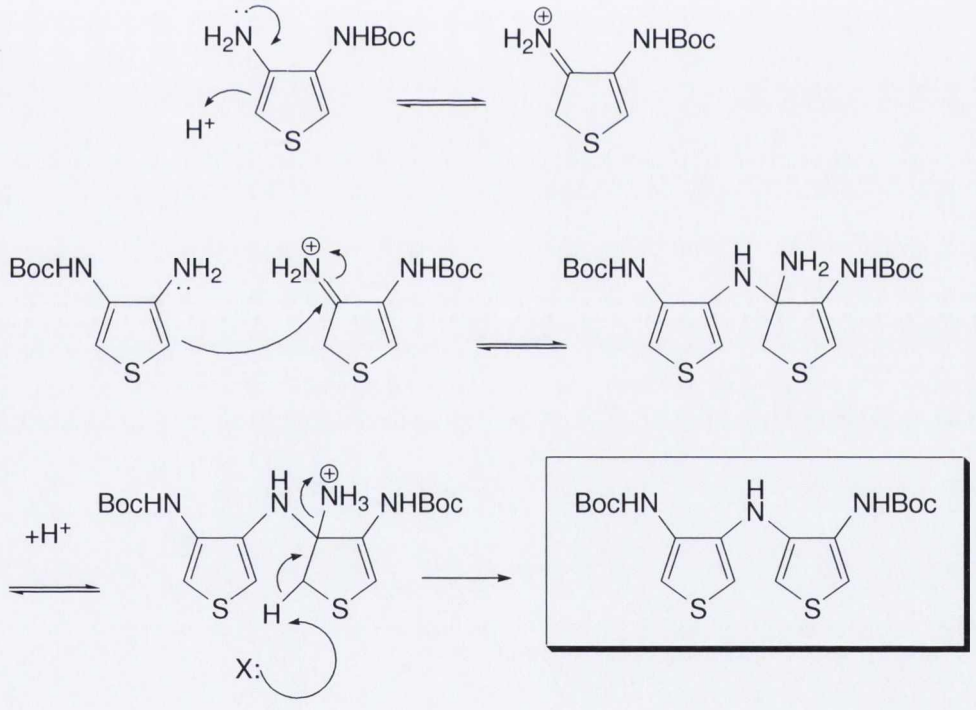
Figure 3.3 – Structure of *O*-*tert*-butyl-*N*-(4-*tert*-butoxycarbonylaminothien-3-yl)carbamate

3.4.1.4 Attempted Preparation of *Bis*-(4-aminothien-3-yl)amine (75)

The fourth step involved the synthesis of **74**, by refluxing **73** in AcOH over 1 h (Scheme 3.22).[140] This step proved difficult since the product decomposes easily. The best yield achieved was 62% (lit.[140] 89%); however, it has been observed that starting material is often still present after the reported reaction time. Control of the reaction time was nevertheless necessary, as longer reaction times

resulted in lower yields with increased quantities of an ash-like black powder isolated, both of these observations were found to be associated with decomposition or polymerisation of the product.

The proposed mechanism for the formation of **75** is a modification of that of a similar reaction hypothesised by Brugier *et al.*[140] and is shown in Scheme 3.25. The lone pair of the nitrogen of the 3-aminothiophene assists in the protonation of the thiophene ring at the 2 position, causing the loss of aromaticity. The iminium ion thus formed is attacked by a neighbouring aminothiophene forming a tetrahedral intermediate. Protonation of the primary amine forms the good leaving group NH_3R^+. Deprotonation of the dihydrothiophene ring at the 2 position with elimination of NH_3 gas results in **74**. The last step is irreversible due to the elimination of ammonia gas. Support for the initial step of the mechanism comes from the isolation of a number of 2-alkyl-3,4-diaminothiophenes formed from the reaction of **72** and **73** with various ketones and aldehydes.[140] Therefore 3-aminothiophenes are known to attack electrophiles in this position.



Scheme 3.25

Deprotection of the diamine with 25% HBr in AcOH as described in the literature was attempted (Scheme 3.22),[140] however no **75** was isolated. This is attributed to the total decomposition or

polymerisation of **74** as observed via ^{1}H NMR spectroscopy. Attempts to purify the remaining **74** by column chromatography failed as an extremely complex mixture of products was observed on TLC. It should be noted that in the literature, compound **74** was only synthesised to confirm the identity of a side product, and that no information on the stability of either **74** or **75** was given.

3.4.2 Alternative Strategies

3.4.2.1 Synthesis of Boc Protected Amidine Intermediates

It was thought to be worthwhile to prepare the Boc-protected-thiophene amidine dimers **77** and **78**, from their respective monomers **79** and **80** respectively, all Figure 3.4. This proposed synthesis would circumvent the preparation of **75**, since **73** could be coupled to Boc-protected guanidylating reagents **62** and **63** forming **79** and **80** respectively. It would in turn allow access to the hydrochloride salts of both **79** and **80**, which being thiophene analogues of lead compound **48** are synthetically interesting in their own right.

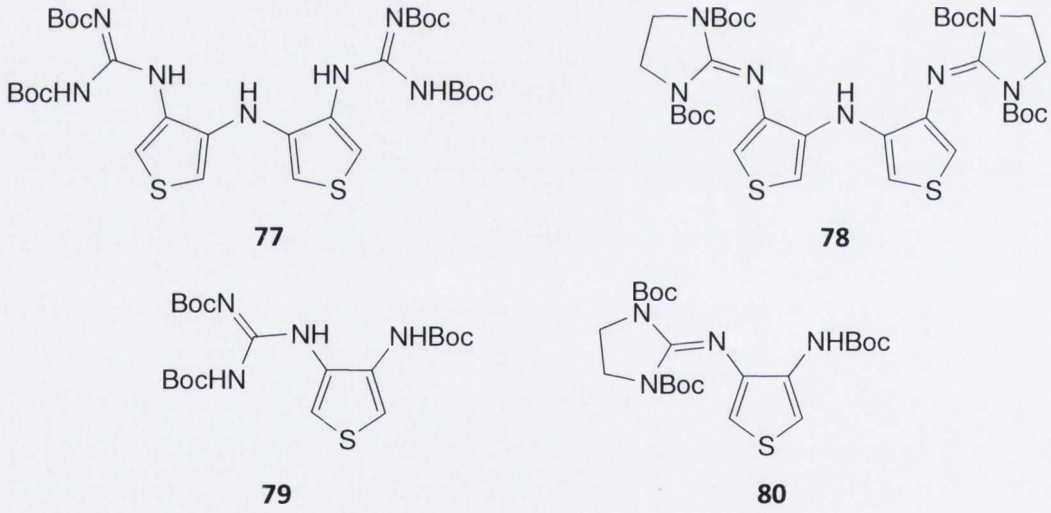


Figure 3.4 - Compounds involved in the hypothesised alternative synthesis.

Compound **80** was synthesised from **73** using the standard 2-iminoimidazolidylation procedure[112] using 1.1 eq. **63**, 1.1 eq. $HgCl_2$ and 4 eq. TEA; however, **80** proved to be inseparable from **63** by TLC using ethyl acetate (EtOAc), DCM, hexane/EtOAc mixtures on silica and hexane/EtOAc mixtures on alumina. Attempted purification by column chromatography using hexane/EtOAc (3:2) did not result in isolation of pure product. Recrystallisation from minimal ether with hexane was attempted, but the

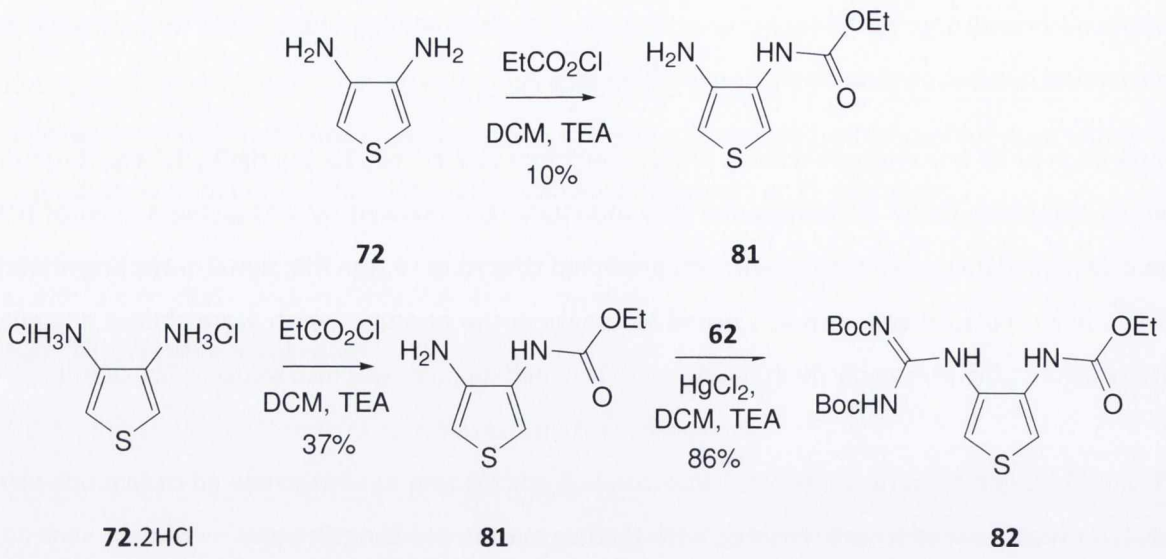
compound proved too insoluble. Recrystallisation from minimal amount of DCM with hexane was then carried out, but no pure crystals were obtained.

Preparation of **79** was attempted using similar conditions (1.2 eq. **62**, 1.2 eq. $HgCl_2$, 3.5 eq. TEA) using column chromatography for purification (hexane:EtOAc 7:3 followed by 7:1) giving a yield of 61%. Again spectral data was consistent with the predicted structure, *i.e.* the NH_2 signal in starting material **73** had been replaced with two less broad NH peaks in the product, which were shifted downfield with respect to the previously mentioned signal. The melting point was also sharp at 200-203 °C.

3.4.2.2 Synthesis of Intermediates with Orthogonal Protecting Groups

Due to the purification difficulties observed with **80**, and the difficulties predicted for the guanidylations of **79** and **80** to generate **77** and **78** due to the fact that removal of the Boc groups would likely occur in the same step, it was decided to explore orthogonal protecting groups. Since the *O*-ethyl carbamate protecting group used by Brugier *et al.*[140] can be cleaved under basic conditions, it was chosen for synthesis along with the classic base-labile protecting group 9-fluoromethyloxy carbonyl (Fmoc), since both groups are stable under acidic conditions. Thus the new target molecules, **82** and **84**, and are depicted in Scheme 3.26 and Figure 3.5.

Synthesis of ethyl *N*-(4-aminothien-3-yl)carbamate (**81**) from **72** according to the method of Brugier et al.[140] using 1 eq. ethyl chloroformate yielded the target molecule as an oil with spectral data in agreement with the literature. However the yield was much poorer than that reported in the literature (10% compared with 64%), so a modification of the procedure using the salt of **72** instead of the free amine and keeping the reaction under argon, lead to the isolation of **81** in a higher yield of 37%.



Scheme 3.26

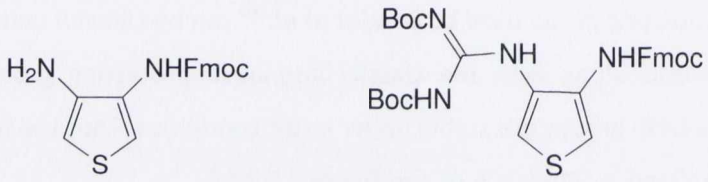


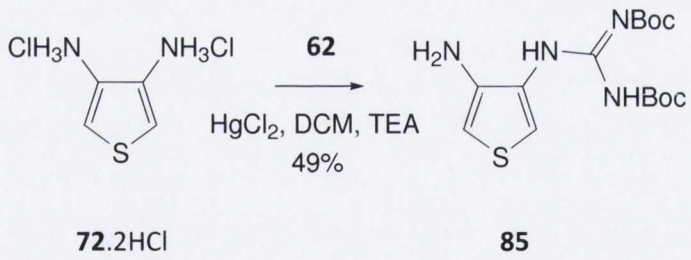
Figure 3.5 – Target molecules with Fmoc protecting group

The Fmoc-protected carbamate (**83**) was attempted via a modification of the method of Schadendorf *et al.*[142] as their substrate was also an aromatic amine. The hydrochloride salt of **72**, having given better results in the synthesis of **81**, was treated with 3.5 eq. NaOH in dry THF, before a solution of 1 eq. Fmoc chloride in the same solvent was added dropwise. The reaction was monitored by TLC for two days, and TLC analysis on a third day showed the formation of five new spots, so the reaction was quenched and a low yield of 8% of almost pure material was obtained after basic extraction. The reaction was repeated over a shorter time using a small excess of TEA as base but after 3 columns (Hexane:EtOAc 3:1) only 2% of **83**, of a similar purity to the previous batch, was isolated. However there was enough material to test the guanidylation reaction. The poor yield of **83** could be caused by the slight excess of base used in its synthesis (3.5 eq. as opposed to 3 eq.) or perhaps Fmoc-Cl was added too soon after the addition of base.

Gratifyingly, after the mediocre yield obtained for **81**, **82** was synthesised cleanly, following the Kim and Qian method, in 86% yield. Spectral data was in agreement with the structure, since in an analogous manner to compounds previously mentioned in this chapter, the NH_2 ^1H NMR signal of starting material **81** had been replaced with two considerably downfield NH signals in the product. Unfortunately the attempted synthesis of **84** did not proceed as cleanly or in good yield, with only trace amounts of impure material having been isolated. It was postulated that the difficulties in the synthesis of **84** was largely due to the steric interference created by the bulky Fmoc group *ortho* to the reacting group, and the synthesis was taken no further.

3.4.2.3 Preparation of [2,3-Di(*tert*-butoxycarbonyl)]-1-(4-aminothien-3-yl) guanidine (85)

Due to the difficulties involved in purifying the various *mono*-protected 3,4-diaminothiophene derivatives (**79**-**84**), it was decided to attempt the synthesis of *mono*-guanidylated aminothiophene **85** (Figure 3.6), in order to prepare the corresponding hydrochloride salt of **85** and that of dimer **77**. The preparation of **85**, from the coupling of **72** with **62**, was achieved smoothly using the standard procedure, giving a yield of 49% after purification (Biotage column), with similar spectral consistencies observed in the product in comparison to the starting material as has been described for similar molecules (e.g. **82**).



72.2HCl **85**

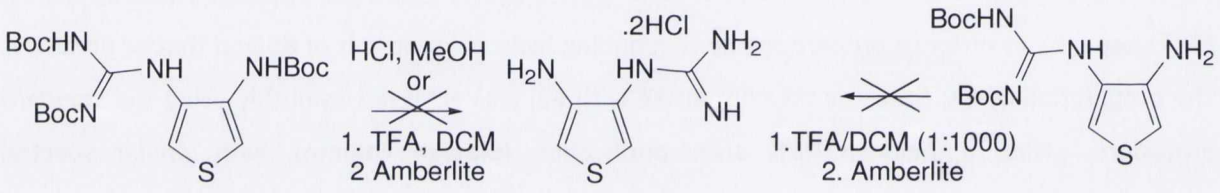
Figure 3.6 – Structure of [2,3-di(*tert*-butoxycarbonyl)]-1-(4-aminothien-3-yl)guanidine

3.4.2.4 Deprotection of the Amidines from the Alternative Synthesis

Deprotection of **79** was attempted using 1.25 M HCl in MeOH, as this is a one-step method of producing hydrochloride salts. Purification was carried out by reverse phase column chromatography (Biotage), however, on removal of solvents a brown residue was obtained which contained no

product consistent with the structure as determined by ^{1}H NMR spectroscopy. It was therefore believed that the conditions using HCl were too strongly acidic to be used on these sensitive aminothiophenes. The method involving a 50:50 TFA/DCM mixture used previously in Section 3.3.4.2 was then attempted along with the use of reverse phase column chromatography but no product was isolated.

The deprotection of **85** in order to generate **86** using the even milder conditions of DCM/TFA (10 mL/0.1 mL) was carried out over 2 h in order to minimise decomposition. ^{1}H NMR data suggested the presence of guanidine dihydrochloride **86**.HCl (Scheme 3.27) and its molecular ion was observed by HRMS. However, it was never isolated in a reasonable purity since, when attempted, reverse phase column chromatography repeatedly produced a highly impure material which was not recrystallisable.

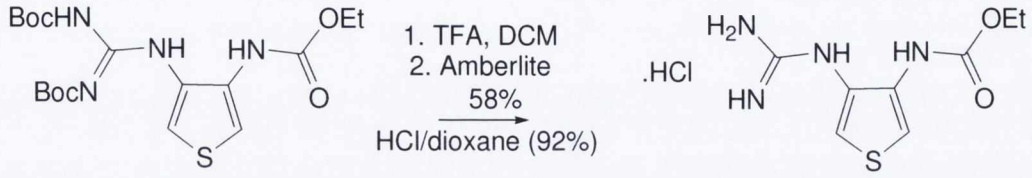


79 **86** **85**

Scheme 3.27

The acid instability of **86** was a disappointing result since it hinted at the acid instability of its derivatives, such as the analogues of **52** (Figure 1.20) which with greater electron density on the nitrogen of the amine would be more susceptible to protonation and polymerisation reactions. Methyl and ethyl methanesulfonate had been used to alkylate diamines in the Rozas group previously.[103,105] The reaction involved reacting one equivalent of each of the alkylating agent, starting amine and TEA in DCM and resulted in the isolation of both the *mono- di*-alkylated amine in moderate yields. Preliminary attempts to alkylate **85**, involving the use of this method had been carried out prior to this result in the hope of making analogues of phenylguanidine derivative **52**. However, the disappointing results when attempting to cleanly isolate **86** indicated that any related alkylated products would also be unstable, therefore, the attempts to synthesise these materials was ceased.

Due to the acid sensitivity of the free 3-aminothiophenes, it was decided to deprotect the Boc groups of **31** to produce a compound from this series of investigations for pharmacological testing. Thus *N*-(4-ethoxycarbonylaminothien-3-yl)guanidine hydrochloride (**87**) was synthesised in 58% yield from TFA/DCM followed by Amberlite resin treatment. A later synthesis using the 4M HCl/dioxane method, which is described in section 4.2.8.2, increased the yield to 92%.



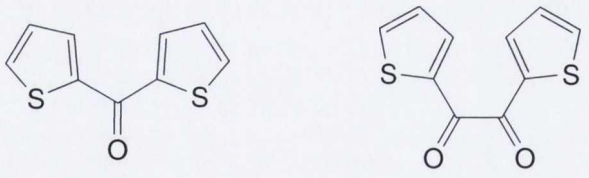
82 **87**

Scheme 3.28

3.5 Towards a General *Bis*-aminothiophene Synthetic Strategy

3.5.1 Introduction

Both the 2,2-(*bis*-thiophenyl)methylketone (**88**) and the 2-(*bis*-thiophenyl)ethylketone (**89**), shown in Figure 3.7, are available commercially so a divergent synthetic strategy to prepare a series of *bis*-thienyl alkyl and ketone linked diamine intermediates from **88** and **89** was proposed.



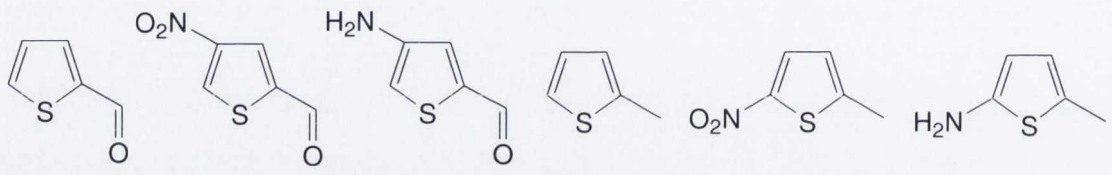
88 **89**

Figure 3.7 - Commercially available *bis*-thienyl compounds.

The inexpensive starting material 2-thiophenecarboxaldehyde (**90**, Figure 3.8) was chosen as a model compound on which to explore reaction conditions, due to the similarity of the functional groups present and the regiochemistry expected. In addition, some of the derivatives that can be obtained

from **90** could be, by themselves, good precursors of thiophene analogues of known α_2-AR antagonists.

In the strategy, by nitrating **90**, 4-nitro-2-thiophenecarboxaldehyde (**91**, Figure 3.8) can be synthesised and this in turn can be used to prepare 4-amino-2-thiophenecarboxaldehyde (**92**, Figure 3.8). Similarly, if the carbonyl moiety of **90** is reduced to a methyl group, thus making 2-methylthiophene (**93**, Figure 3.8), a similar nitrating and reduction scheme can be followed, producing 5-nitro-2-methylthiophene (94, Figure 3.8) and 5-amino-2-methylthiophene (95, Figure 3.8) respectively.



90 **91** **92** **93** **94** **95**

Figure 3.8 – Derivatives of 2-thiophenecarboxaldehyde

3.5.2 2-Thiophenecarboxaldehyde (90) and its Derivatives

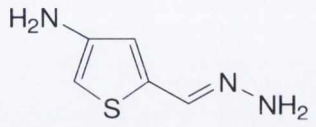
3.5.2.1 Nitration of 2-Thiophenecarboxaldehyde (90)

Nitration of **90** using H_2SO_4 with 1 eq. of KNO_3[141] and double recrystallisation the crude material from hexane/ether (1:1) gave **91** as the major product in 55% yield. Recrystallisation from either hexane or MeOH proved unsuccessful. It was later observed that purification by silica column (hexane/EtOAc 2:1) also yielded pure **91** in a yield of 74%. Melting point data was in good agreement with literature values at 54-56 °C (Lit.[143] 55-56 °C). The corresponding 5-nitro-2-thiophenecarboxaldehyde was also present as a minor product, but was not isolated. The reactivity of **90** is not consistent with that generally reported in the literature for thiophenes with electron withdrawing groups in the 2 position. In such cases the ratio of the 4 and 5 substituted products is generally more evenly distributed.[144]

3.5.2.2 Reduction using Zinc and Hydrazine

The reduction of nitro compound **91** was initially attempted using zinc powder (2 eq.) and excess hydrazine hydrate in MeOH, via the method of Gowda and Gowda.[145] This unusual nitro group reduction was chosen due to the high yields and good selectivity reported.

Due to the presence of the aldehyde moiety, which could be derivatised to a hydrazone during the 10 minutes reaction timescale, it was not certain whether the reduction would give 4-amino-2-thiophenecarboxaldehyde (**92**, Figure 3.8) or its hydrazone (**96**, Figure 3.9) as the major product.



96

Figure 3.9 - 4-Amino-2-thiophenecarboxaldehyde hydrazone (**96**)

None of product **92** was observed by ^{1}H NMR examination of the crude residue. Attempted purification was made difficult by the high polarity of the crude material obtained, and its poor solubility in most organic solvents. This made obtaining the hydrazone and subsequent hydrolysis to **92** unfeasible.

Upon completion of the experiment, a yellow material, which was insoluble in MeOH, was always observed. It was postulated that this was the quaternary ammonium salt of **92**. Attempted formation of the ammonium salt and isolation by alumina column chromatography gave negative results. In a further experiment, washing the celite during workup with NaOH saturated MeOH successfully dissolved this material. Subsequent evaporation of solvent resulted in a highly complex mixture of products as observed by TLC.

By filtering the crude residue and washing with EtOAc and MeOH in another experiment, 20 mg of a dark sticky compound was isolated. The pure compound had ν_{max} of 3408 cm^{-1}, characteristic of a hydrazone group[146] and ^{1}H and ^{13}C NMR spectra were in agreement with the assignment of the product as **96**. Compound **96** proved extremely difficult to manipulate due to its poor solubility in organic solvents so other methods of reduction were sought.

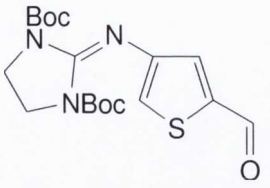
3.5.2.3 Reduction using Tin Reagents

A selective reduction of the nitro compound **91** to the amine **92** was then attempted using Sn (3.5 eq.) in conc. HCl via the method of Brugier discussed earlier.[132] Compound **91** was found to be poorly soluble in HCl, which prevented the reaction from reaching completion. Therefore, it was decided to modify the reaction conditions in order to improve results. This was attempted by adding additional HCl, minimal ether or by heating the reaction mixture to 70-80 °C. In the first two cases, basic extraction in ether and removal of solvent at room temperature produced a white product, which quickly darkened to orange and became insoluble in ether and acetone. Only solvent was observed by NMR spectroscopy. A black precipitate was observed in the heated reaction mixture and it was not possible to isolate any product after work up.

It was postulated that the harshly acidic reaction conditions involved could cause the decomposition of the amine as was previously observed for amine **66**, or could catalyse the polymerisation of any compound **92** formed. In order to avoid the strong acid conditions and to counteract the poor solubility of **91** in HCl, it was decided to use the milder reagent $SnCl_2$, one of the active reducing agents in Sn/HCl reductions, in subsequent reactions.

Results using $SnCl_2$ (5 eq.) in EtOH via the method of Bellamy and Ou[147] proved to be similar; with difficulty isolating any soluble material. However, solution tests for aromatic amines carried out on ice did show promising initial results. According to Vogel's Textbook of Practical Organic Chemistry,[148] the following method is a standard test for the presence of aromatic amines. Addition of HCl and $NaNO_2$(aq) solutions to a mixture suspected of containing aromatic amine **92** resulted in the formation of nitrous acid as determined using starch-iodide paper. Subsequent addition of basic 2-napthol solution resulted in the formation of a strong orange colour, indicative of the formation of an azo-dye; heating of the nitrous acid solution resulted in gas evolution. A further test for amines is staining with ninhydrin. Therefore a small amount of the original solution was spotted on a silica plate, showing a faint ring under UV and a short lived orange ring after treatment with ninhydrin solution.

Since these tests indicate the presence of an aromatic amine in solution, isolation of **92** by the slow evaporation of solvents[149] under vacuum at -20 °C was also attempted. It was hoped that use of the crude solution directly in a standard coupling with **63** would result in the formation of Boc-protected thienylimidazolidine **97** (Figure 3.10) as this is similar to the procedure carried out by Malicorne *et al.*[150] However, no desired product was observed.

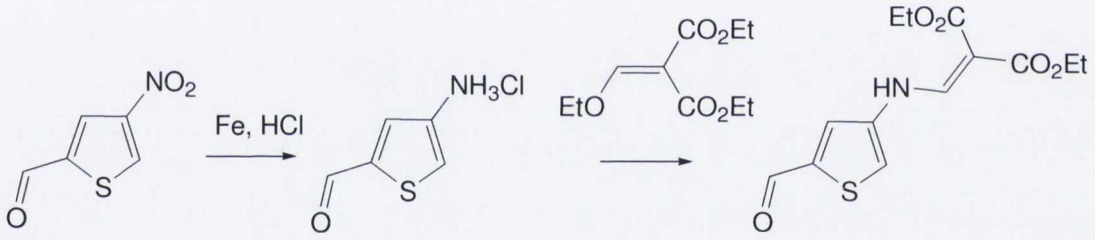


97

Figure 3.10 - {*N*-[1,3-di-(tert-butoxycarbonyl)]-Imidazolidin-2-ylidene}-4-amino-2-thiophene-carboxaldehyde

3.5.2.4 Reduction using Iron in HCl

The reduction of **91** was attempted by emulating the synthesis described by Malicorne *et al.*[150] This involves the crude salt of **92** being isolated, but not characterised, before being taken to the next step of the reaction sequence: the reaction with diethyl ethoxymethylenemalonate (Scheme 3.29).

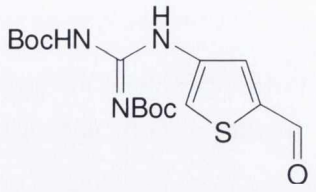


91 **92**.HCl

Scheme 3.29

In a manner analogous to that just described, the preparation of Boc-protected thienylketone **98** (Figure 3.11) was thus attempted starting from the reaction of **91** with Fe (6 eq.) in conc. HCl in 50% ethanol. To what was hoped was the salt of **92** were added sequentially: commercial guanidylating agent 1,3-*bis*(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (1.2 eq.), TEA (5 eq.) and $HgCl_2$ (1.5 eq.). Analysis of the crude material obtained using low resolution mass spectrometry (LRMS) showed a species with a molecular ion at 360, which was not any of the expected products or intermediates. This indicates that the product which was sought-after was not synthesised by this method. A possible reason for the failure of the reaction, under the conditions which afforded the intermediate salt mentioned in the literature, is the long reaction time (overnight) of the subsequent guanidylation.

If compound **92** is highly unstable, which all experimental evidence indicates, then it may be too short lived to furnish desired product **98** under conditions similar to those described.



98

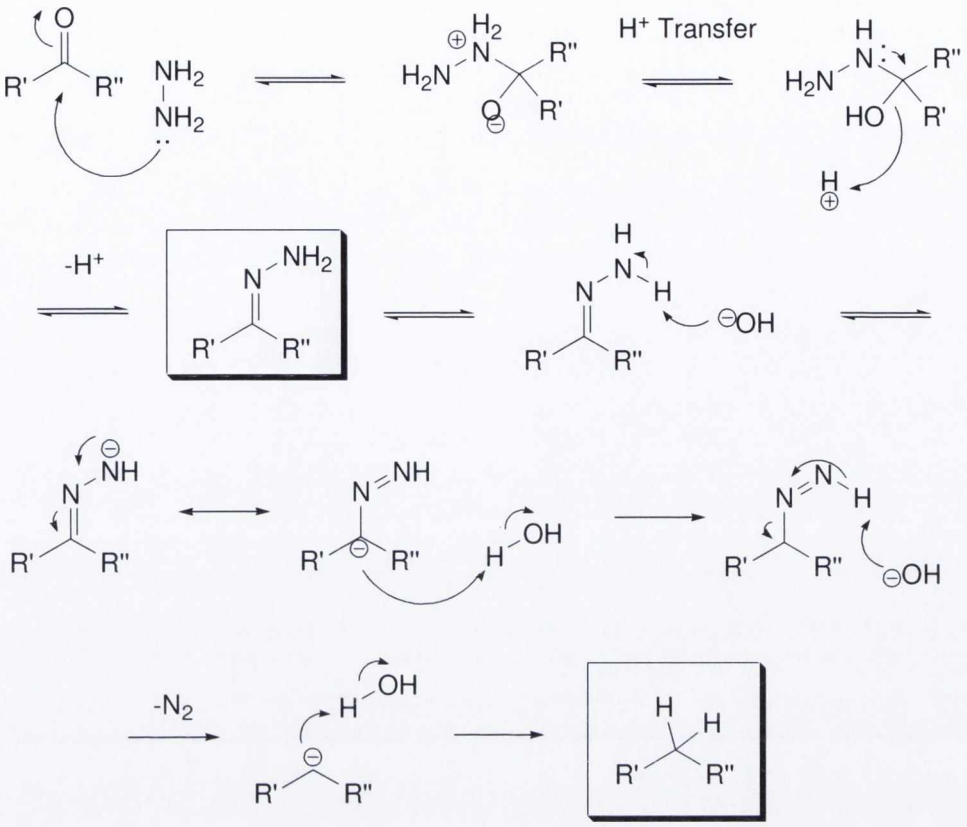
Figure 3.11 - 4-[2,3-Di(*tert*-butoxycarbonyl)]guanidino-2-thiophenecarboxaldehyde

3.5.3 Formation of 2-Methylthiophene (93) and its Derivatives

3.5.3.1 Wolff-Kishner Reductions of 2-Thiophenecarboxaldehyde (90) Derivatives

The mechanism of the Wolff-Kishner reduction is depicted in Scheme 3.30 and commences with the addition of hydrazine to the carbonyl carbon of the aldehyde or ketone via one of the nitrogen atoms. This generates a tetrahedral zwitterionic intermediate, which undergoes hydrogen transfer to produce a neutral species. The hydroxyl group picks up a proton from solution, allowing for the nitrogen assisted elimination of water followed by the loss of a proton resulting in the formation of a hydrazone.

Deprotonation of the hydrazone moiety results in the formation of a N=N bond, with the C=N bond taking up a proton from solution. A further deprotonation of the diazene results in the formation of a N≡N bond, which can allow for the elimination of gaseous nitrogen, with the concomitant abstraction of a proton from solution to form a methyl or methylene group.



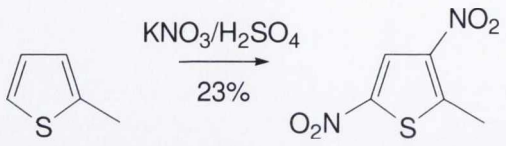
Scheme 3.30

The Wolff-Kishner reduction of **90** using excess hydrazine hydrate and KOH in ethylene glycol[151,152] resulted in the isolation of pure **93** in the presence of some EtOAc in a yield of 55%, This is lower than the 78% reported in the literature,[151] due to the lack of microscale distillation apparatus. The boiling point at 112 °C, though, was consistent with the literature (112-113 °C).[151] A Wolff-Kishner reduction of **91** was attempted although 4-nitro-2-methylthiophene was not isolated.

3.5.3.2 Nitration of 2-Methylthiophene (93)

Nitration of **93** using 1 eq. KNO_3 in H_2SO_4[141] resulted in the formation of a dinitrothiophene, which was thought to be 3,5-dinitro-2-methyl thiophene (**99**, Scheme 3.31) based on the theoretical reactivity of 2-methyl thiophene and IR data. The presence of an electronegative substituent alpha to an aromatic nitro group is expected to result in an increase in the NO_2 asymmetrical stretch and a decrease in the symmetrical stretch beyond the respective ranges of 1550-1500 and 1360-1290 cm^{-1}.[146] These peaks for both nitro groups occur within the standard range (ν_{max} 1550, 1508, 1340 and 1318 cm^{-1}), and support the assignment of the product as **99**, the only possible isomer with no

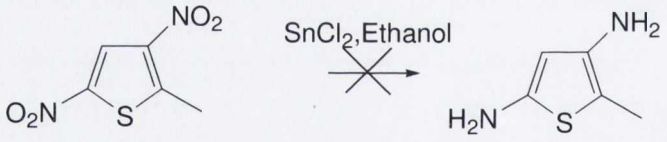
adjacent nitro groups. This hypothesis was confirmed using spectral data, which matched that previously describing **99** in the literature.[153] The melting point (98 °C) was in agreement with the assignment of the product, as it almost matched the literature value of 98-99 °C.[154] The reaction was low yielding with only 23% isolated based on the quantity of 2-methylthiopene used.



93 **99**

Scheme 3.31

Compound **99**, being a related species to **94**, was used as a model compound on which to test possible reduction of the latter. Attempted reduction of **99** using the procedure of $SnCl_2$ (Scheme 3.32) based procedure of Bellamy and Ou[147] resulted an orange material, which when analysed by ^1H NMR spectroscopy showed only baseline peaks of a mixture of aromatic and aliphatic signals. No broad peaks characteristic of amino groups were observed indicating that 3,5-diamino-2-methylthiophene (**100**) was not formed.

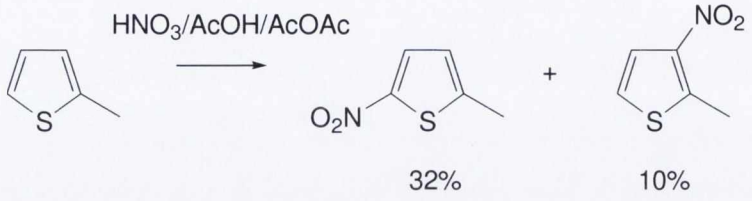


99 **100**

Scheme 3.32

Nitration of **93** using fuming HNO_3 and AcOH in the presence of acetic anhydride (AcOAc),[155] or modification using KNO_3 as nitrating agent did not yield desired products **94** and **101**. However, when carried out at temperatures in the range -10 to -40 °C, the HNO_3 procedure[153] yielded a mixture of two *mono*-nitrothiophene isomers in a combined yield of 42%, in a 3:1 ratio. Since the methyl group is expected to direct substituents to the 3- or 5- positions, and thiophenes with electron donating

groups in the 2-position undergo EAS preferentially in the 5-position,[144] 5-nitro-2-methylthiophene (**94**) is the expected major product, with 3-nitro-2-methylthiophene (**101,** Scheme 3.33) the expected minor product. Boiling points of the two materials were 60 and 110 ℃ both at 20 mbar with the literature boiling point of **94** at 104 ℃ at 15 mm Hg[153] (approximately 20 mbar), indicating that the compound boiling at 110 ℃ is **94**. To the best of our knowledge there is no data currently on the boiling point of **101**.

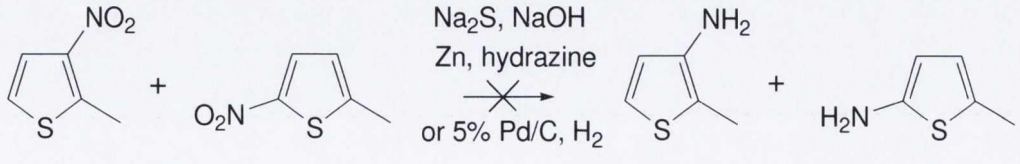


93 **94** **101**

Scheme 3.33

3.5.3.3 Attempted Reduction of Nitromethylthiophenes

Next the reduction of the mixture of **94** and **101** was attempted in the hopes of generating 3-amino-2-methylthiophene (**102**) and 5-amino-2-methylthiophene (**95**). First, Na_2S was chosen as it had given good results previously, however no product was isolated. Employment of the Zn/hydrazine method[145] resulted in a dimethyl sulfoxide (DMSO)-d_6 ^1H NMR spectrum containing signals which appeared to be NH_2 peaks, however no product was isolated and the results were not reproducible. Hydrogenation using 5% Palladium carbon[156] which had not been tried previously as cleavage of the thiophene nucleus was anticipated, gave results that were far from those expected with the starting material isolated unchanged at the end of the reaction. Scheme 3.34 shows the reactions attempted for the reduction of **94** and **101**.



101 **94** **102** **95**

Scheme 3.34

3.6 Conclusions

Due to the lack of commercial thiophene amines with few substituents, the synthesis of thiophene amines from nitrothiophenes, for guanidylation under Kim and Qian conditions was explored.

Di-Boc-protected guanidylating and imidazolidylating agents **62** and **63** were successfully prepared in good yields via the method used previously in our laboratory. Initial investigations of strategies for guanidylation starting from nitrothiophenes were promising, given that the reduction of nitrothiophene **67** to aminothiophene **66** worked very well, and the subsequent guanidylation of the latter to produce Boc-protected guanidine **69** worked satisfactorily. The synthesis of nitrothiophene **67** from acyclic starting materials 1,4-dithiane-2,5-diol and nitrostyrene followed by oxidation and dehydration using DDQ worked well also and, due to the greater quantity of Bo-protected guanidine **69** produced subsequently, allowed for the deprotection of the latter to yield guanidine hydrochloride salt **70** quantitatively.

Attempted synthesis of Boc-protected-amidino dimers **77** and **78** using dimeric aminothiophene **75** as an intermediate proved unsuccessful due to the acid instability of the latter. A modified synthetic strategy with the scope to generate **77**, **78** and related molecules, by leaving the acid-catalysed coupling reaction till later in the reaction sequence, was devised based on the successful preparation of **79**, **80**, **82** and **84**. The strategy was, however, deemed unsuccessful due to the instability of hydrochloride salt **86**, which prevented its clean isolation. However, related salt **87**, which bears some structural resemblance to **52** was synthesised for testing.

A strategy to generate simple and twin alkyl-substituted thiophene amines for guanidylation, based on the derivatisation of aldehyde **90** was devised. Wolff-Kishner reduction of **90** to generate methylthiophene **93** proceeded smoothly, which was also true of the nitrations of **90** and **93** to produce nitrothiophenes **91** and **94** respectively. However, due to the difficulties encountered in the reduction of the nitroderivatives involved in the *bis*-aminothiophene strategy, it was decided to explore alternative methods for the generation of thiophene amines for guanidylation.

4 Guanidine-like Thiophenes: From Commercial and Gewald Aminothiophenes

4.1 Introduction

Due to the fact that the cyclohexane fused derivative **54** (Figure 4.1) is an α_2-AR antagonist[104] with good α_2-AR affinity (pKi = 7.11)[104] and shows some structural similarities to 5-HT, the possibility exists that compounds of this type could act at both the α_2-AR and at 5-HT autoreceptors resulting in dual activity. It was therefore decided to synthesise the thiophene analogue of compound **54** shown in Figure 4.1.

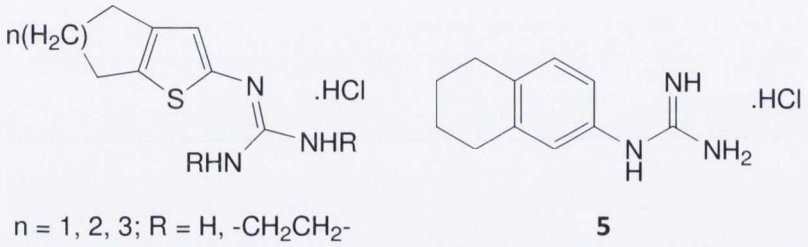
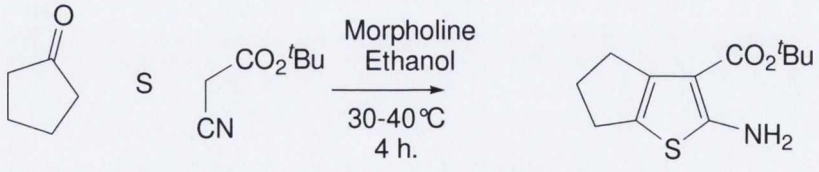


Figure 4.1 - Target compound analogues (left) of the lead compound **54** (right)

Since the structurally related compound *tert*-butyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (**103**, Scheme 4.1) has been previously described,[157] and *tert*-butyl α cyanoacetate, from which it is derived, was readily available, it was chosen as a synthetic starting point.



103

Scheme 4.1

It is then hoped that the Gewald amines thus generated could be decarboxylated directly, or guanidylated prior to decarboxylation, using standard conditions.

4.2 Alkyl-2-aminothiophene-3-carboxylates via the Gewald Reaction

4.2.1 History and Mechanism of the Gewald Reaction

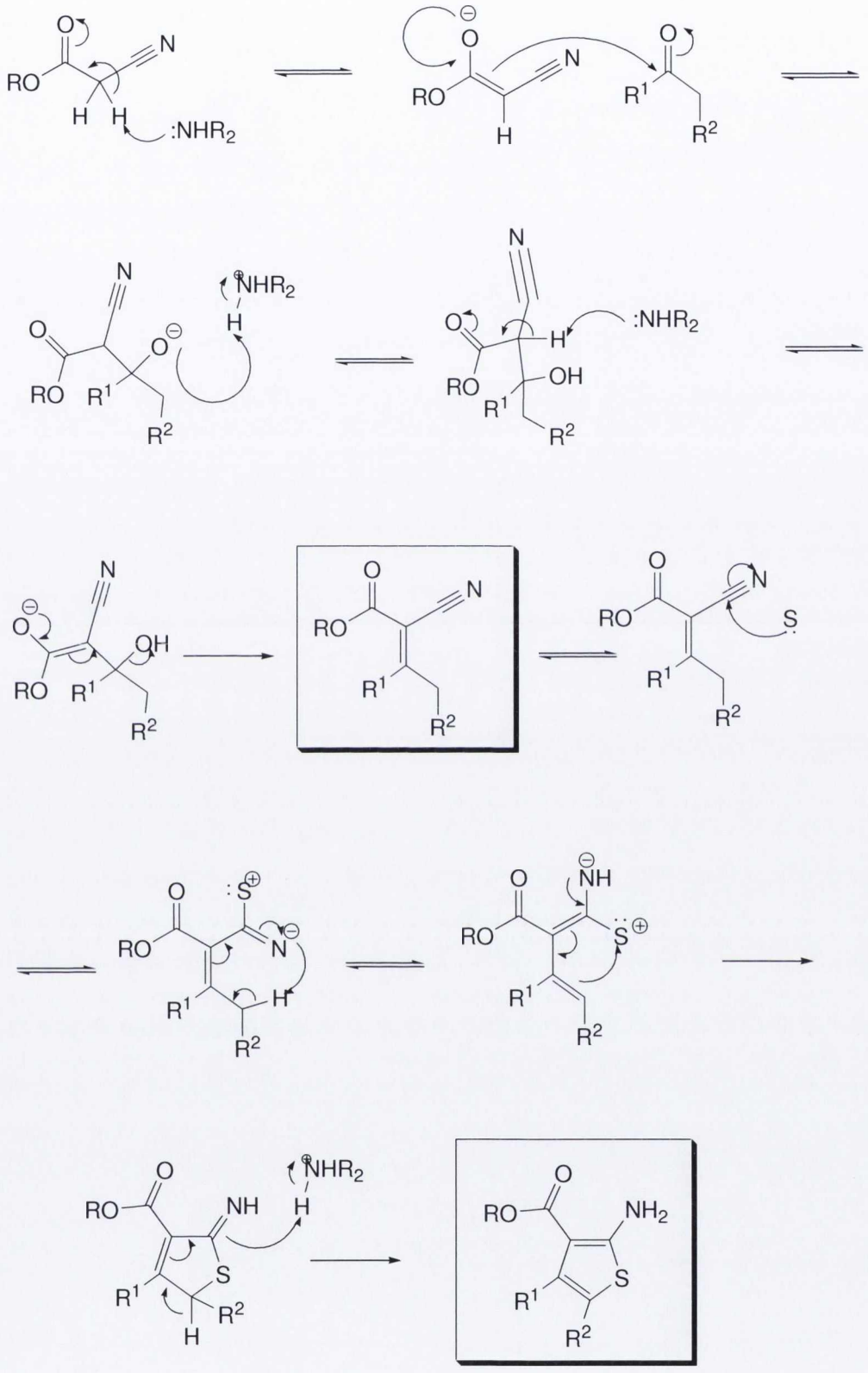
The Gewald reaction was first discovered by Karl Gewald in 1966[158] and is the reaction between an aliphatic ketone, aldehyde or 1,3-dicarbonyl compound with a methylene-activated nitrile and elemental sulfur in the presence of base to furnish a poly-substituted 2-aminothiophene. In particular, the methylene-activated nitrile is either an α-cyano ester or malononitrile, with the former giving a carboxylic ester at the 3-position, and the latter a cyano group in the same position.

The reaction is a two-step one-pot multicomponent reaction with the first step being a Knoevenagel condensation and the second being the thiophene forming step. The reaction mechanism is depicted in Scheme 4.2.

The Knoevenagel condensation occurs as follows: the methylene-activated nitrile (an α-cyano ester in the mechanism shown in Scheme 4.2) is deprotonated α to the nitrile group, and the anion thus formed is stabilised by resonance due to conjugation with the carbonyl and cyano groups. In the example shown the anion, which is an enolate, then reacts at the α-position with the ketone or aldehyde present to form a tetrahedral intermediate which deprotonates the conjugate acid of the base to form an alcohol, in this case an aldol. A second deprotonation at the α-position forms a new anion, and subsequent elimination of a hydroxide ion forms the stable Knoevenagel intermediate.

The exact mechanism of the addition of sulfur is not known. It is postulated that the elemental sulfur attacks the cyano group, forming the anion of an imine. This anion can then deprotonate the position α to the C=C, which could occur via a 6-membered transition state, causing the rearrangement of the double bonds involved and again placing a negative charge on the nitrogen. A second deprotonation is not possible here, therefore the negative charge feeds back in to the conjugated system, allowing carbon to attack sulfur in the cyclisation step. Tautomerisation then occurs to form the stable aminothiophene (See Scheme 4.2).

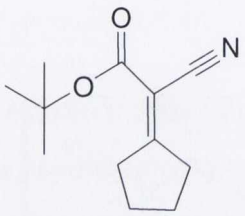
This cyclisation would be 5-endo-trig according to Baldwins rules, which though usually disallowed, would not be in this case as the cyclisation involves both a cation *and* an atom in the second row of the periodic table (sulfur) both of which are known to cause exceptions to this rule.



Scheme 4.2

4.2.2 Synthesis of *tert*-Butyl 2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (103)

The Gewald synthesis of **103** (Scheme 4.1) was carried out using cyclopentanone, sulfur powder and *t*-butyl cyanoacetate in ethanol at 40 °C with morpholine as catalyst (Scheme 4.1).[114] It was observed that the reaction was not going to completion, with separation of the Knoevenagel intermediate, *tert*-butyl (1-cyclopentylidene)-2-cyanoacetate (**104**, Figure 4.2) from the desired product, proving difficult. Use of excess of sulfur and/or morpholine or increasing temperature failed to push the reaction forward.



104

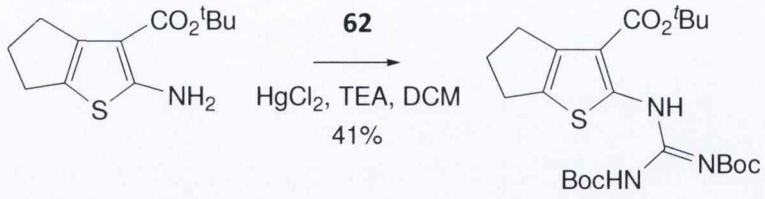
Figure 4.2 - *tert*-Butyl (1-cyclopentylidene)-2-cyanoacetate

A repeat reaction in the absence of sulfur afforded **104** in 14% yield, and this sample was used to optimise the separation of **103** and **104** on TLC. The compounds were found to have identical R_f values, with no possible separation using mixtures of hexane/EtOAc and DCM/MeOH in a variety of ratios.

Efforts at purification via column chromatography using neutral, acidified or basified solvents failed. Several recrystallisations were attempted from EtOAc/hexane, MeOH/hexane and hexane after columning. Compound **103** was successfully isolated in an 18% yield using intermediate **104** as starting material, along with sulphur and morpholine, followed by an acidic workup and column chromatography purification.

Due to the poor yields obtained for **103**, it was decided to use the mixture of **103** and **104** directly in the subsequent guanidylation, as no functional group in **104** was thought to be nucleophilic enough to compete with the amine nucleophile in **103**. This would eliminate the need for complete purification which invariably resulted in excessive product loss. Guanidylation was carried out,[115] after estimating the quantity of **103** present in the mixture via ^{1}H NMR spectroscopy, resulting in the

isolation of pure thienyl guanidine **105** (Scheme 4.3) in 41% yield, which, though only moderate, was in line with yields for other thiophene obtained previously.

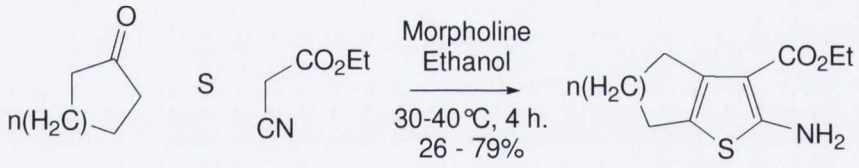


103 **105**

Scheme 4.3

4.2.3 Synthesis of Ethyl 2-Aminocycloalkyl[*b*]thiophene-3-carboxylates

Due in part to the relative expense of *tert*-butyl α-cyanoacetate relative to the ethyl ester and also to the difficulties in purifying **103**, it was decided to pursue the synthesis of the cycloalkylthiophene carboxylate series as the ethyl esters (**106**-**108**, Scheme 4.4).



106, n = 1

107, n = 2

108, n = 3

Scheme 4.4

Similar to the synthesis of **103**, cyclopentane-fused ethyl ester (**106**, Scheme 4.4) also proved difficult to purify, with a mixture of the final compound and the corresponding Knoevenagel intermediate obtained. Attempts to use the 2010 method of Mojtahedi *et al.*[159] using at a 0.4 mmol scale under sonication for 5-7 min with either morpholine or TEA, not only failed to provide the clean product

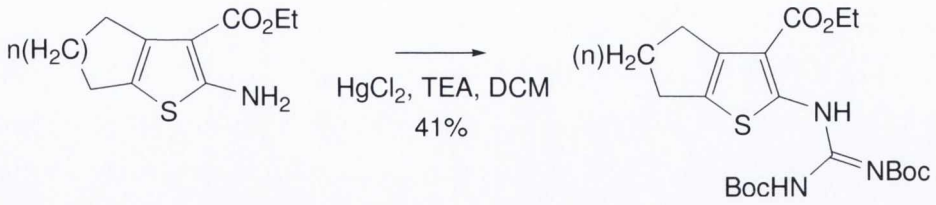
106, but resulted in only the Knoevenagel intermediate in the case of morpholine and only starting materials when TEA was used (both of which were observedetermined by ^{1}H NMR spectroscopy).

In the thesis of Isabel Rozas,[160] the reaction which produced **103** was carried out on a 100 mmol scale, and the product was reported as having crystallised out of the reaction mixture on cooling in a freezer overnight. Therefore, it was decided to scale up this Gewald reaction to a 40 mmol scale to see if this would result in easier purification. Unfortunately, this did not result in easier isolation of the product, or a higher yield of the mixture of Gewald and Knoevenagel products (13% vs. 26%); however, it did provide enough material for use in the following reactions.

Similarly, small scale reactions using cyclohexanone and cycloheptanone also gave poor results. Fortunately, when the 40 mmol reaction conditions were employed using cyclohexanone and cycloheptanone, the reactions occurred as reported by Rozas, with ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**107**, Scheme 4.4) and ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepto[*b*]thiophene-3-carboxylate (**108**, Scheme 4.4) isolated cleanly in 79 and 61% yields respectively. It was observed that even dropwise addition of morpholine to the rest of the reaction mixture at the beginning of the reaction results in the build up of substantial heat, which could be an important factor in initialising the reaction, thus explaining why the reaction worked better when scaled up.

4.2.4 Synthesis of 2,3-[Di(*tert*-butoxycarbonyl)]-1-(3-ethoxycarbonylcycloalkyl[*b*]-thiophen-2-yl)guanidines

The corresponding cycloalkyl-fused Boc-protected-guanidino esters **109**, **110** and **111**, Figure 4.3) were obtained using the standard reaction conditions, with yields of 36, 52 and 49% respectively. The poor yield of **109** possibly could be due to inaccuracy in the calculation of the yield of **106** by ^{1}H NMR spectroscopy, or the presence of the Knoevenagel product, which may slow down or interfere with the reaction.



106, n = 1 **109**, n = 1

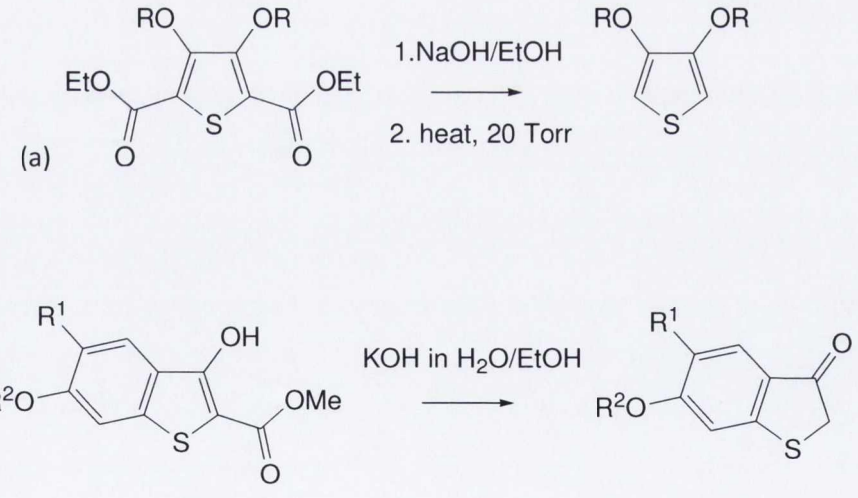
107, n = 2 **110**, n = 2

108, n = 3 **111**, n = 3

Figure 4.3 – Structures of 2,3-[di(*tert*-butoxycarbonyl)]-1-(3-ethoxycarbonylcycloalkyl[*b*]thiophen-2-yl)guanidines

4.2.5 Attempted Basic Decarboxylation of Ethyl 2-Aminothiophene-3-carboxylates and Related Derivatives.

Decarboxylation at the 2-position of thiophene derivatives has been described under basic conditions. Scheib *et al.* reported a decarboxylation in the 2-position using NaOH in EtOH followed by heating at reduced pressure (Scheme 4.5 (a)),[161] whereas Dinescu and co-workers reported decarboxylation of a thiophene with a hydroxyl group in the 3-position using 15% KOH in 1:1 H_2O/EtOH alone (Scheme 4.5 (b)).[162]



Scheme 4.5

Decarboxylation of **107** was attempted due to the ease with which it was synthesised, and since its derivatives are of foremost interest. Firstly, it was heated to reflux in 15% potassium hydroxide (KOH) in 1:1 H_2O/Ethanol at 95 °C. This temperature was chosen as it was hoped that under these conditions the sensitive aminothiophenes would be stable. The reaction merely resulted in the hydrolysis of the ester to the carboxylic acid. However, no decarboxylation was observed when the reaction was monitored by checking for the emergence of an aromatic signal in the ^{1}H NMR spectroscopy.

After this initial attempt, **107** was heated to reflux using the same proportion of KOH only in water to allow for the use of a higher temperature (115 °C) since it is common for high temperatures to be necessary for decarboxylation to occur. The starting material was only sparingly soluble in water and, due to this factor, neither decarboxylation or hydrolysis was observed.

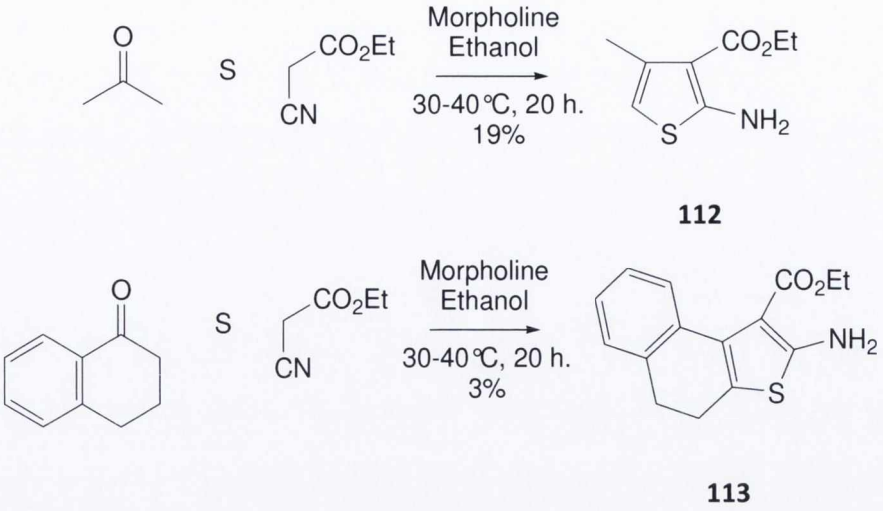
It was thought that by using guanidylated molecule **110**, decarboxylation could be achieved since even higher temperatures could be used and thus the instability of thiothiophene amine **107** could be circumvented. However, decarboxylation of aminothiophene **110** using 15% KOH in 1:1 H_2O/Ethanol at 190 °C[161,162] proved unsuccessful, with no hydrolysis or decarboxylation.

4.2.6 Attempted Synthesis of 2-Iminoimidazolidine Derivatives of Ethyl 2-Amino-cycloalkyl[*b*]thiophene-3-carboxylates

Due to the relative ease of the synthesis of the Boc-protected guanidine derivatives of the ethyl 2-amino-cycloalkyl[*b*]thiophene-3-carboxylates it was decided to prepare the corresponding 2-iminoimidazolidine derivatives. However, when Dardonville and Rozas conditions (3.2), which involve imidazolidylating agent **63**, $HgCl_2$ and TEA in DCM, were carried out on **106** and **107**, only starting materials were observed in the crude mixture via ^{1}H NMR spectroscopy and MS.

4.2.7 Synthesis of Other Ethyl 2-Aminothiophene-3-carboxylates and their Derivatives

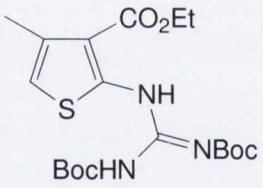
Due to the relative ease in producing the Gewald thiophenes discussed in section 4.2 in comparison to the syntheses carried out in Chapter 3, it was decided to use other ketones which were already present in our lab to generate further products. Thus the Gewald reaction was attempted using acetone and α-tetralone to afford ethyl 2-amino-4-methylthiophene-3-carboxylate (**112**, Scheme 4.6) and ethyl 2-amino-4,5-dihydro-naphtho[2,1-*b*]thiophene-1-carboxylate (**113**, Scheme 4.6) respectively.



Scheme 4.6

Unfortunately, the syntheses of **112** and **113** did not prove as straightforward as that of **107** and **108**, with respective yields of 19% and 3% having been obtained after extensive columning. Due to the difficulty in obtaining **113** in useful quantities, the compound was not taken any further.

Guanidylation of **112** resulted in the formation of 2,3-[di(*tert*-butoxycarbonyl)]-1-(3-ethoxycarbonyl-4-methylthien-2-yl)guanidine (**114**, Figure 4.4) in 68% yield.



114

Figure 4.4 – 2,3-[Di(*tert*-butoxycarbonyl)]-1-(3-ethoxycarbonyl-4-methyl thiophene-2-yl)guanidine

4.2.8 Towards (3-Ethoxycarbonylthiophen-2-yl)guanidines

4.2.8.1 Premise

Due to the initial difficulties encountered in decarboxylating the Gewald products **106-108** and the Boc-protected guanidines made from them, **109-111**, and the structural similarities that the salts of the latter family of compounds would have with known α_2-AR antagonists such as RX821002,

atipamezole and fipamezole (all Figure 4.5), it was decided to synthesise these salts for biological testing. Atipamezole is approved for use in the reversal of the sedative and analgesic effects of dexmedetomidine and medetomidine in dogs, and fipamezole is in phase III clinical trials as an antiparkinsonian drug for humans.

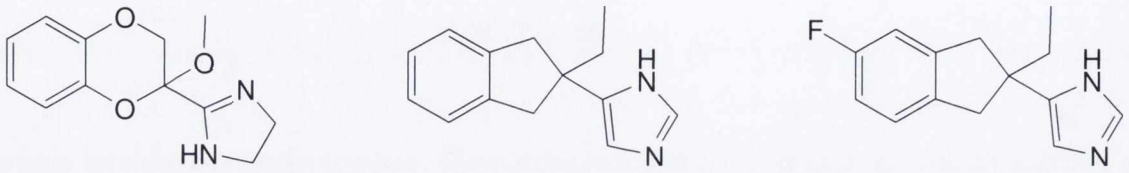
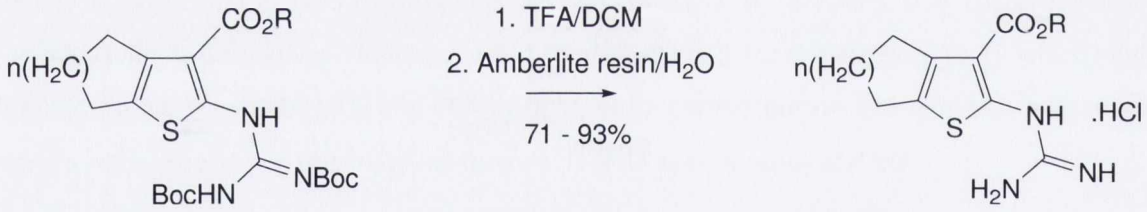


Figure 4.5 – Structures of RX821002 (left), atipamezole (centre) and fipamezole (right)

4.2.8.2 Deprotection of 2,3-[Di(*tert*-butoxycarbonyl)]-1-(3-alkoxycarbonyl-alkylthiophen-2-yl)guanidines

Boc deprotection of **109-111** using 50% TFA/DCM results in the formation of trifluoroacetate salts, which were treated directly with IRA400 Amberlyte Resin in its chloride activated form to yield the more soluble hydrochloride salts (Scheme 4.7).[115] This afforded the guanidine hydrochloride salts **115**, **116**, **117** and **118** (Scheme 4.7) in respective yields of 84, 90, 93 and 71%.



105, R = ^{t}Bu, n = 1

109, R = Et, n = 1

110, R = Et, n = 2

111, R = Et, n = 3

115, R = H, n = 1

116, R = Et, n = 1

117, R = Et, n = 2

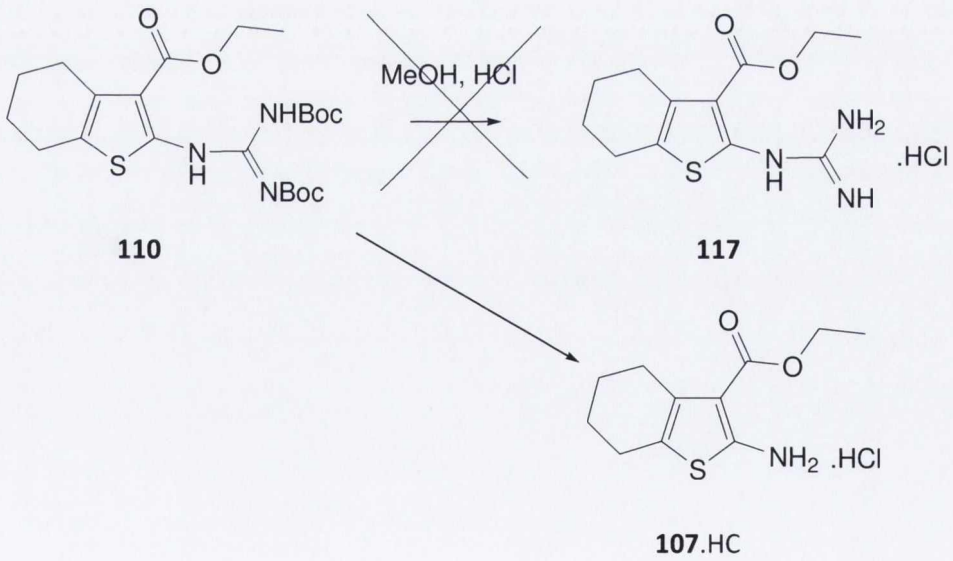
118, R = Et, n = 3

Scheme 4.7

As could be expected, the *tert*-butyl ester moiety in **105** was hydrolysed during deprotection, as observed using ^{1}H and ^{13}C NMR spectroscopy and HRMS; however, the ethyl ester moieties in **109-111** were not.

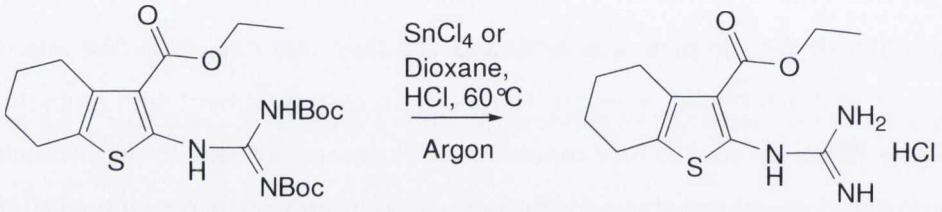
Deprotection of **114** via TFA/DCM and treatment with Amberlite resulted in the presence of a contaminant which was also found in other samples deprotected at around the same time. By process of elimination and ^{1}H NMR spectroscopy, the contaminant was determined to originate from the Amberlite resin. The level of contaminant present in these samples could not be controlled, and its presence appeared to occur randomly in samples treated with Amberlite from a number of product batches. Even though the company was contacted and they sent new resin, the impurity was still present although in much smaller amounts. However, because treatment with Amberlite was often needed multiple times, in order to fully convert the trifluoroacetate salts to the hydrochloride salts, this procedure was deemed unsuitable for further use. Thus an alternative method of deprotection was sought.

Compound **110** was chosen as a model due to the ease with which it could be synthesised. Deprotection of **110** using 4M HCl in methanol resulted in the cleavage of the guanidine moiety from the molecule, thus providing only the hydrochloride salt of amine **107**. This was determined by NMR and HRMS data.



Scheme 4.8

Use of 4 eq. of tin(IV) chloride ($SnCl_4$),[163] which necessitates anhydrous conditions to prevent the formation of solid $SnCl_4.5H_2O$, was also attempted and fortunately resulted in the desired product after 4 h (Scheme 4.9) as determined by ^1H and ^{13}C NMR spectroscopy. However, the sample was observed to be quite impure by ^1H NMR and since the use of a heavy metal at this late stage of synthesis could be problematic and considering that 4M HCl in anhydrous dioxane under argon had provided the guanidine salts of related molecules it was decided to examine this method instead.



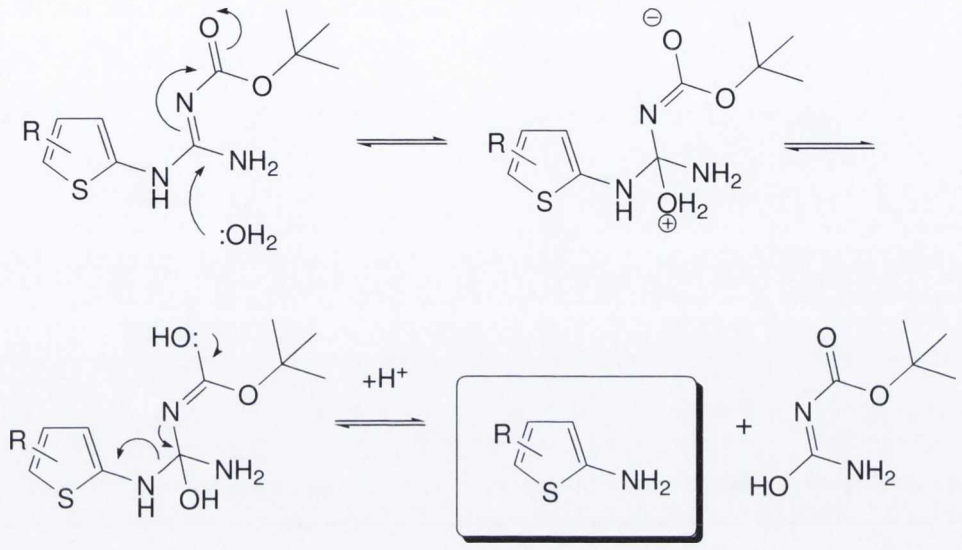
110 **117**

Scheme 4.9

Using the mild conditions of 5 eq. HCl in dioxane, the cleavage of one of the Boc groups was observed via LRMS after 3.5 h at r.t. After longer periods of time there was still a substantial quantity of *mono*-Boc protected guanidine and after 20 h, when the reaction was stopped and the crude was examined by ^1H NMR spectroscopy, the product contained substantial impurities. The reaction was then repeated and was heated to 60 ℃, which resulted in a greater ratio of product to amine salt after 20 h. Experiments carried out into the kinetics of Boc-deprotection by Ashworth *et al.* in 2010[139] suggested that the rate of decomposition increased after approximately 6 h, therefore the use of 25 eq. of HCl in conjunction with heating over a period of 4-6 h was tried next (Scheme 4.9). Gratifyingly, this resulted in a substantially pure sample by ^1H NMR spectroscopy, which could be purified using a 2.5 – 3.5 cm reverse phase pencil column with H_2O and acetonitrile as solvents as is described in the experimental section.

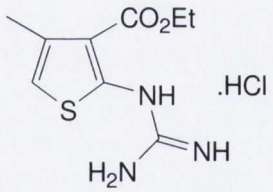
The fact that HCl induced deprotection occurred successfully under anhydrous conditions suggests that water is somehow involved in the cleavage of the guanidine group when methanol was used as solvent. It is possible that after the first Boc group is cleaved, tautomerisation leads to the formation of a pseudo-Michael acceptor which could be attacked by any nucleophile present (i.e. water or methanol) to form a zwitterionic intermediate, which could tautomerise and eliminate the thiophene

amine in a process which would probably involve resonance stabilisation through the aromatic thiophene ring. A hypothetical mechanism is depicted in Scheme 4.10.



Scheme 4.10

Using the optimised HCl/dioxane method, **114** was successfully converted to *N*-(3-ethoxycarbonyl-4-methylthien-2-yl)guanidine hydrochloride (**119**, Figure 4.6) in 93% yield.



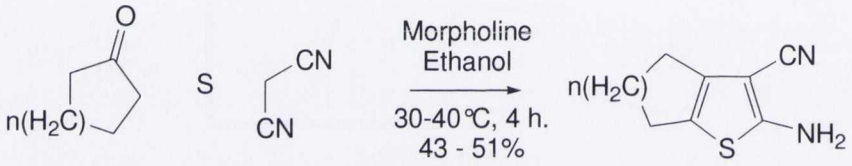
119

Figure 4.6 - (3-Ethoxycarbonyl-4-methylthiophen-2-yl)guanidine hydrochloride

4.3 2-Aminothiophene-3-carbonitrile Derivatives

4.3.1 Gewald Synthesis of 2-Aminothiophene-3-carbonitriles

Due to the relative ease of the synthesis of the alkyl 2-amino-alkylthiophene-3-carboxylates and their successful conversion to Boc-protected thienyl guanidines and their corresponding guanidine hydrochloride salts, it was decided to attempt the Gewald synthesis of the corresponding 2-aminothiophene-3-carbonitriles and their guanidine derivatives. Using 40 mmol quantities of malononitrile, sulfur and the requisite ketone in EtOH, with dropwise addition of morpholine resulted in the formation of Gewald carbonitriles **120**, **121** and **122** (Scheme 4.11) from each of cyclopentanone, cyclohexanone and cycloheptanone, in respective yields of 46, 51 and 43%.



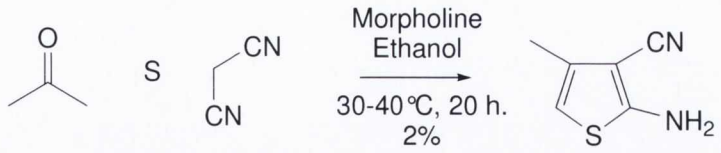
120, n = 1

121, n = 2

122, n = 3

Scheme 4.11

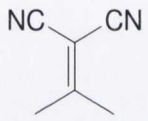
In a similar manner to the synthesis of the ethyl 2-amino-thiophene-3-carboxylates discussed in 4.2.6, acetone and α-tetralone were treated with malononitrile, sulfur and morpholine in EtOH. No product was detected in the case of α-tetralone, with only starting materials observed by ^{1}H NMR spectroscopy and HRMS. In the case of acetone, the desired product 2-amino-4-methylthiophene-3-carbonitrile (**123**, Scheme 4.12) was obtained in a poor yield of 2%.



123

Scheme 4.12

It was hoped that the poor yield of **123** could be improved by carrying the reaction out in two steps, with the corresponding isopropylidene (**124**, Figure 4.7) as the first product. Synthesis of **124** was carried out under Dean Stark conditions using 4 eq. acetone, 1 eq. malononitrile, 0.225 eq. sodium acetate trihydrate and 0.21 eq. AcOH in refluxing benzene as in the method of Chen *et al.*[164] A satisfactory yield of 72% was obtained, for this step of the reaction sequence.



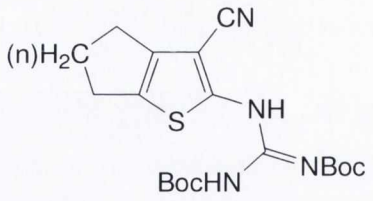
124

Figure 4.7 – Isopropylidenemalononitrile

Fortunately, the yield of **123** was improved and the work up simpler when **124** was used as starting material giving 15% for the second step of the reaction sequence and 11% across both steps.

4.3.2 Synthesis of 2,3-[Di(*tert*-butoxycarbonyl)]-1-(3-cyanoalkylthiophen-2-yl)guanidines

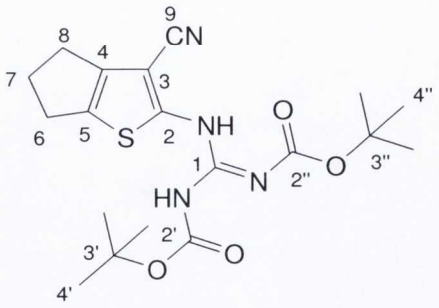
Synthesis of the Boc-protected guanidines was carried out using the standard method of Kim and Qian[112] as was described previously in 3.2. Thus Boc-protected guanidines **125**, **126** and **127** Figure 4.8) were prepared in yields of 20, 36 and 61% respectively. The yield improved substantially as the number of atoms in the cycloalkyl ring increased.



125, n = 1 **126**, n = 2 **127**, n = 3

Figure 4.8 – Structures of the [2,3-di(*tert*-butoxycarbonyl)]-1-(3-cyano-cycloalkylthien-2-yl)guanidines prepared

Detailed NMR analysis of these compounds via ^{1}H, ^{13}C, heteronuclear single quantum correlation (HSQC), heteronuclear multiple-bond correlation spectroscopy (HMBC) and selective total correlation spectroscopy (TOCSY) were used to fully assign each signal. Interestingly, in both **125** and **127** the CH_2 and CH_3 signals on the ^{13}C spectra overlap completely. For **125**, this occurs in two instances, with an overlapping CH_2 and CH_3 at 28.0 ppm and also at 28.1 ppm on the carbon spectrum (Figure 4.9), while for **127** this occurs once (Figure 4.10). The HSQC experiments carried out for compounds **125** and **127** confirm that this is indeed the case as the carbon signals at 28.0 and 28.1 can be seen to couple to both a CH_2 and CH_3 signal. This phenomenon does not occur for **126**.



125

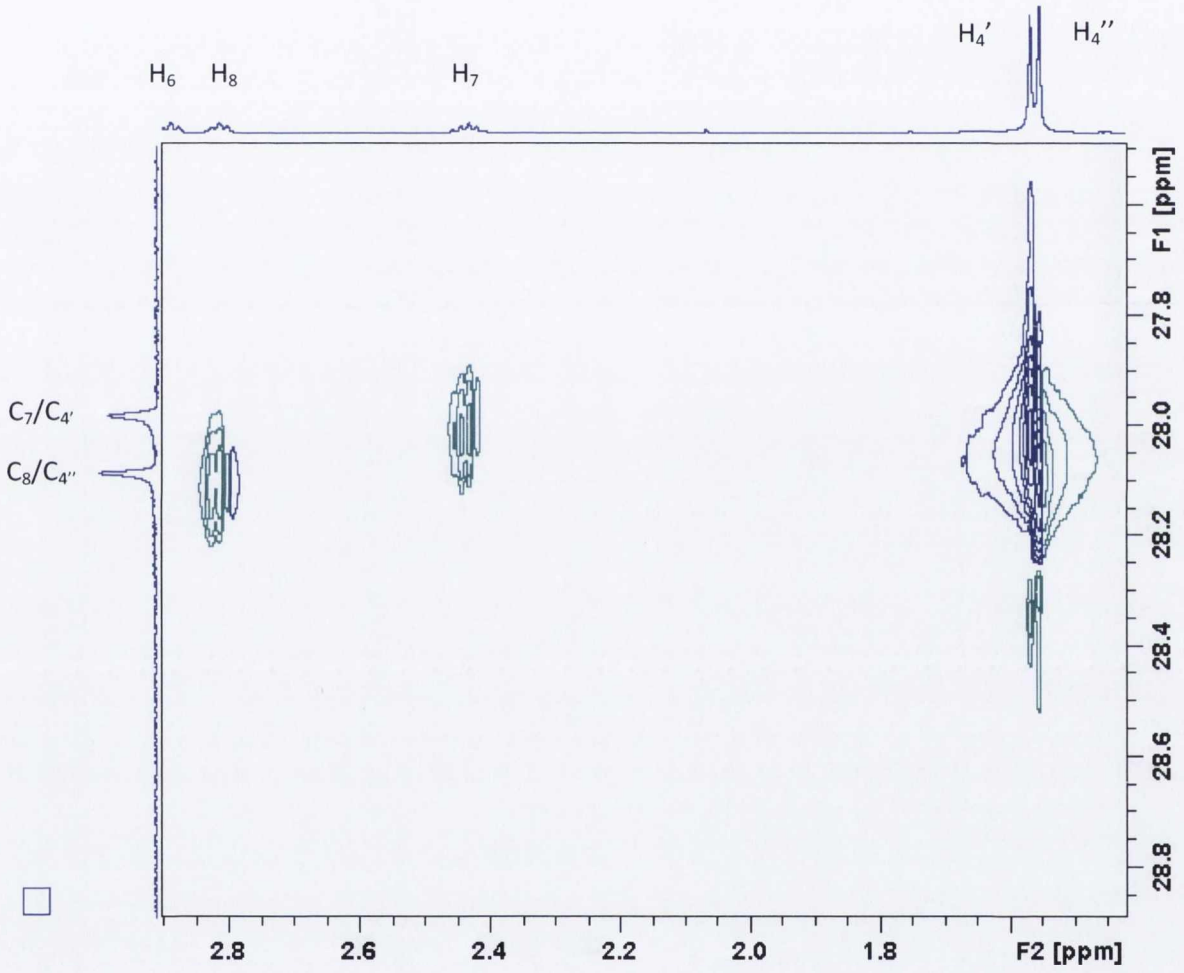
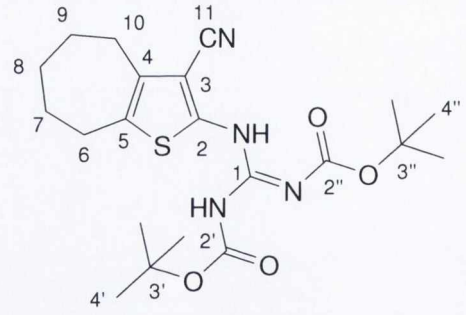


Figure 4.9 – HSQC showing overlapping CH_2 and CH_3 carbon signals at 28.0 and 28.1 ppm in **125**



127

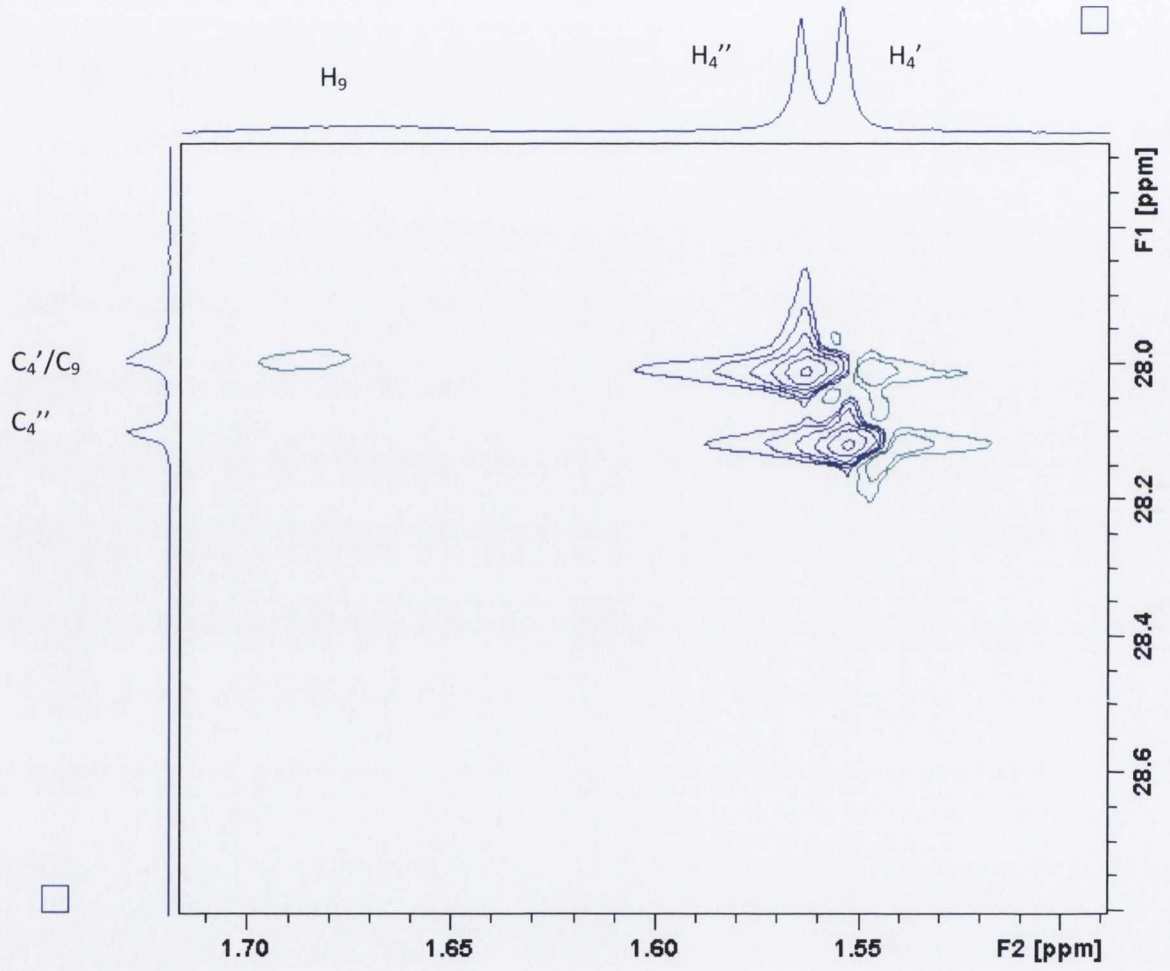
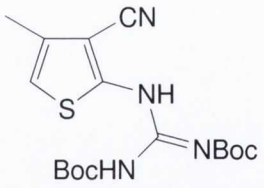


Figure 4.10 – HSQC showing overlapping CH_2 and CH_3 signal at 28.0 ppm in **127**

The Kim and Qian method[112] also worked successfully with compound **123** to give Boc-protected guanidine **128** (Figure 4.11) in low yield (29%). Spectroscopic data was consistent with the structure.



128

Figure 4.11 - 2,3-[Di(*tert*-butoxycarbonyl)]-1-(3-cyano-4-methylthien-2-yl)guanidine

4.3.3 NMR characterisation of [2,3-Di(tert-butoxycarbonyl)]-1-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]thien-2-yl)guanidine (127)

The structure of all molecules was fully assigned where possible by ^{1}H NMR, ^{13}C NMR, HMBC, HSQC and in some cases selective TOCSY. An example of the characterisation carried out is given for **127**, a labelled structure of which is depicted in Figure 4.12.

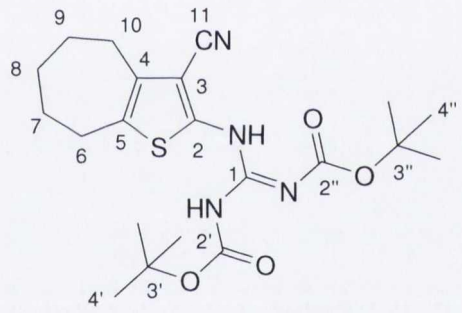


Figure 4.12 – Carbon and proton assignment of [2,3-di(*tert*-butoxycarbonyl)]-1-(3-cyano-cycloalkylthien-2-yl)guanidine **127**

The characterisation process is demonstrated as follows.

Firstly, on the HMBC, the NH signal at 11.48 ppm couples to the carbon signals at 97.6, 143.4 and 162.3 ppm therefore this must be the NH signal between C_1 and C_2 since this can feasibly couple to the three carbons C_1, C_2 and C_3 whereas the second NH signal couples only to the carbonyl carbon at 143.4 ppm (Figure 4.13). By first principles the signal at 162.3 ppm is the guanidine carbon as this is the only signal in the range expected. Of the aromatic carbon signals, the one at 143.4 ppm couples more strongly to the first NH signal than does the signal at 97.6 ppm, therefore the former must be C_2

and the latter must be C_3. The fact that the NH signal at 11.31 also couples quite strongly to this carbon indicates that intramolecular hydrogen bonding is taking place within the molecule. The NH at 11.31 ppm also seems to couple more strongly with the signal at 151.0 ppm therefore this must be $C_{2'}$ and the other signal in the carbonyl region (152.8 ppm) must be $C_{2''}$ the other carbonyl carbon. This order of coupling is also observed more strongly in other di-Boc-protected guanidines.

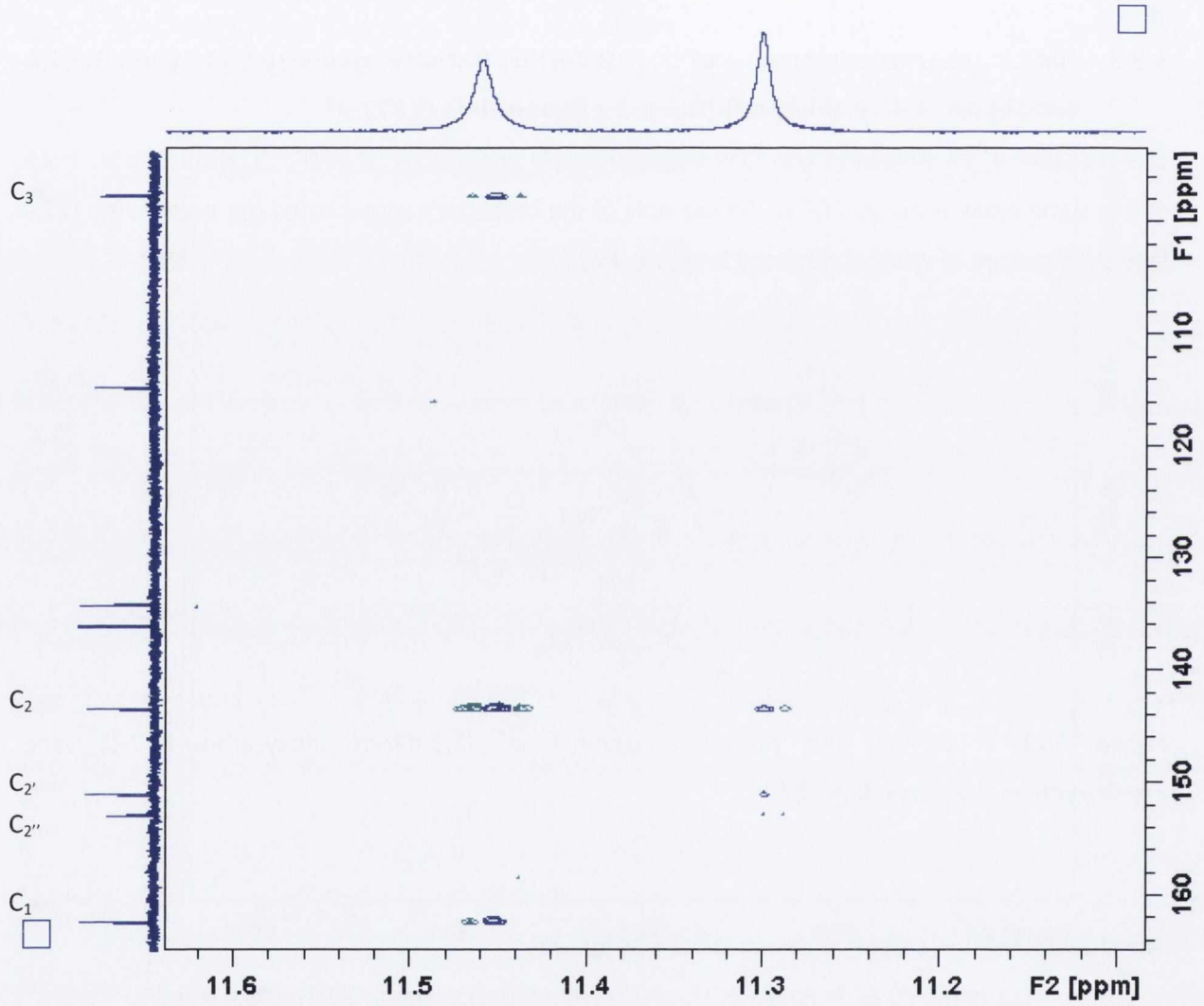


Figure 4.13 – HMBC showing NH signal coupling

Still on the HMBC, the signal at 1.56 ppm couples strongly to the carbon signal at 152.8 ppm therefore since this peak corresponds to $C_{2''}$, the CH_3 signal is therefore caused by $H_{4''}$, and thus by process of elimination, the downfield CH_3 signal at 1.57 must be $H_{4'}$ (Figure 4.14).

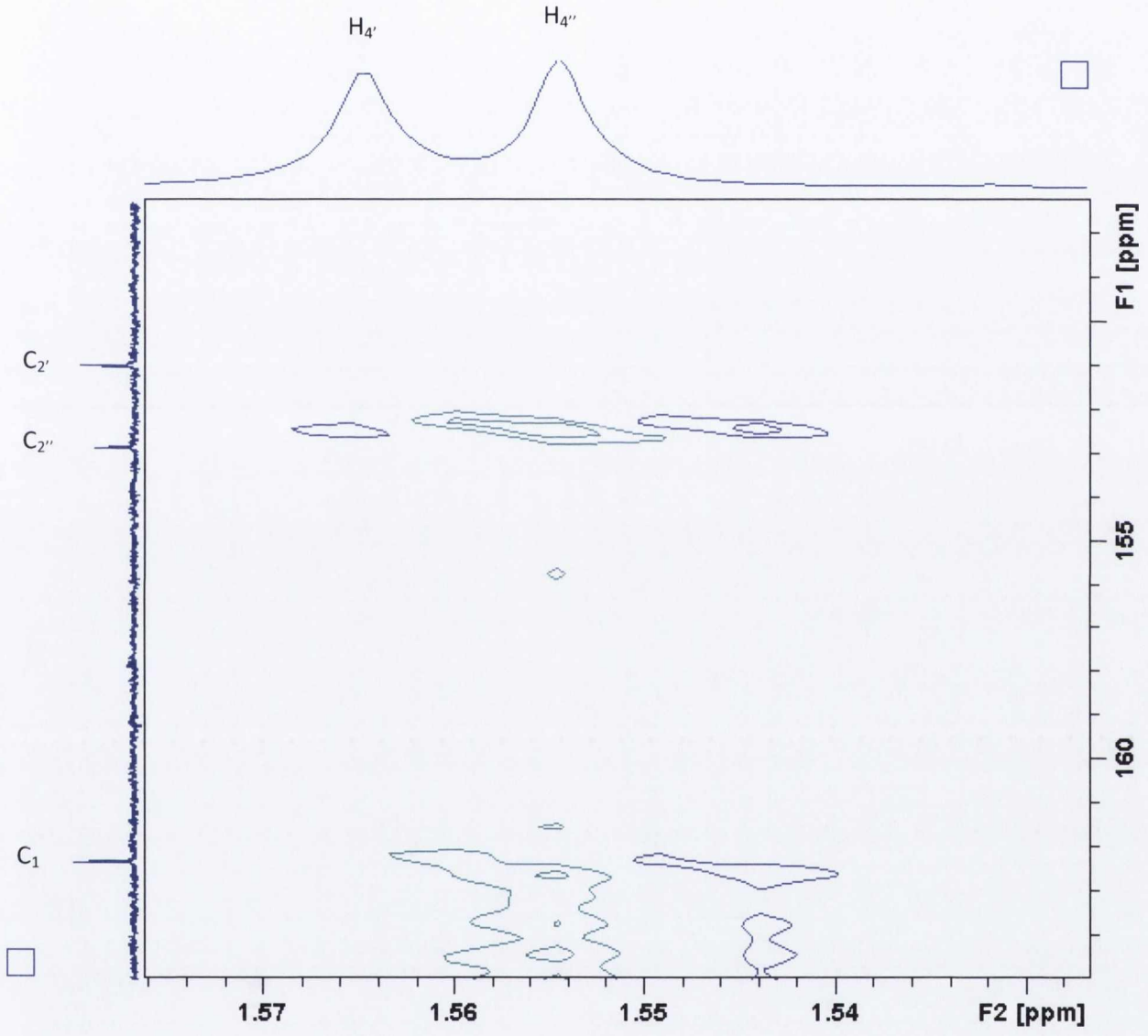


Figure 4.14 – HMBC showing CH_3 coupling

Secondly, the HSQC signal at 1.56 ppm couples to signal at 28.1 ppm and the more downfield signal at 1.57 ppm couples to signal at 28.0 ppm. This means that the signal at 28.1 ppm is $C_{4''}$ and that the signal at 28.0 ppm is $C_{4'}$ (Figure 4.15).

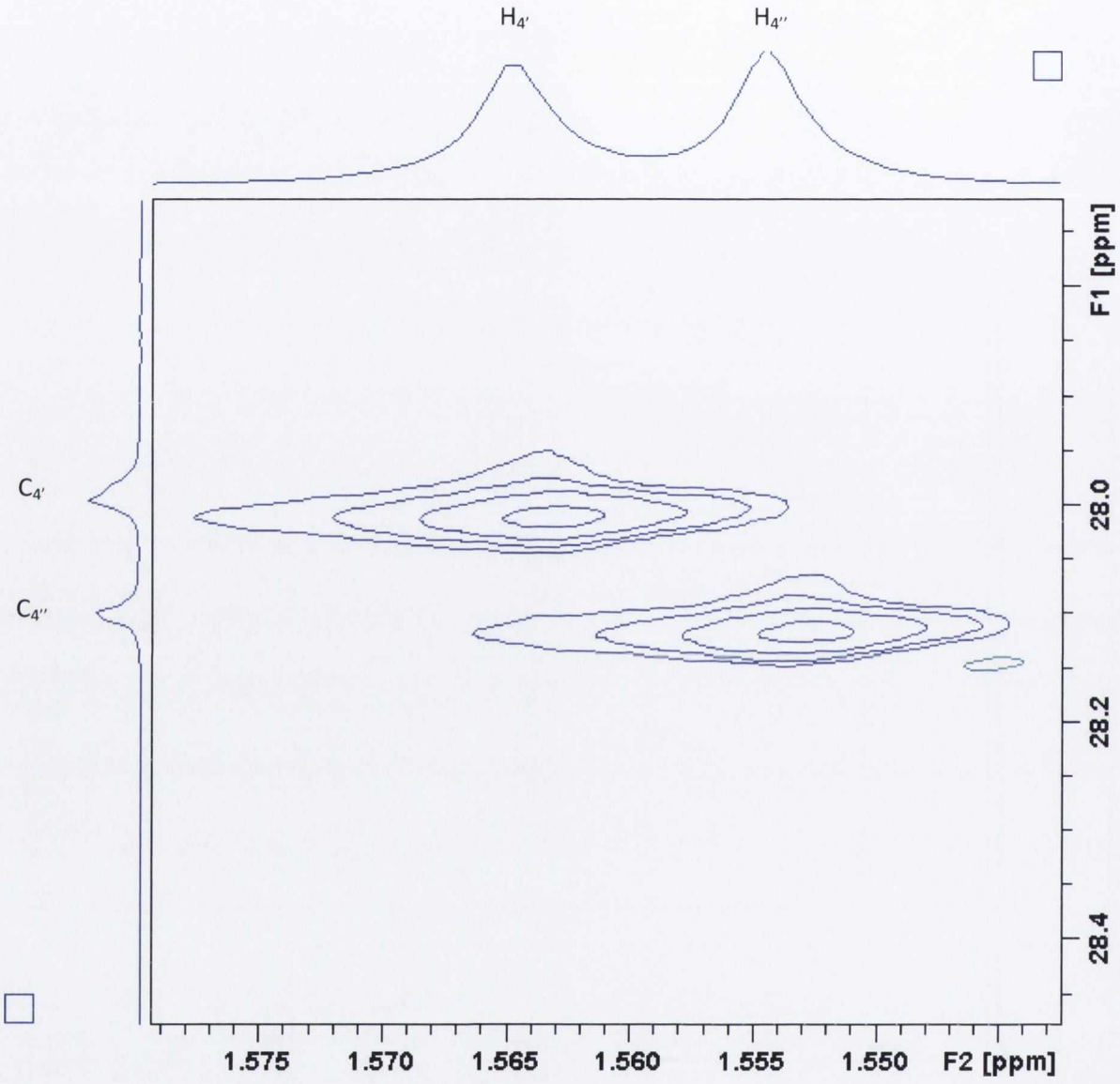


Figure 4.15 – HSQC showing CH_3 carbon and proton signals

Again on the HMBC, the signal at 1.56 ppm couples to 80.4 ppm, whereas the signal at 1.57 ppm couples to signal at 84.8 ppm. This indicates that the signal at 80.4 ppm is $C_{3''}$, whereas that at 84.8 ppm is $C_{3'}$ (Figure 4.16).

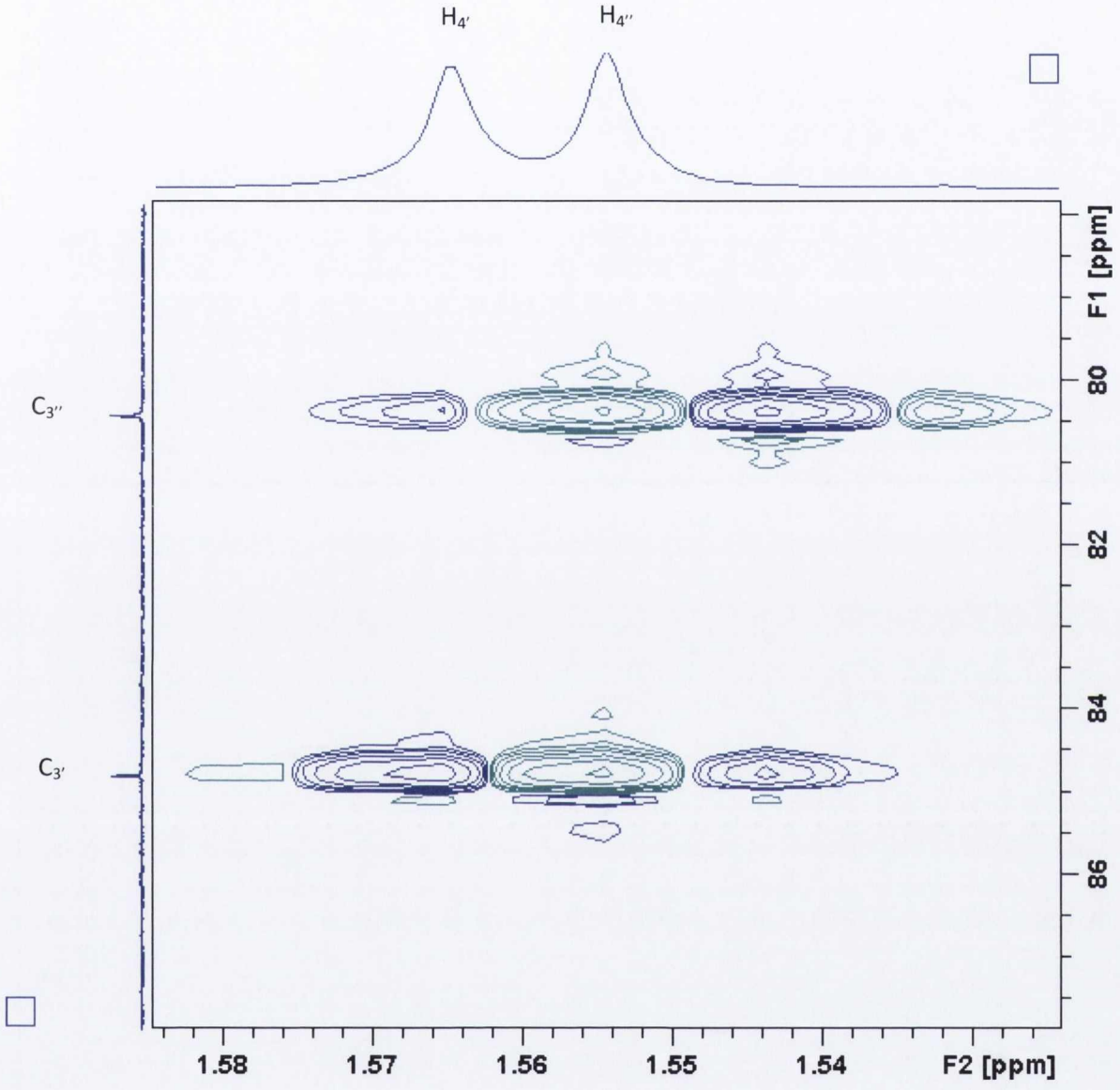


Figure 4.16 – HMBC showing coupling of CH_3 protons to aliphatic quaternary carbons

Figure 4.17 shows that the proton signal at 2.75 – 2.73 ppm couples most strongly to the signals at 97.6 (C_3), 136.0 and 134.1 ppm on the HMBC, therefore the latter 2 signals must be carbons C_4 and C_5. This proton signal must therefore correspond to H_6 and H_{10}. The protons signals at 1.68 – 1.66 ppm couple weakly to these signals, therefore these must be H_7 and H_9. The signal at 1.87 ppm does not couple with any of the quaternary carbons therefore it must be H_8. The upfield portion of the signal at 1.68 – 1.66 ppm couples to the carbon at 136.0 ppm whereas the downfield portion couples to the carbon at 134.1 ppm. By process of elimination the carbon signal at 114.6 ppm is C_{11}.

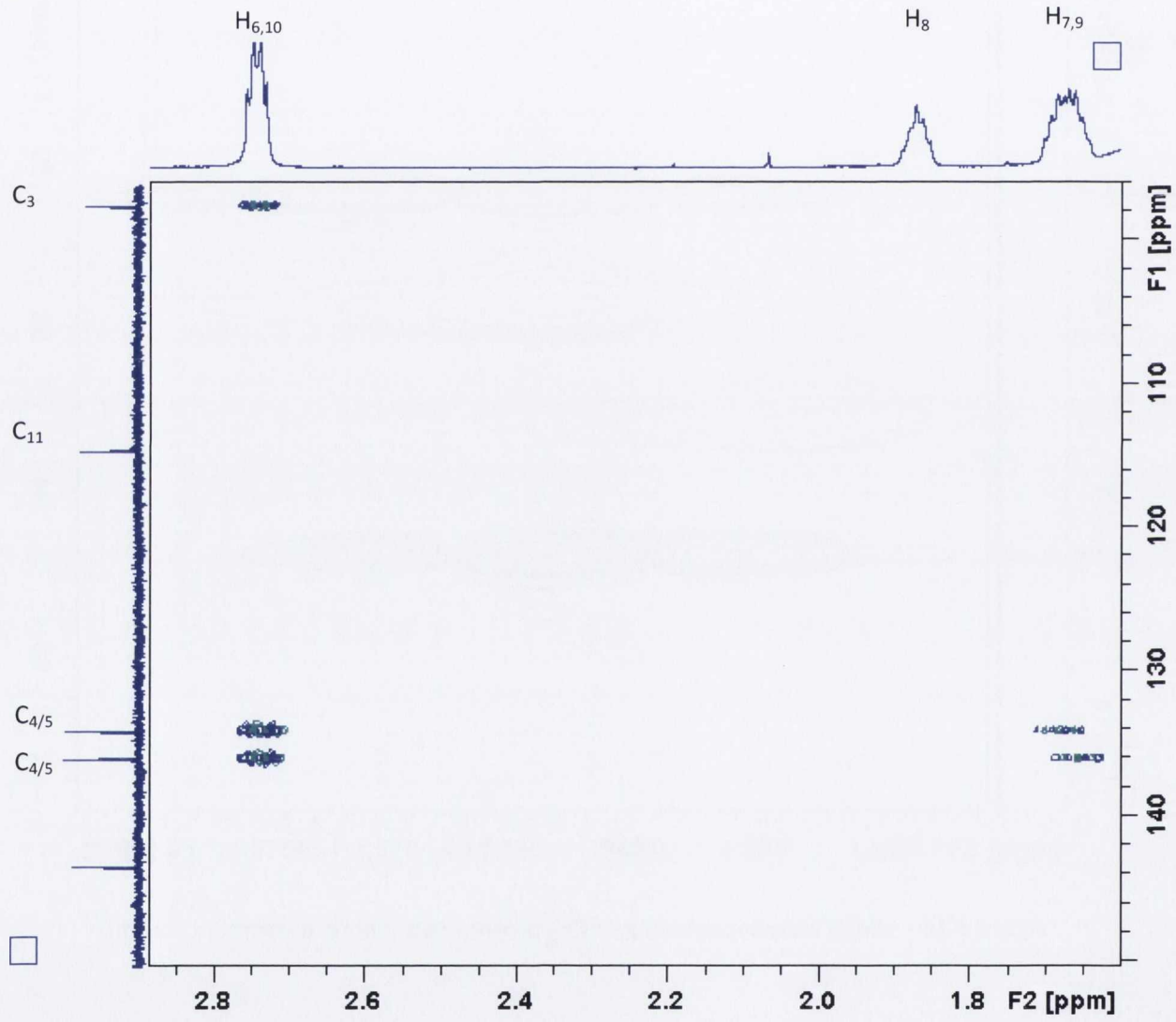


Figure 4.17 – HMBC coupling of CH_2 protons

Still on the HMBC, the upfield portion of the signal at 2.75 – 2.73 ppm couples more strongly to C_3 and is therefore corresponds to H_{10}, while the downfield portion is H_6 (Figure 4.18).

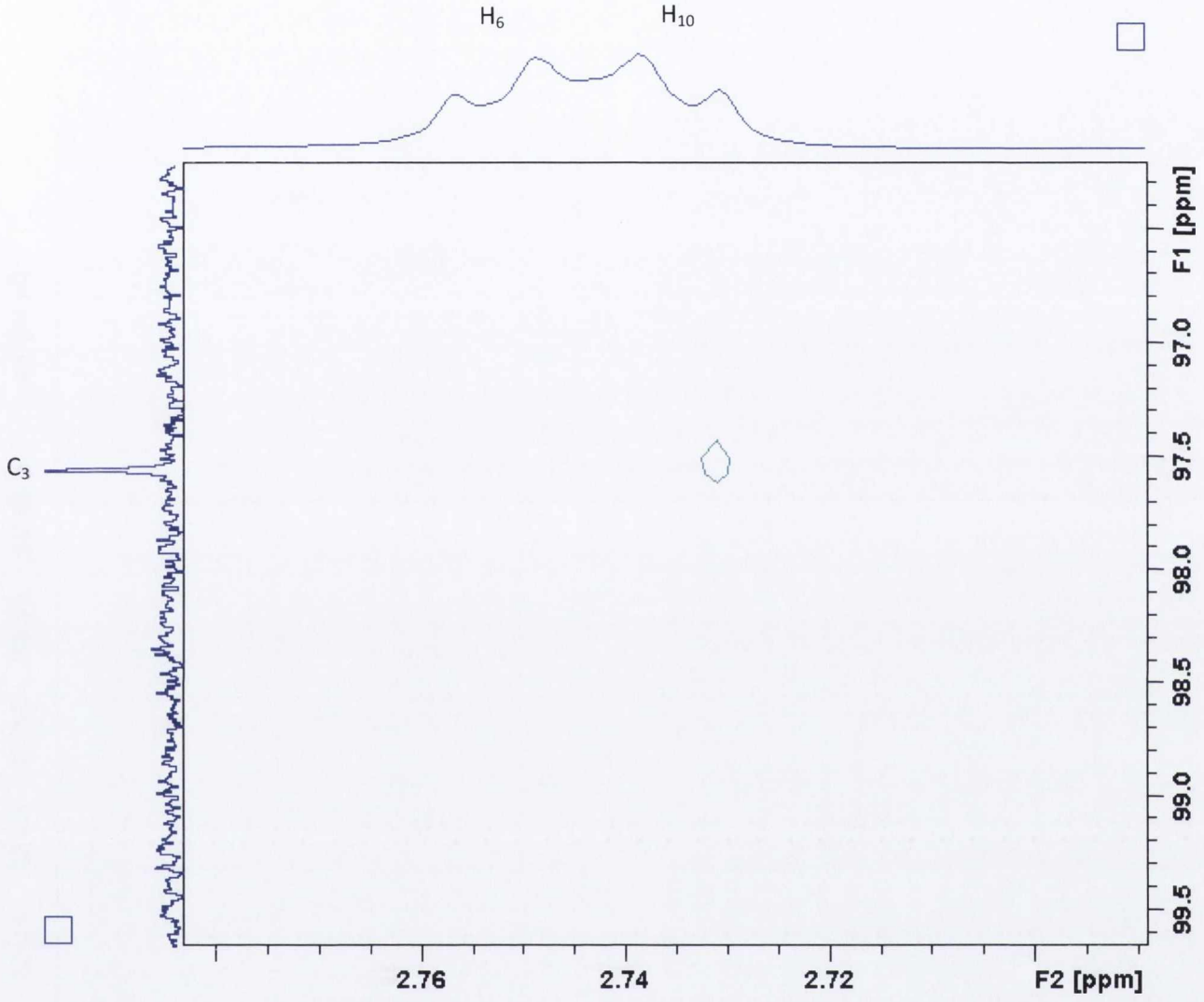


Figure 4.18 – HMBC showing coupling between C_3 and H_{10}

On the HSQC, The multiplet at 2.75 – 2.73 ppm couples to the signals at 29.1 ppm on the HSQC, with the upfield portion of the proton signal coupling to the downfield carbon and vice versa. Since the upfield portion of the proton multiplet is H_{10}, the downfield carbon signal is therefore C_{10} similarly the upfield carbon signal is C_6 (Figure 4.19).

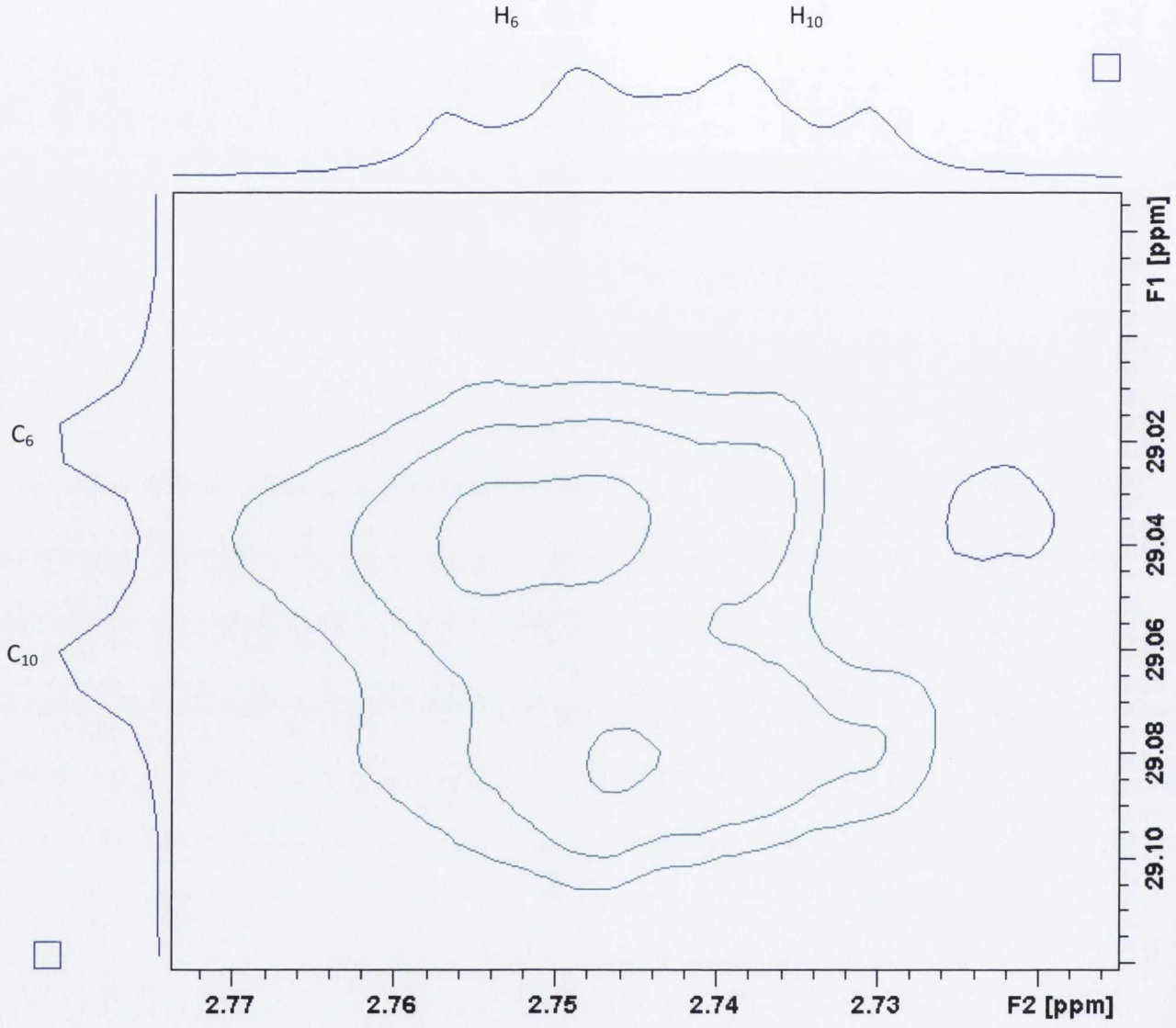


Figure 4.19 – HSQC coupling of protons H_6 and H_{10} with C_6 and C_{10}

The proton signal at 1.87 ppm couples with that at 32.1 ppm on the HSQC and therefore both correspond to H_8 and C_8 respectively (Figure 4.20).

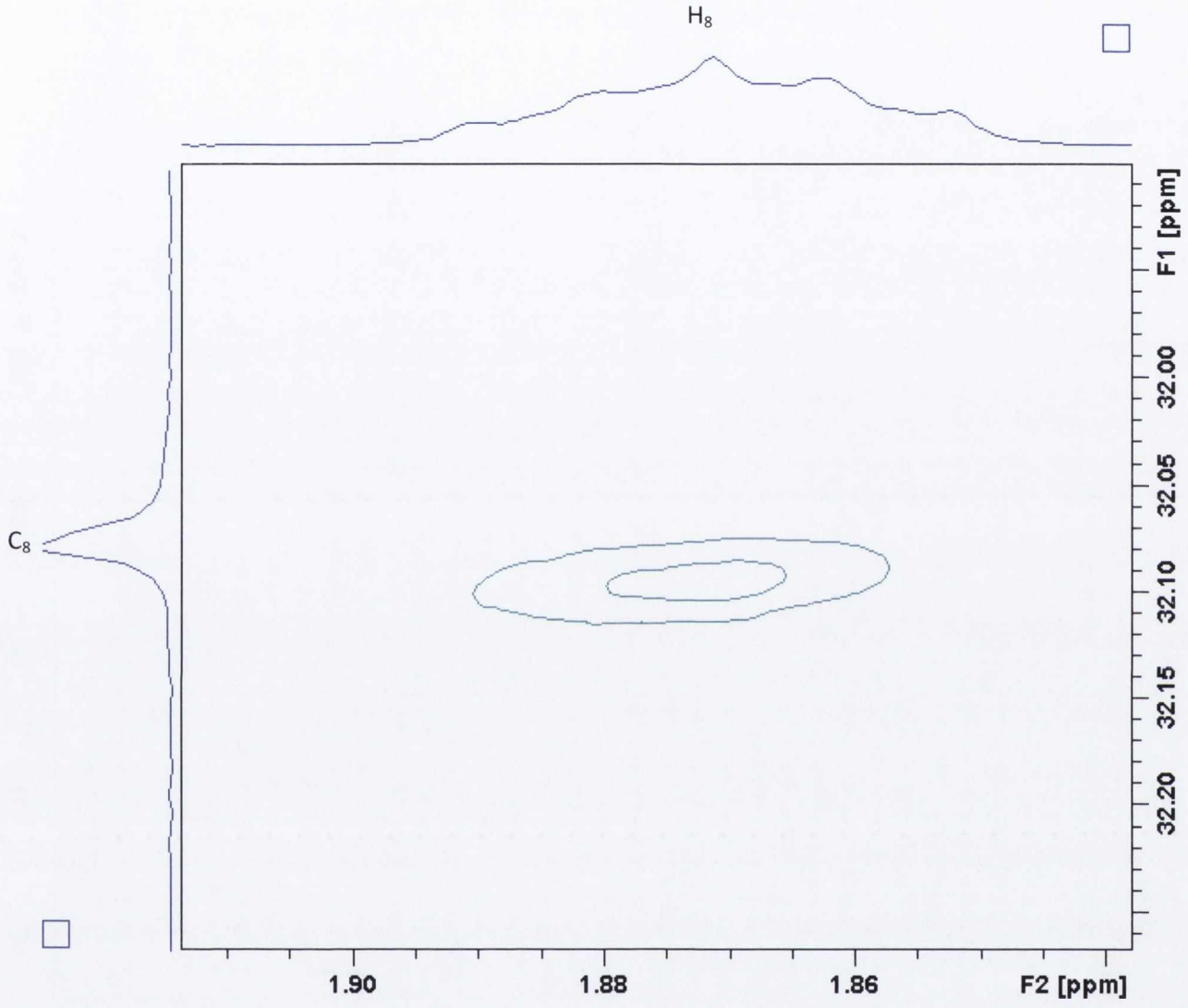


Figure 4.20 – HSQC coupling of H_8 and C_8

Back to the HMBC, the upfield side of the signal at 2.75 – 2.73 ppm (H_{10}) couples to the CH_2 carbon at 28.0 ppm, while the downfield side (H_6) couples to the carbon at 27.4 ppm therefore these two carbons are C_9 and C_7 respectively (Figure 4.21). The protons also couple to C_8 as would be expected.

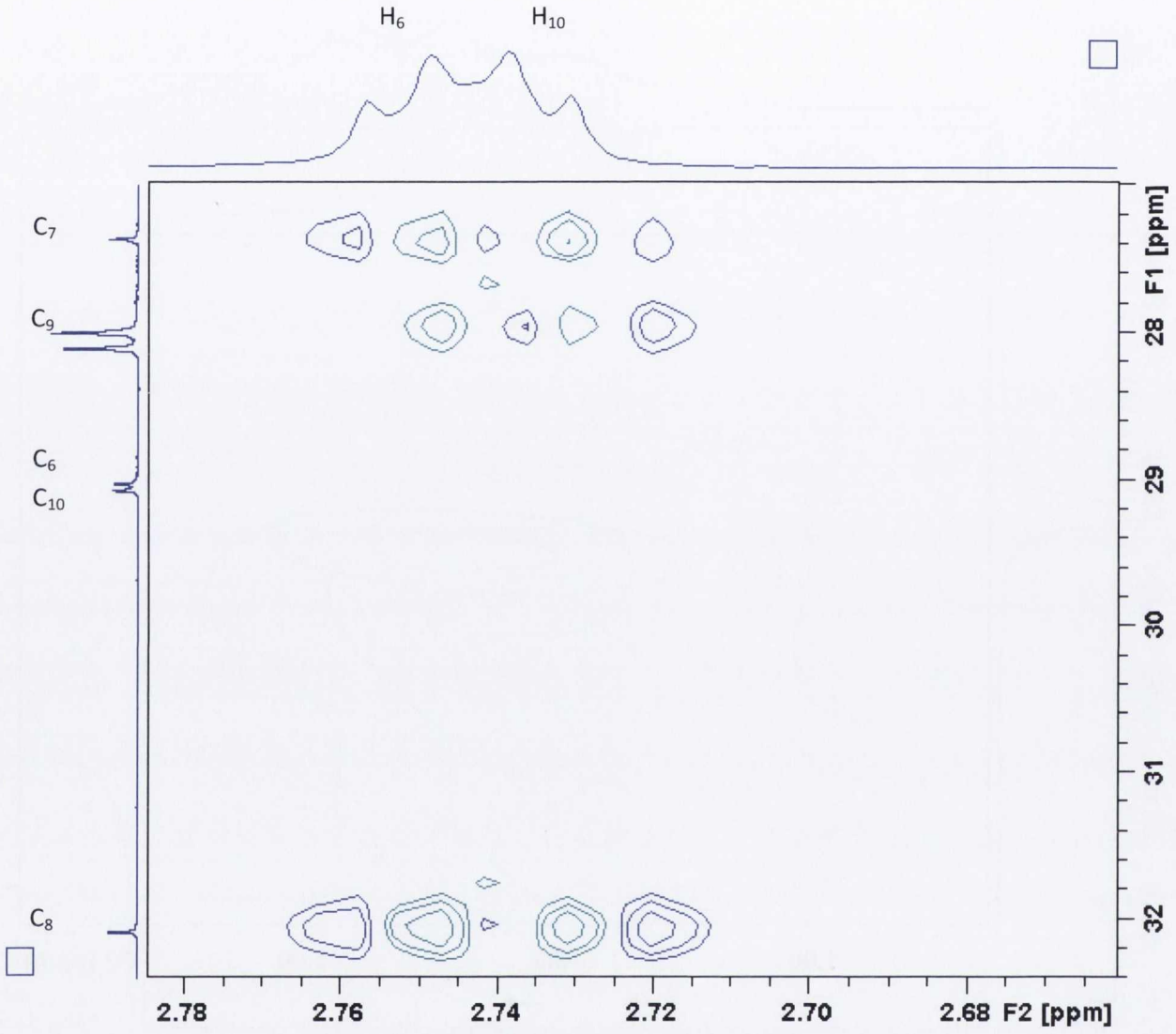


Figure 4.21 – HMBC coupling of H_6 and H_{10}

On the HSQC, the downfield portion of the signal at 1.68 -1.66 ppm couples to the signal at 28.0 ppm whereas the upfield portion couples to the signal at 27.4 ppm (Figure 4.22).

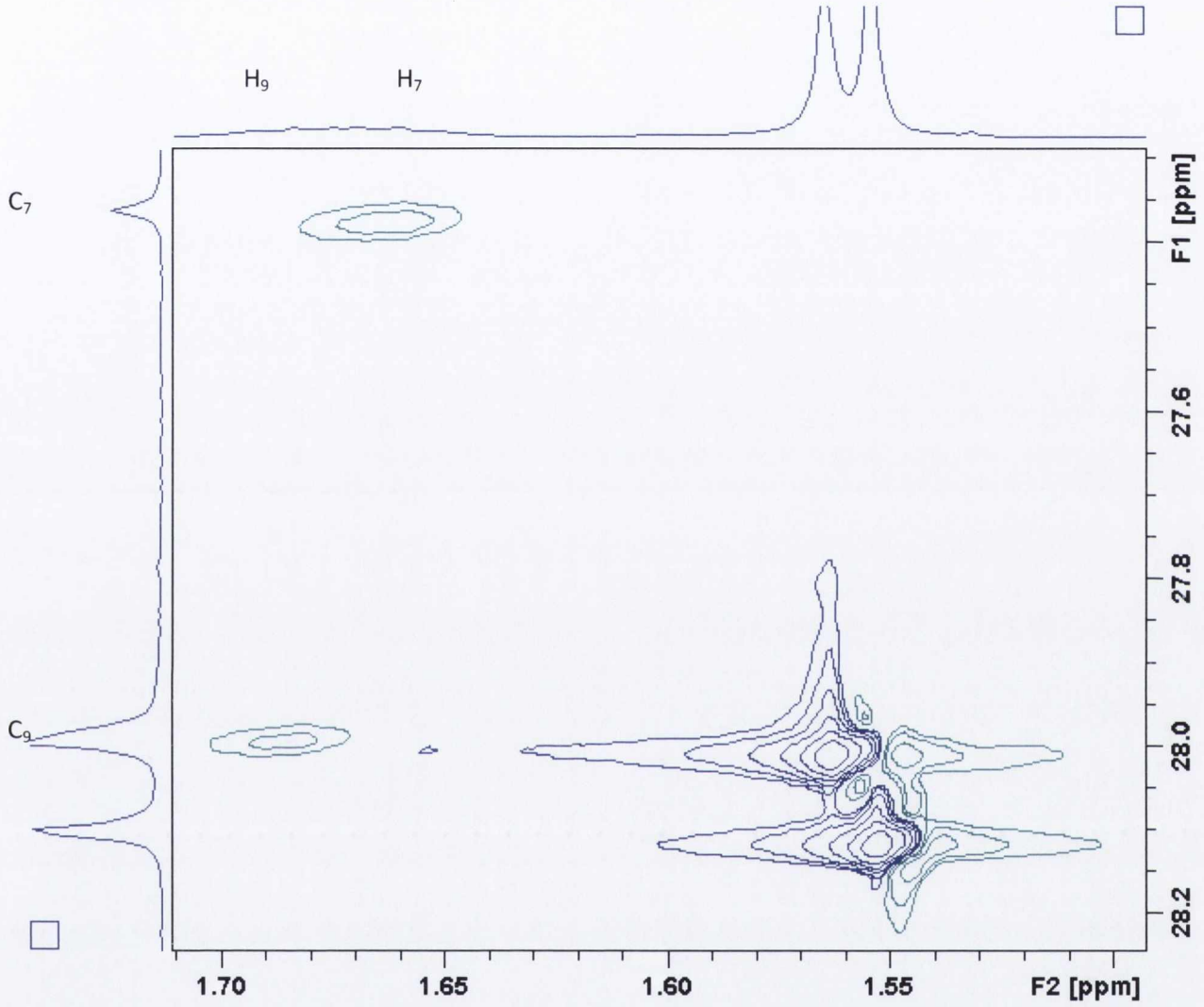


Figure 4.22 – HSQC coupling of C_7 with H_7 and C_9 with H_9

Figure 4.23 shows the Selective TOCSY carried out on the signal at 1.87 ppm at a delay time of 0.03 s, which shows close correlation with the signal at 1.68 - 1.66 ppm. Also the signal at 2.75 – 2.73 ppm correlates to it strongly when the delay is larger (0.06 s). This confirms the HMBC results.

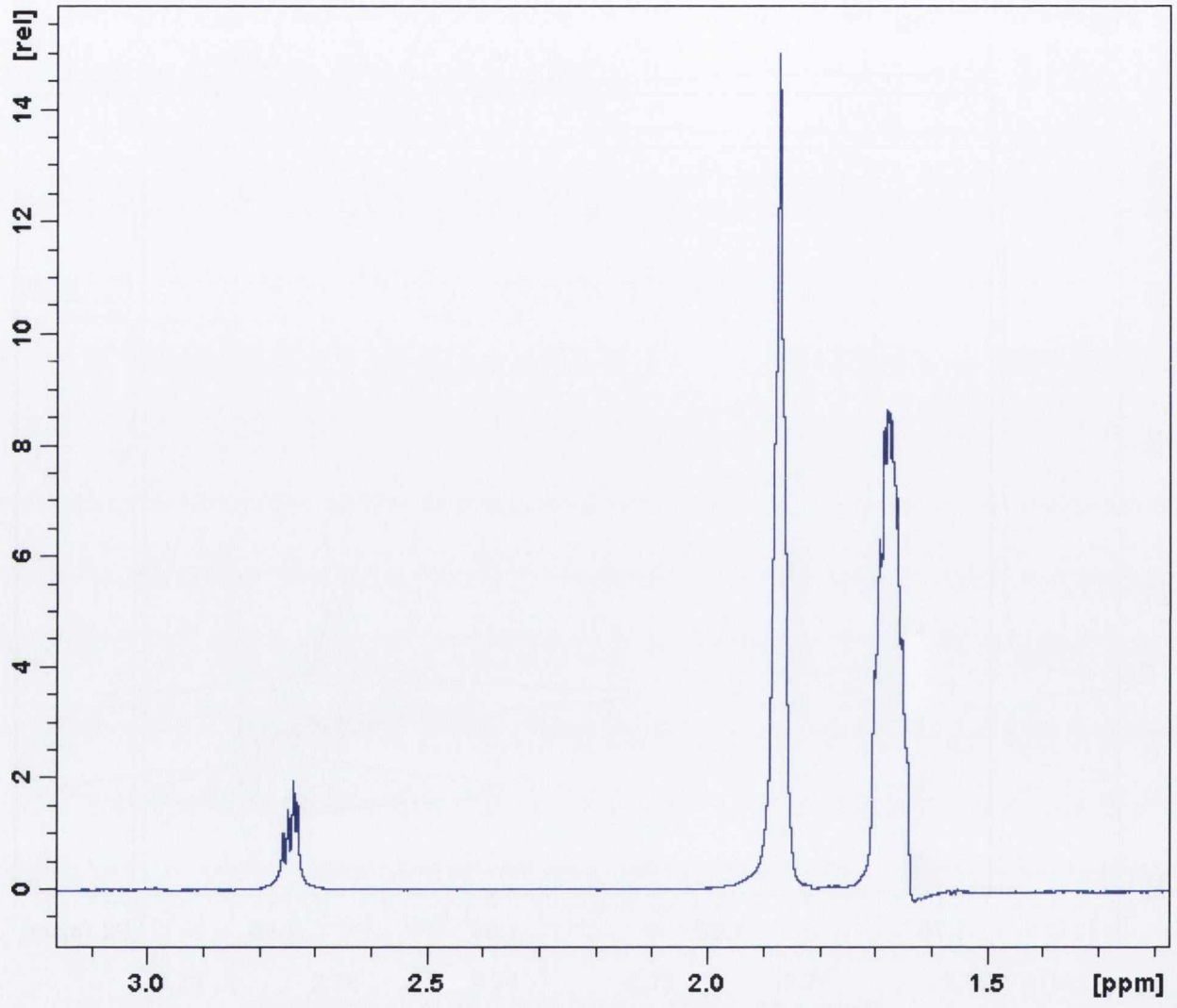
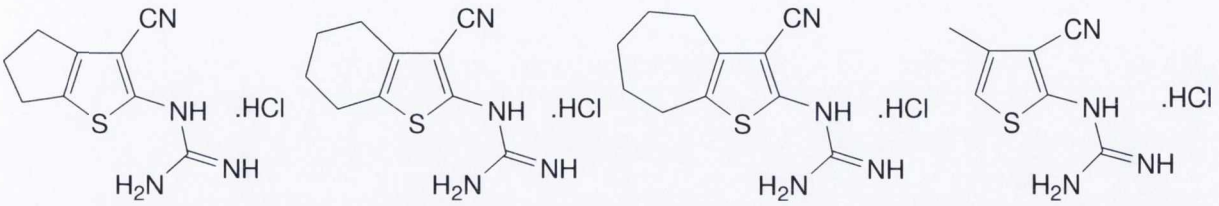


Figure 4.23 – Selective TOCSY at the H_8 signal at 1.87 ppm carried out at a delay of 0.03 s

4.3.4 Deprotection of 2,3-[Di(*tert*-butoxycarbonyl)]-1-(3-cyanoalkylthiophen-2-yl)guanidines

Deprotection of compounds **126**, **127** and **128** was initially carried out using the TFA/DCM and Amberlite method.[115] However the same contaminant as before was observed in samples prepared via this method and could not be removed by carrying out reverse phase chromatography or by

recrystallisation using water or water/EtOH mixtures. Therefore, the HCl/dioxane method discussed in 4.2.8.2 was used to prepare pure samples for biological testing. The expected products of this deprotection reaction are shown in Figure 4.24.

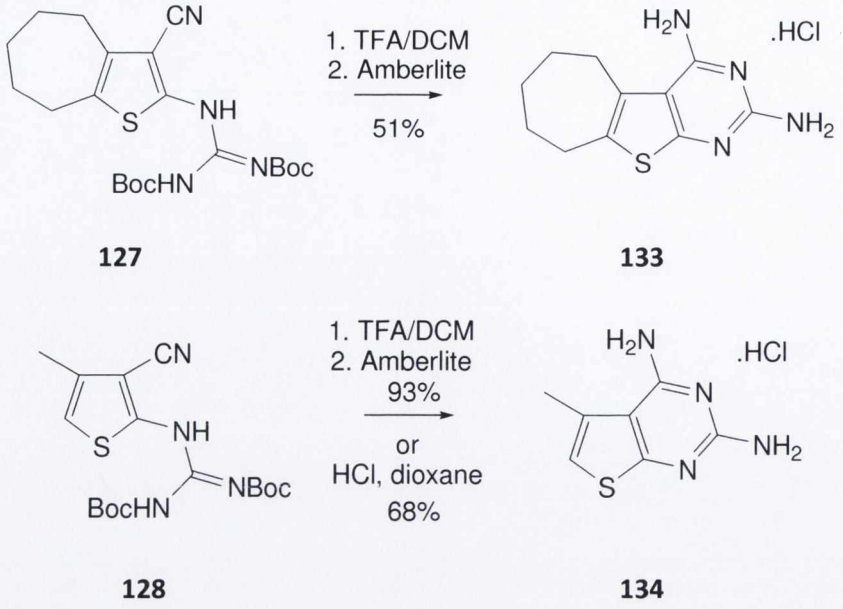


129 **130** **131** **132**

Figure 4.24 - Products expected from the deprotection of the 2,3-[di(*tert*-butoxycarbonyl)]-1-(3-cyano-alkylthiophen-2-yl)guanidines

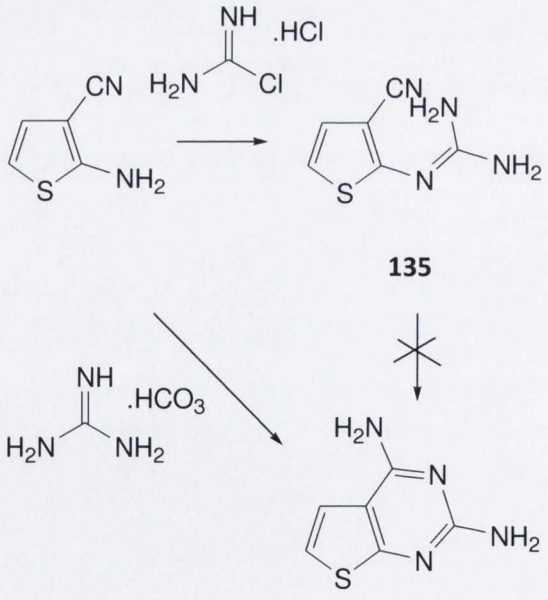
HRMS and ^{1}H and ^{13}C NMR spectroscopic data were consistant with the structures of **129-131**, with no Boc peaks observed via ^{1}H NMR and with ^{13}C NMR confirming the absence of peaks from the Boc groups, while retaining the guanidine carbon. IR characterisation of **129-131** showed nitrile signals at 2224 and 2225 cm^{-1} respectively. This confirmed that these three products were guanidine hydrochloride salts **129**, **130** and **131** (Figure 4.24) respectively, and they were obtained in yields of 39, 43 and 58%. These three products needed to be purified quickly and were not left in aqueous solution for long periods of time (eg. in an NMR tube).

Spectral data from NMR (in D_2O) and HRMS experiments were in agreement with the expected salt structures, however, when IR spectroscopy was carried out on some samples of what was believed to be **131-132**, no nitrile signal was observed. Since NMR and HRMS data were consistent with the structure, it was postulated that the products must be isomers of the expected structures. Since protons bonded to nitrogen atoms do not show up via ^{1}H NMR when D_2O is used due to proton-deuterium exchange, it is possible that the products correspond to cycloalkylpyrimidine hydrochlorides **133** and **134** (Scheme 4.13). Yields for **134** were 93% using TFA/DCM and 68% using HCl/dioxane; the yield of **133** was 51% using the former method.



Scheme 4.13

When looking for further evidence to support these structures, a 1990 paper by Link[165] was found in which free guanidine reacted with 2-amino-thiophene-3-carbonitrile to form 2,4-diaminothieno[2,3-*d*]pyrimidines (Scheme 4.14) which confirms that such a transformation is possible.

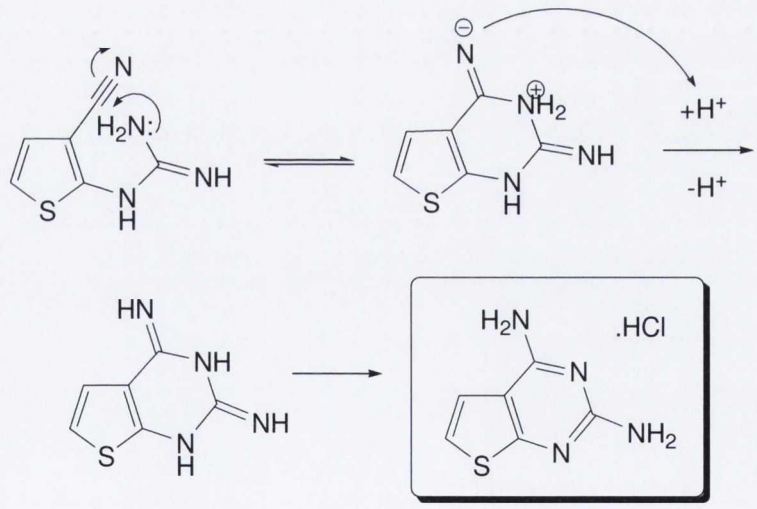


Scheme 4.14

The *N*-(3-cyano-thiophen-2-yl)guanidine (**135**, Scheme 4.14), which is a tautomer of the originally expected product from the deprotection, was made using chloroformamidine hydrochloride, however, interestingly 2,4-diaminothieno[2,3-*d*]pyrimidines did not form from **135** cleanly under the basic, neutral or acidic conditions used by Link.

However, under the experimental procedures described in this document, it was possible to isolate the 2,4-diaminothieno[2,3-*d*]pyrimidines perhaps due to the availability of reverse phase chromatography, which was not available at the time of the paper by Link. Also of note is that the cyclisation reactions to form the 2,4-diaminothieno[2,3-*d*]pyrimidines gave reasonable yields (43 - 91%), in contrast to the difficulties encountered by Link. This was perhaps due to the choice of solvent used to solubilise **129**-**132**, since the cyclised products are poorly soluble in aqueous solution they precipitate out of solution which may shift the equilibrium via Le Chateliers principle. To test this theory, pure **131** was left in aqueous solution for a week after which time the corresponding 2,4-diaminothieno[2,3-*d*]pyrimidine, cyclohepta-fused thienylpyrimidine hydrochloride (**133**, Scheme 4.13), was observed by ^{1}H and ^{13}C NMR spectroscopy and IR.

Nitriles are less basic than guanidines,[138] therefore the nucleophile must be the guanidine moiety in the molecule. A mechanism in which the cyclisation does occurs after both Boc groups are removed, *i.e.* when a *N*-(3-cyano-thiophen-2-yl)guanidine is formed, is shown in Scheme 4.15.



Scheme 4.15

The guanidine can attack the nitrile group at the carbon atom, leaving the nitrogen to pick up a proton from solution. This 6-exo-trig cyclisation is allowed by Baldwin's rules. Tautomerisation to the more stable aromatic product can then occur.

4.4 Commercial Amines

4.4.1 Synthesis of Derivatives of Commercial Amines

Due to the difficulties encountered in attempting to synthesise thiophene analogues of the lead compound **54** (Figure 4.1), it was decided to make guanidine analogues of some commercially available aminothiophenes. Thus, the Boc protected guanidines of 2-methoxycarbonylthiophene (**136**), 3-methoxycarbonylthiophene (**137**), 2-ethylthiophene (**138**) and 2-methylthiophene (**139**) were synthesised in respective yields of 57, 37, 80 and 94% (Figure 4.25).

It is of note that the low yield of **137** is largely due to difficulties in purification; in particular, it was necessary to recrystallise the semi-crude product after an initial column. Compounds **138** and **139** gave significantly higher yields than their aromatic counterparts, because aliphatic amines are substantially more nucleophilic than aromatic ones.

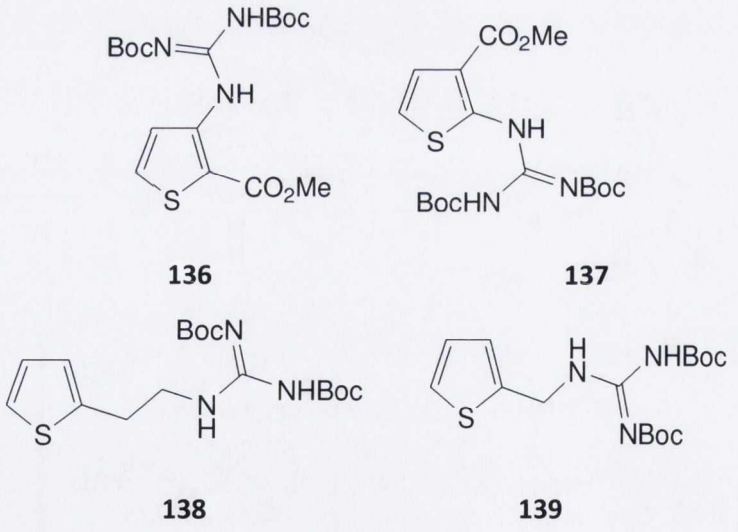
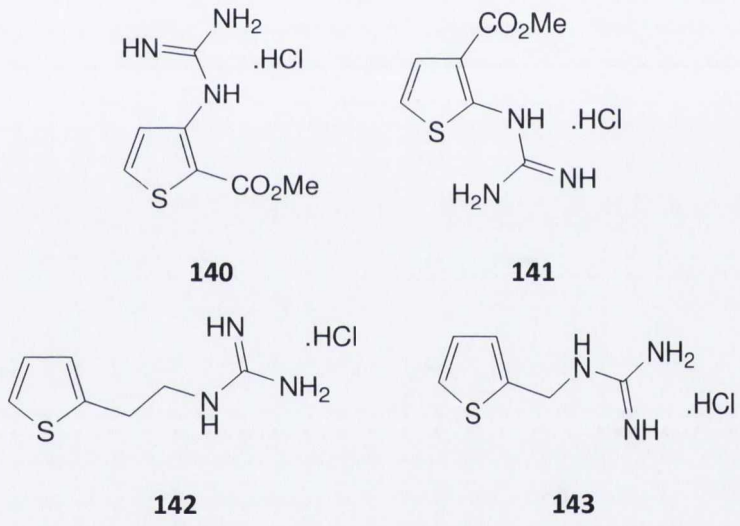


Figure 4.25 – Boc-protected guanidine derivatives of commercial amines

Deprotection of the Boc-protected guanidines **136** to **139** was carried out initially using the TFA/DCM method described initially in 3.3.4.2 giving the corresponding hydrochloride salts: (**140**, **141**, **142** and **143**; Figure 4.26) in yields of 99, 51, 43 and 54% respectively. However, the Amberlite contaminant was present in **140** to **142** and the samples were deemed insufficiently pure for biological testing, therefore deprotection of the corresponding Boc-protected guanidines was again carried out using 4M HCl/dioxane which gratifyingly give higher respective yields of 99, 90 and 95%.

Attempted 2-iminoimidazolidylation of the commercial amines resulted in similar results to those discussed in 4.2.6, with only imidazolidating agent **63** isolated upon purification of the reaction mixture; the synthesis of imidazolidine containing analogues of **136**-**139** was therefore not pursued any further.



140 **141**

142 **143**

Figure 4.26 – Guanidine hydrochloride salts of commercial amines

4.4.2 Crystal Structures

Data from X-ray crystallography was collected for compounds **140** and **141** and the structures are shown in Figure 1.19. Analysis of these structures leads to some interesting results. In the crystal structure of **140**, the ester group and the guanidine moiety are only slightly out of plane with each other, whereas in isomer **141**, the two moieties are so far out of plane that they are close to perpendicular.

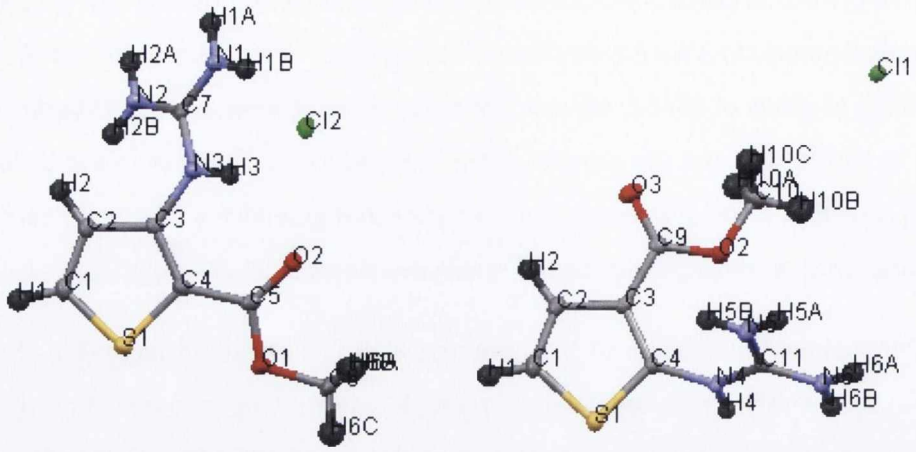


Figure 4.27 – Crystal structures of *N*-(2-methoxycarbonylthien-3-yl)guanidine hydrochloride (**140**) and *N*-(3-methoxycarbonylthien-2-yl)guanidine hydrochloride (**141**)

Both molecules occupy a similar volume in the structures; with **140** having 4 molecules in a unit cell, which has a cell volume of 1018.89 $Å^3$ and with **141** having an 8 molecule unit cell which has a cell volume of 2035.71 $Å^3$, roughly twice that of the former unit cell (Table 4.1 and Table 4.2).

Table 4.1- Unit cell parameters for **140**

Space Group	Cell Lengths	Cell Angles	Cell Volume	Z, Z'	R-Factor (%)
P 2_1 2_1 2_1	a 7.361 Å	α 90°	1018.89 $Å^3$	Z: 1	2.27
	b 10.015 Å	β 90°		Z': 0	
	c 13.821 Å	γ 90°			

Table 4.2 – Unit cell parameters for **141**

Space Group	Cell Length	Cell Angles	Cell Volume	Z, Z'	R-Factor (%)
C 2/c	a 11.739(4) Å	α 90.00°	2035.71 $Å^3$	Z: 1	3.37
	b 9.691(3) Å	β 107.522° (4)		Z': 0	
	c 18.765(6) Å	γ 90.00°			

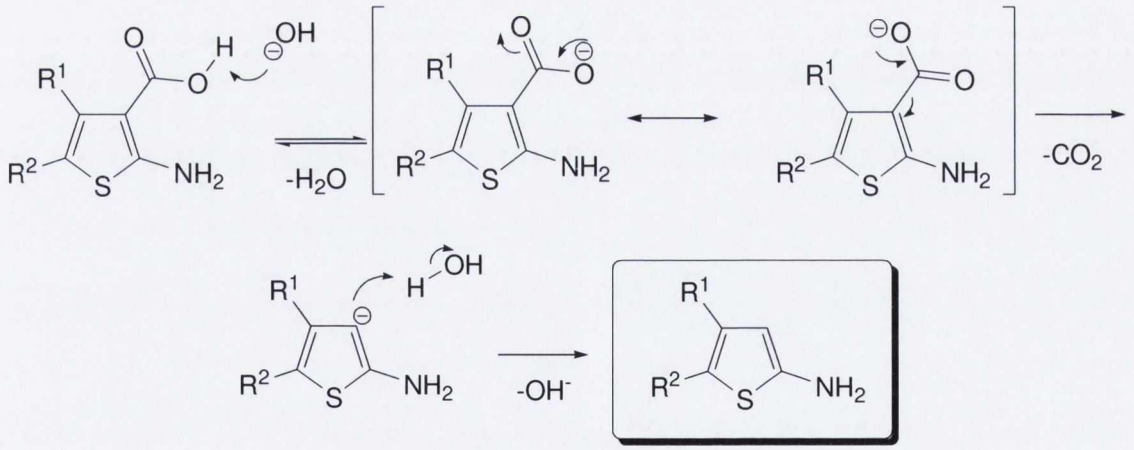
4.5 Synthesis of (Thiophen-2-yl)guanidine Derivatives

4.5.1 Background to Acidic Decarboxylation

After the initial poor results when attempting to decarboxylate **107** and **110** under basic conditions, it was decided to try to perform this reaction under acidic conditions such as those described by Barker *et al.*[166] In particular, Barker and co-workers used a two-step ester hydrolysis of methyl-3-amino-2-thiophene carboxylate under basic conditions followed directly by decarboxylation using oxalic acid at 38 ℃ to generate the oxalate salt of 3-aminothiophene, which was used quickly in the next reaction to prevent decomposition. It was thus envisaged that this procedure could be attempted on all the aminothiophene esters described in this chapter in order to generate thiophene guanidine analogues of **54**.

4.5.2 Comparison of the Mechanisms of Basic and Acidic Decarboxylation on Thiophene

Earlier, in Section 4.2.5, the basic decarboxylation of thiophenes in the 2-position was described, and the procedures used were attempted on **107** and **110**; however, the mechanism of such a transformation at the 3-position was not discussed. Thus a mechanism for possible decarboxylation is depicted in Scheme 4.16 and is discussed thereafter.

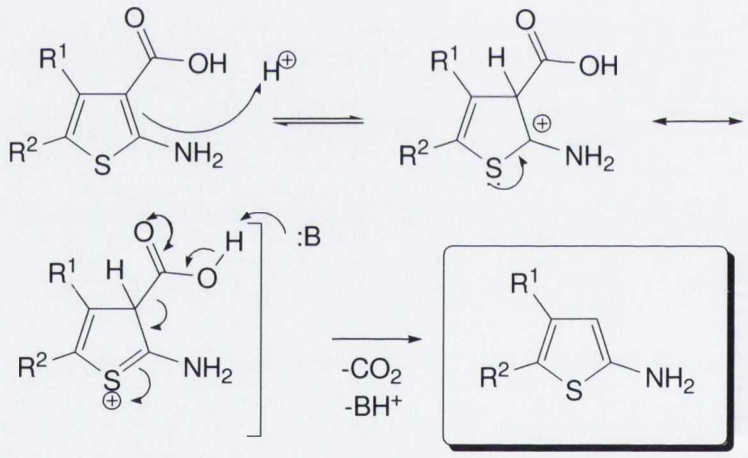


Scheme 4.16

In a basic decarboxylation, the carboxylic acid is deprotonated first by a hydroxide ion, then the negatively charged oxygen on the carboxylate moiety feeds electrons into the adjacent carbon, with

the end result being decarboxylation, and the generation of a negatively charged ion. In the case of a thiophene-2-carboxylate, we can see that this negatively charged ion is in fact quite unstable, since the negative charge cannot be delocalised across the aromatic ring, thus making high temperatures necessary to affect the decarboxylation as the intermediate would be very high in energy.

By contrast, the decarboxylation mechanism of an acidic decarboxylation is examined in Scheme 4.17. Firstly, the mechanism begins in a manner analogous to the first step of EAS, in that an acidic proton is attacked by a double bond of the thiophene molecule. The proton adds to the carbon adjacent to the acid moiety, as this places a positive charge on the carbon β to the carbonyl which can be stabilised by resonance due to the adjacent sulfur atom. Also the electron poor carbon of the acid would destabilise the positive charge if it were adjacent. Finally, the conjugate base of the acid can deprotonate the acid resulting in the decarboxylated thiophene. Therefore, due to the more stable charged intermediate of an acidic decarboxylation on a thiophene-3-carboxylic acid in comparison to a basic one, the former seems more probable.



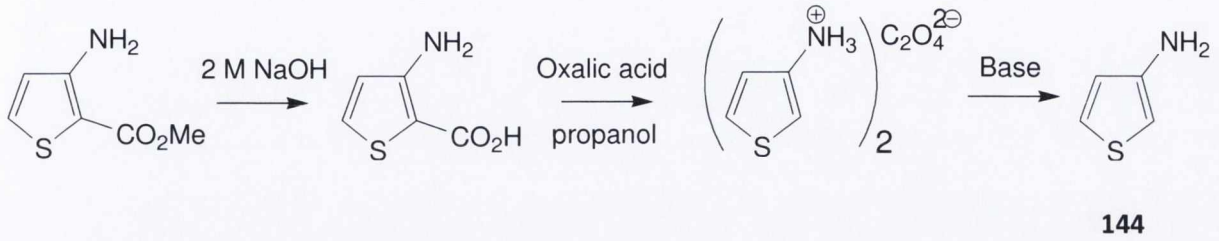
Scheme 4.17

4.5.3 Synthesis and Derivatisation of 3-Aminothiophene (144)

4.5.3.1 Synthesis of Boc-protected 3-Aminothiophene Derivatives

The decarboxylation method of Barker[166] involves the basic ester hydrolysis of methyl-3-aminothiophene-2-carboxylate using the weakly basic conditions of aqueous 2 M NaOH was heated to reflux over 30 min. The solution is then acidified to pH 3 to precipitate out the acid, and the acid is collected by filtration pressed dry and dissolved in propanol along with the addition of a small excess

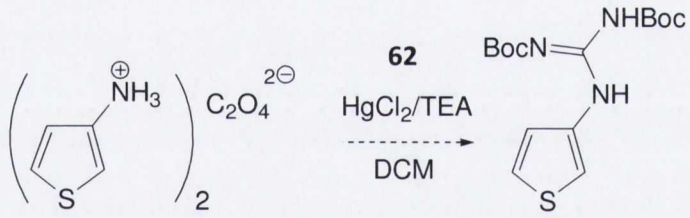
of oxalic acid and gentle heating to 38 °C over 45 min. The crude oxalate salt is then collected via precipitation with ether and filtration. Treatment with base, best done immediately prior to the next reaction step to prevent decomposition, reportedly gives 3-aminothiophene (**144**, Scheme 4.18).



144

Scheme 4.18

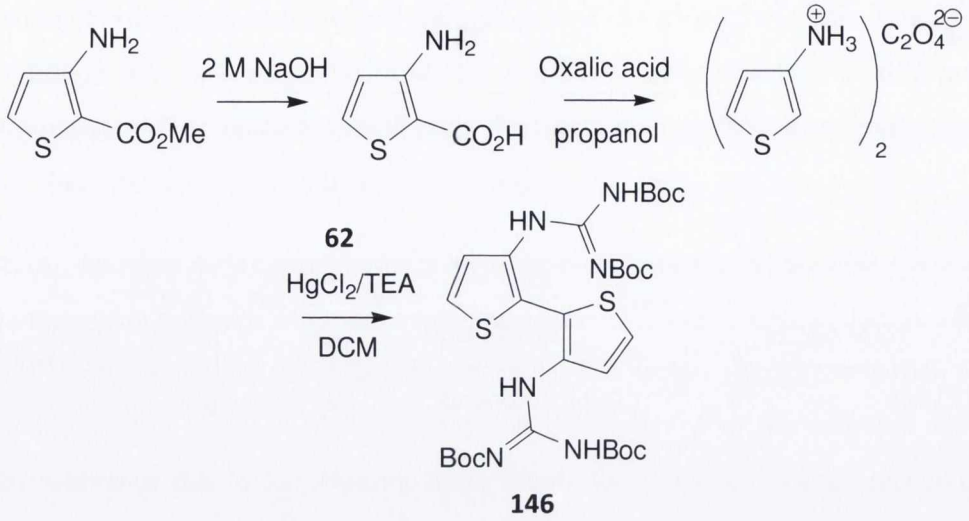
Initial use of what was believed to be the oxalic acid salt of **144**, directly in Kim and Qian[112] guanidylation conditions, was attempted, as had been successful in Section 3.4.2.2 for the conversion of **72** to **81**. This involved treatment of the supposed salt with **62**, $HgCl_2$ and TEA in DCM. However when the isolated product was fully characterised it became apparent that the intended product **145** (Scheme 4.19) had not been obtained.



145

Scheme 4.19

On the ^1H NMR spectroscopy, the product had one less aromatic proton and one quaternary carbon more than expected. This ruled out the possibility that the decarboxylation did not in fact occur as **136**, the product expected if the decarboxylation did not take place, has two more carbons than **145**. ESI^+ HRMS analysis showed a molecular ion at *m/z* 681, which suggested that the product was in fact the dimer (**146**, Scheme 4.20).



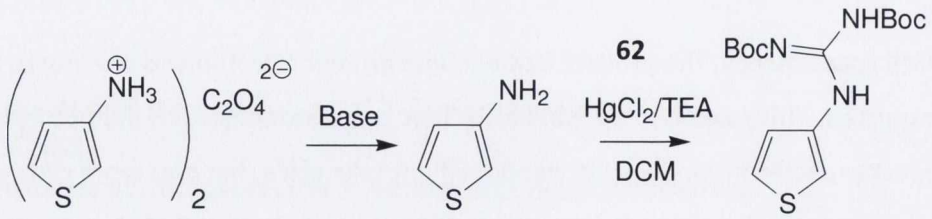
146

Scheme 4.20

The ^{1}H and ^{13}C NMR data were also consistent with this structural assignment, with the ^{1}H NMR spectrum showing one less aromatic proton than would be expected and the ^{13}C NMR spectrum containing 11 distinct carbon species as would be expected for molecule **146**.

4.5.3.2 Proposed Mechanism of the Dimerisation of 3-Aminothiophene

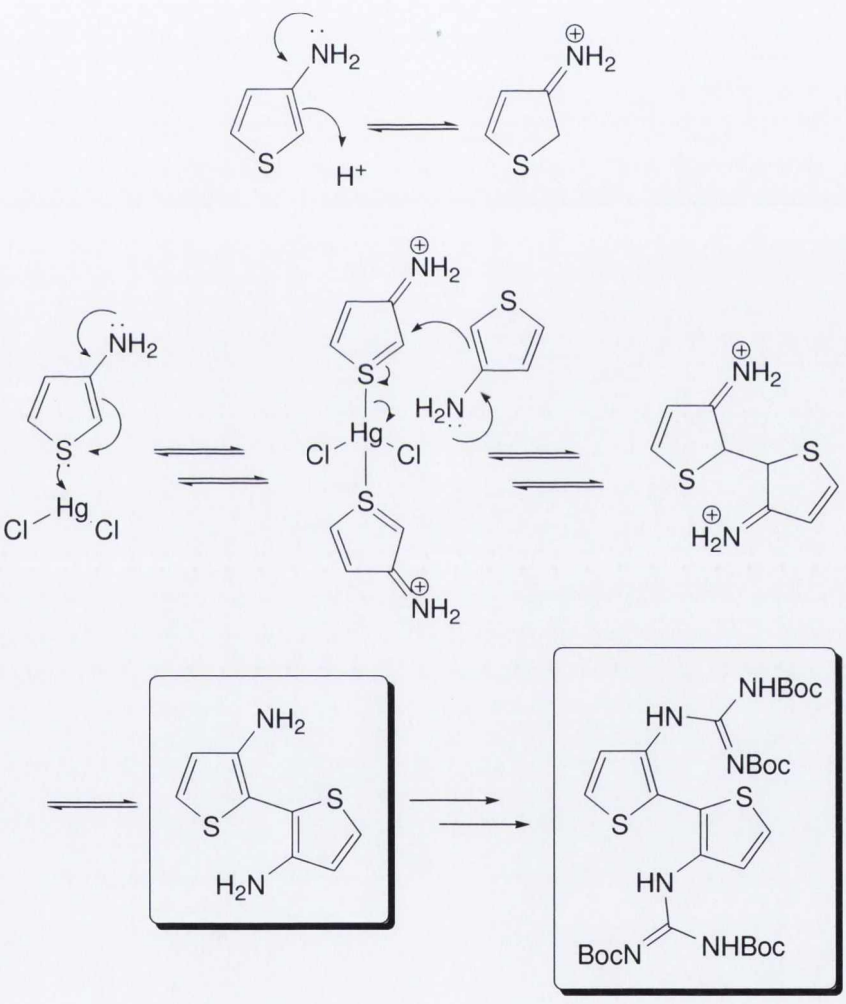
This brought up the question as to whether the dimerisation occurred before or during the guanidylation step, so the synthesis was repeated, but this time the ^{1}H NMR of the oxalate salt was obtained. The ^{1}H NMR was consistent with the oxalate salt of **144** and the product was guanidylated under the standard conditions, this time after free-basing the amine. Gratifyingly, desired product **145** (Scheme 4.21) was obtained, but in the low yield of 5%, with the rest preseumably having decomposed.



144 **145**

Scheme 4.21

A hypothesised mechanism for the formation of the side product is depicted in Scheme 4.22. It is proposed that the enamine character of the 3-aminothiophene is exploited when the thiophene oxidatively adds the mercury centre. Addition of another molecule of the thiophene forms a complex, in which the carbon in the 2 position is electrophilic enough for a further thiophene molecule to attack at this position. Reductive elimination of the thiophene results in the formation of a dimer, which can then be guanidylated directly. It is supposed that the presence of either both acid and base or acid alone is necessary for the reaction to occur since none of this product was observed when the guanidylation was carried out on the free amine.



Scheme 4.22

Support for the initial oxidative addition of the thiophene to the mercury centre comes from the known ability of mercury to complex with sulfur containing molecules in a similar fashion as is exemplified by the isolated complex $[HgI_2(\eta^1\text{-}S\text{-imidizolidine-2-thione-}H_2)_2]$ (Figure 4.28).[167] The possibility of formation of complexes involving mercury and the thiophene nucleus could also be responsible for the poor to average yields of the Boc-protected guanidines obtained.

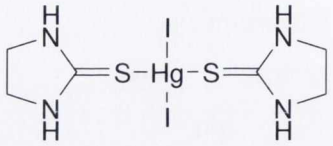


Figure 4.28 – Structure of $[HgI_2(\eta^1\text{-}S\text{-imidizolidine-2-thione-}H_2)_2]$

There is also support for the ability of 3-aminothiophenes to attack electrophiles through the 2-position due to the enamine character of 3-aminothiophenes and their ability to alkylate easily at this position under acid catalytic conditions as described by Brugier *et al.* (Figure 4.29).[140]

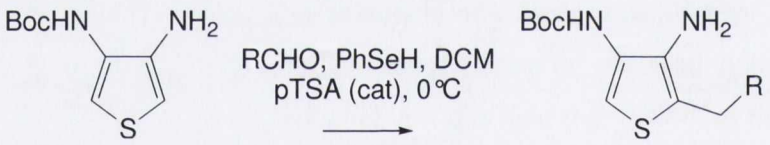
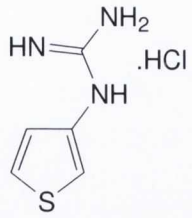


Figure 4.29 – Acid catalysed alkylation of 3-aminothiophenes at the 2 position

4.5.3.3 Synthesis of the 3-Aminothiophene Derived Hydrochloride Salts

Attempts to deprotect **146** to form its hydrochloride salt using the TFA/DCM and Amberlite procedure resulted in a complex mixture from which the intended product could not be isolated. Deprotection of **145** was attempted next, with HCl/dioxane followed by reverse phase column chromatography resulting in the clean isolation of *N*-(thien-3-yl)guanidine hydrochloride (**147**, Figure 4.30) in a satisfying yield of 88%.

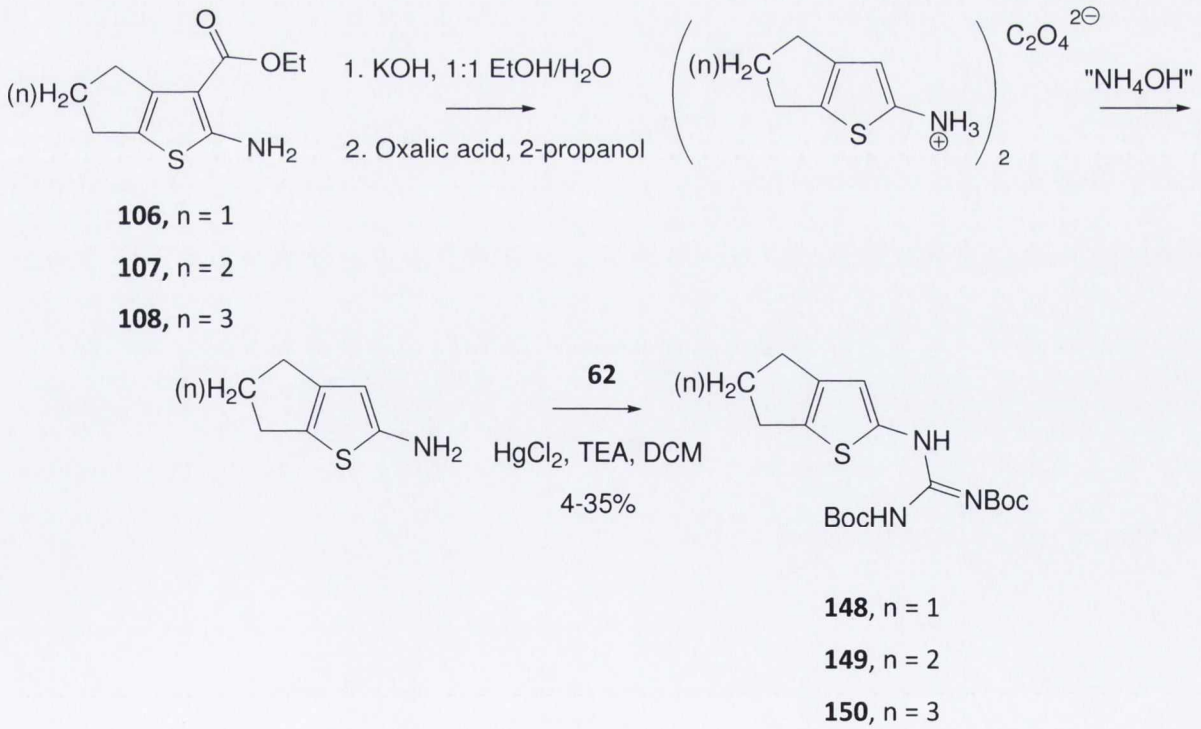


147

Figure 4.30 – Structure of (thiophen-3-yl)guanidine hydrochloride

4.5.4 Synthesis and Derivatisation of (Cycloalkyl[*b*]thiophen-2-yl)guanidine Hydrochloride Salts

The method of Barker was applied to **106** to **108**, but in the first step, the ester hydrolysis, no reaction occurred when a pure aqueous solution was used, so it was postulated that this was due to the poor solubility of the starting materials in basic water. A modification using KOH in 50% ethanolic H_2O and the subsequent removal of the excess ethanol in vacuo after the reaction had reached completion resulted in the clean isolation of the Boc-protected guanidines **148**, **149** and **150** in respective yields of 4, 23 and 35% (Scheme 4.23). All data was consistent with the desired stuructures.



106, n = 1

107, n = 2

108, n = 3

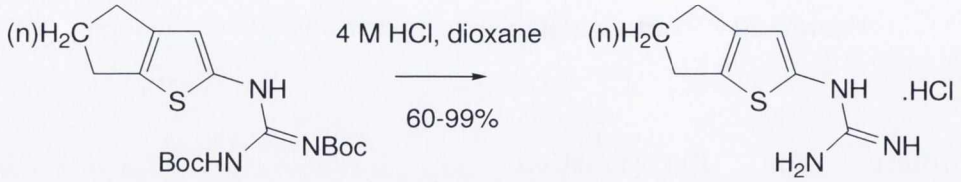
148, n = 1

149, n = 2

150, n = 3

Scheme 4.23

Application of the HCl/dioxane deprotection method to **148** to **150** followed by reverse phase column chromatography resulted in the clean isolation of the corresponding hydrochloride salts **152**, **151**, and **153** in yields of 60, 79 and 99% respectively (Scheme 4.24).



148, n = 1

149, n = 2

150, n = 3

151, n = 2

152, n = 1

153, n = 3

Scheme 4.24

4.6 NMR Spectroscopic Analysis

The NH signals on the ^{1}H NMR spectrum of **136** have been assigned so that the most downfield signal is ascribed to the NH adjacent to the aromatic ring in the opposite manner to related compounds **82** and **145**. This assignment is based on the fact that on the HMBC spectrum for this molecule, the NH proton at 11.92 ppm can be seen to couple with the aromatic carbon at 124.6 ppm, indicating that it is in fact the NH proton adjacent to the aromatic ring (Figure 4.31).

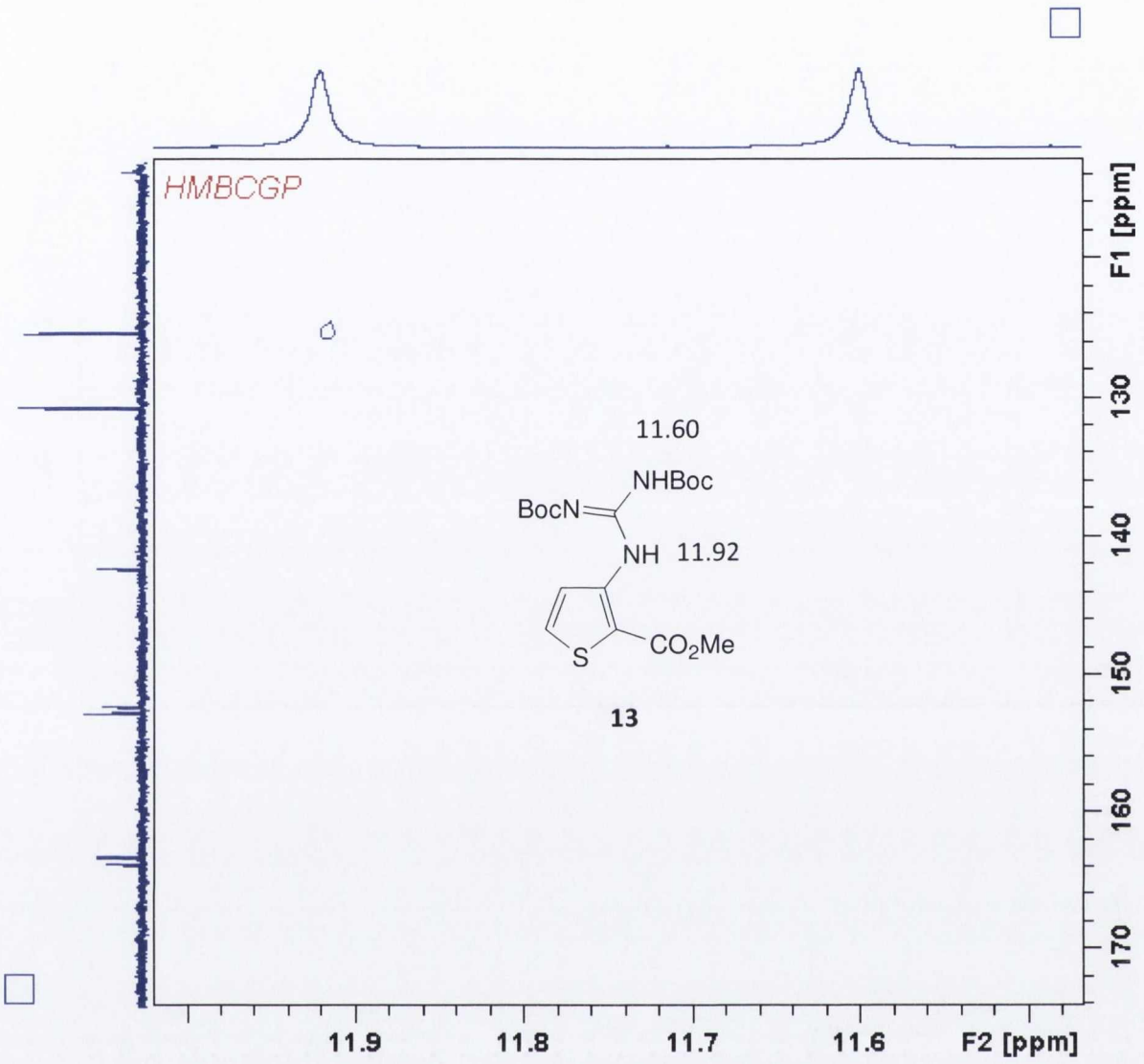


Figure 4.31 – HMBC of **136** showing coupling of the NH proton at 11.92 ppm with the aromatic carbon at 124.6 ppm

For molecules **82** and **145**, however, the NH protons are in the opposite order (Figure 4.32 and Figure 4.33). This appears to be because the NH adjacent to the aromatic ring shifts downfield in **136**, possibly due to the electron withdrawing effect of the ester group deshielding or hydrogen bonding interactions.

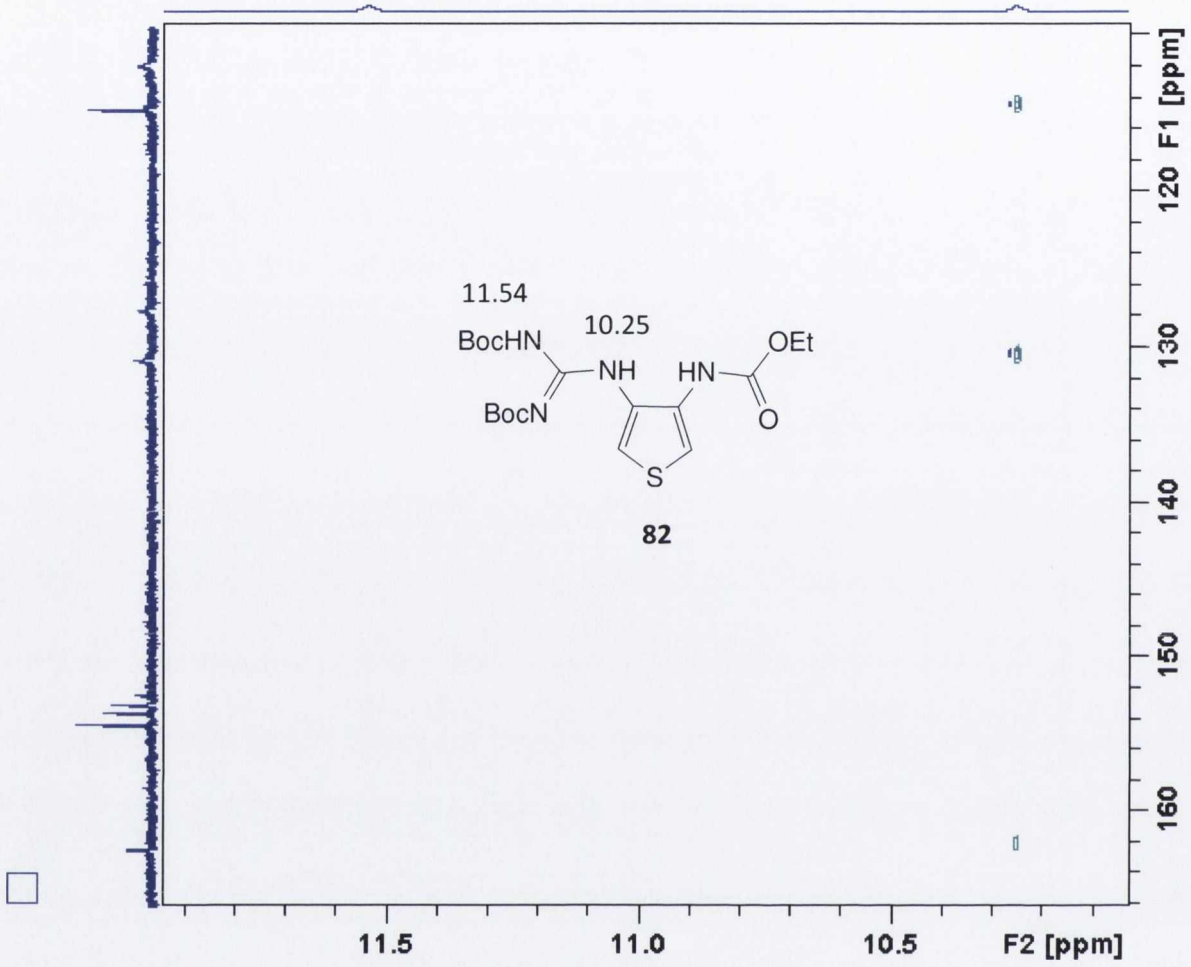


Figure 4.32 – HMBC spectrum of **82** showing coupling of the NH proton at 10.25 ppm with the aromatic carbons at 114.9 and 130.9 ppm. It is also seen to couple to the guanidine carbon at 162.6 ppm

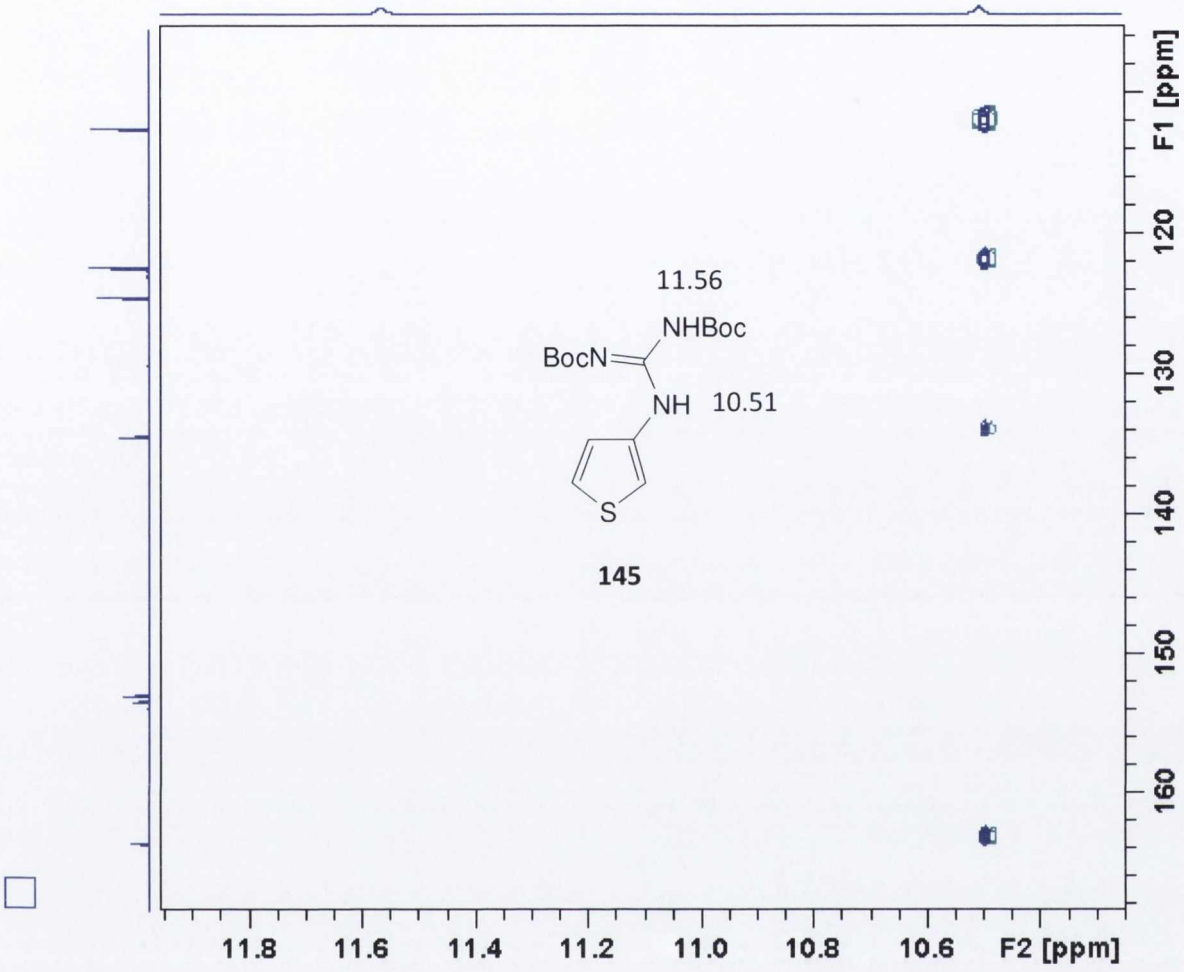
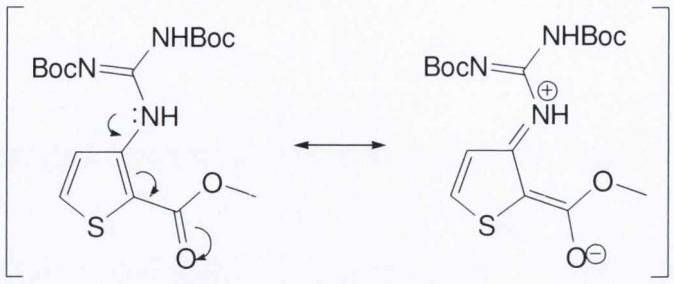


Figure 4.33 – HMBC spectrum of **145** showing coupling of the NH proton at 10.51 ppm with the aromatic carbons at 112.3, 122.2 and 134.2 ppm. It is also seen coupling to the guanidine carbon at 163.4 ppm

Scheme 4.25 shows the resonance structure involved in the deshielding of the nitrogen atom adjacent to the aromatic ring and thus the proton which is bonded to that atom in compound **136**.



Scheme 4.25

In Figure 4.34, we see the modes of hydrogen bonding interaction possible for compound **136**. It is apparent that it is a normal hydrogen bonding interaction occurring between 6 linked atoms. Neither this hydrogen bonding interaction nor the resonance structure depicted in Scheme 4.25 are possible for molecules **82** and **145**, which accounts for the downfield shifted NH signal for the nitrogen bonded to the aromatic ring in **136**.

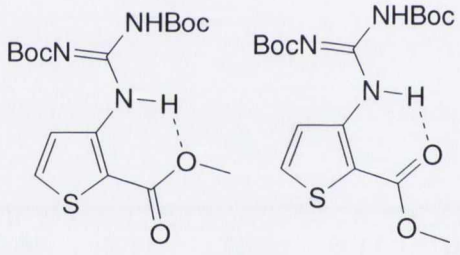


Figure 4.34 – Hydrogen bonding interactions in **136**

4.7 Conclusion

In this chapter, the synthesis of thiophene guanidine hydrochloride salts derived from Gewald and commercial amines was explored.

After some initial difficulties, Gewald products **103**, **106-108**, **112-113** and **120-123** were successfully synthesised. From these compounds di-Boc guanidines **105**, **109-111**, **114** and **125-128** were prepared. Hydrochloride salts of these derivatives were successfully made using either 1:1 TFA/DCM and Amberlite resin or 4M HCl/dioxane, with the latter method replacing the former due to the presence of contamination from the Amberlite resin. The salts prepared from the Gewald derivatives are **115-119**, **129-131** and **133-134**.

Di-Boc-protected molecules, **136**-**139**, derived from commercial thiophene amines and their guanidine hydrochloride salts **140**-**143** were synthesised.

Successful acid decarboxylation of methyl-3-aminothiophene-2-carboxylate and **106**-**108** and subsequent guanidylation led to **145** and **148**-**150**. These were further derivatised to hydrochloride salts **147** and **152**-**153**. Direct guanidylation of the oxalate salt of 3-aminothiophene led to **146,** a dimer of **145**.

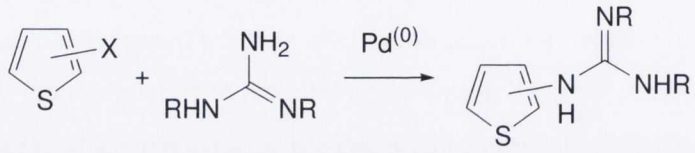
Overall a number of thiophene guanidine hydrochloride salts have been successfully synthesied.

5 Alternative Guanidylation Methods for Heterocycles

5.1 Exploratory Use of the Buchwald-Hartwig Reaction

5.1.1 Premise and History of the Buchwald-Hartwig Reaction

Given the difficulty in working with thiophene amines and therefore the few amidine-like thiophenes synthesised thus far, it was decided to try alternative strategies to synthesise these compounds. In particular, the transition metal catalysed coupling of both un-protected and protected guanidine derivatives to halothiophenes was attempted (Scheme 5.1).



X = Cl, Br, R = H, Boc

Scheme 5.1

The Buchwald-Hartwig reaction is a palladium catalysed cross coupling reaction involving an aromatic halide or pseudo halide and an amine, resulting in the formation of an aromatic amine. The reaction was first published in 1995 by the laboratories of both Stephen L. Buchwald and John F. Hartwig on a variety of simple aryl halides and alkyl amines using tri-*ortho*-tolylphosphine.[168,169] Subsequent work by Buchwald focussed on Palladium complexes with 2,2'-*bis*(diphenylphosphino)-1,1'-binaphthyl[170] (BINAP, Figure 5.1) and Hartwig focussed on organometallic ligands such as 1,1'-*bis*(diphenylphosphino)ferrocene[171] (Figure 5.1).

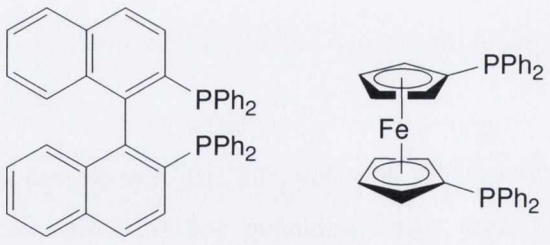
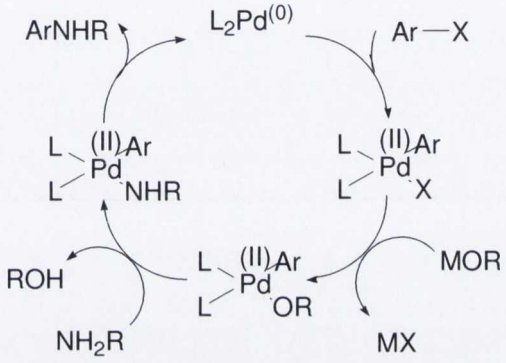


Figure 5.1 – Structures of BINAP and 1,1'-*bis*(diphenylphosphino)ferrocene

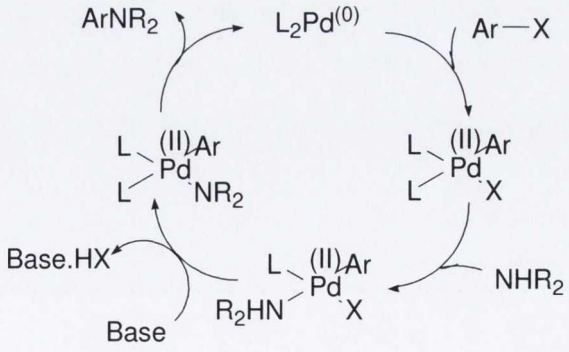
5.1.2 Mechanism of the Buchwald-Hartwig Reaction

The mechanism of the Buchwald-Hartwig reaction is still not fully understood - and a number of possible mechanisms are reported. However, it is thought that the catalytic cycle begins with a Pd(0) complex, which in the case of the bidentate ligands discussed in this chapter is believed to be the $L_1Pd(0)$ species.[172] The mechanism begins as follows: the aryl halide or pseudo halide oxidatively adds to the Pd(0)-ligand complex (Scheme 5.2). The halide (X) can then be replaced by base such as a metal alkoxide (MOR), eliminating a salt (MX). Addition of the amine then results in the formation of alcohol, and reductive elimination results in the aryl amine.



Scheme 5.2

According to the hypothesis of Buchwald,[173] however, for dialkylbiaryl phosphane ligands, amine binding takes place directly after oxidative addition (Scheme 5.3). Deprotonation by a base such as a carbonate also results in the removal of the halide from the Pd(II) centre and this is followed by reductive elimination.



Scheme 5.3

5.1.3 Experimental Design and Results

Using the methodologies of Jonkers *et al.*[174] we tested both guanidine hydrochloride (Gua HCl) and di-*tert*-butoxycarbonylguanidine (DiBocGua) as the amine in Buchwald-Hartwig cross coupling reactions using 2- or 3- bromo and chlorothiophenes as the aromatic halide. For the base either K_2CO_3, KO^tBu or KO^tBu and NaH were used with BINAP in toluene or 2-(dicyclohexylphosphino)biphenyl (DCHPB, Figure 5.2) in dioxane as ligand. The conditions tried are summarised in Table 5.1.

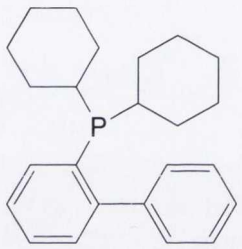


Figure 5.2 – Structure of DCHPB

The initial guanidine source used was DiBocGua as the presence of protecting groups was expected to prevent basic guanidine from permanently binding to the metal centre. However, when initial experiments with DiBocGua proved unsuccessful, with not even trace amounts of the desired product having been formed (MS and ^1H NMR spectroscopy), it was decided to test Gua HCl also but with the addition of an extra equivalent of base. Unfortunately neither source of guanidine gave positive results in initial tests using 2- and 3-bromo and -chlorothiophenes. Since the strong basicity of guanidine was believed to be interfering with the reaction going to completion, it was decided to try using NaH as base along with sodium *tert*-butoxide (NaO^tBu) as the former is a very strong base and is capable of deprotonating the similar substrate thiourea (Section 3.2.2.1). Unfortunately, analysis by both MS and ^1H NMR spectroscopy of the material obtained under these reaction conditions indicated that the reaction failed to yield any of the desired products.

Table 5.1 – Buchwald Hartwig reaction conditions

Reaction	Halothiophene	Base	Solvent	Ligand	Guanidine Source
1	2-Br	K_2CO_3	Toluene	BINAP	DiBocGua
2	3-Br	K_2CO_3	Toluene	BINAP	DiBocGua
3	2-Br	K_2CO_3	Toluene	BINAP	Gua HCl
4	3-Br	K_2CO_3	Toluene	BINAP	Gua HCl
5	2-Br	KOtBu	Toluene	BINAP	Gua HCl
6	3-Br	KOtBu	Toluene	BINAP	Gua HCl
7	2-Br	KOtBu	Toluene	BINAP	DiBocGua
8	3-Br	KOtBu	Toluene	BINAP	DiBocGua
9	2-Br	KOtBu	Dioxane	DCHPB	DiBocGua
10	3-Br	KOtBu	Dioxane	DCHPB	DiBocGua
11	2-Br	KOtBu	Dioxane	DCHPB	Gua HCl
12	3-Br	KOtBu	Dioxane	DCHPB	Gua HCl
13	2-Cl	KOtBu	Toluene	BINAP	DiBocGua
14	3-Cl	KOtBu	Toluene	BINAP	DiBocGua
15	2-Cl	KOtBu	Toluene	BINAP	Gua HCl
16	3-Cl	KOtBu	Toluene	BINAP	Gua HCl
17	2-Cl	KOtBu	Dioxane	DCHPB	DiBocGua
18	3-Cl	KOtBu	Dioxane	DCHPB	DiBocGua
19	2-Cl	KOtBu	Dioxane	DCHPB	Gua HCl
20	3-Cl	KOtBu	Dioxane	DCHPB	Gua HCl
21	2-Br	KOtBu/NaH	Toluene	BINAP	DiBocGua
22	3-Br	KOtBu/NaH	Toluene	BINAP	DiBocGua
23	2-Br	KOtBu/NaH	Toluene	BINAP	Gua HCl
24	3-Br	KOtBu/NaH	Toluene	BINAP	Gua HCl

HRMS was also carried out in ES^- mode, however, this invariably led to peaks which could not be satisfactorily differentiated from the baseline peaks.

5.1.4 Discussion

Looking at the MS results, the most commonly observed ions are either from the starting materials themselves or from oxidised ligands. For example in Reactions 1-4 and 13-16 oxidised BINAP (Figure 5.3) was observed as either a molecular ion peak of $[M + H]^+$ or $[M + Na]^+$ at 655 or 677. Oxidised DCHPB (Figure 5.3) was observed as molecular ion $[M + H]^+$ at 367 in all reactions involving this ligand.

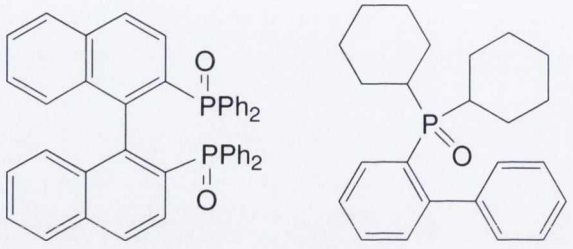


Figure 5.3 – Oxidised ligands BINAP and DCHPB

All ligands were used straight after opening and strict anaerobic conditions were adhered to hence it is likely that the ligands became oxidised in the presence of methanol carrier solvent and air after the reactions had run their course. Di Boc Gua was also often observed at 260 for $[M + H]^+$. However, the identities of the other molecular ions observed could not be satisfactorily determined as Pd complexes of any of the reaction components. Nevertheless, some possibilities exist such as $[M + H]^+$ for palladium complexed to Di Boc Gua at 366 for Reaction 13. Combinations examined included complexes involving ligands such as thiophene, thiophene bromide and chlorides, thiophene guanidines and di-Boc protected guanidines, $^tBuO^-/H$, AcO^-/H, dioxane, toluene, Gua and DiBocGua and guanidinates thereof where relevant; however, due to the large number of combinations, some may not have been examined.

The disappointing results could be explained by analysis of data already in the literature. In particular, a 2001 review of guanidine coordination chemistry by Bailey *et al.* is relevant.[175] When looking at the coordination ability of guanidine, it is of note that, due to it's basicity, guanidine usually exists as a cation, and it has poor Lewis basic ability; however, monoanionic ($(R_2N)_2C=NR^-$) and dianionic guanidinates ($(R_2N)_2C=NR^{2-}$) can bind to metal centres in a number of coordination modes. Typically, guanidines coordinate in the outer sphere of the complex and not to the metal centre.[176]

A palladium complex, which incorporates a guanidine based ligand, has recently been used by Yang *et al.*,[177] under the relatively green conditions of 50% aqueous ethanol, to generate high yields in Suzuki coupling and in the oxidation of alcohols to aldehydes or ketones. The ligand used was 1,1,3,3-tetramethyl-2-(3-trimethoxysilylpropyl)guanidine (Figure 5.4), and the complex, once formed, was grafted onto the solid support SBA-16.

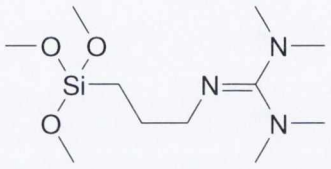


Figure 5.4 – Structure of 1,1,3,3-tetramethyl-2-(3-trimethoxysilylpropyl)guanidine

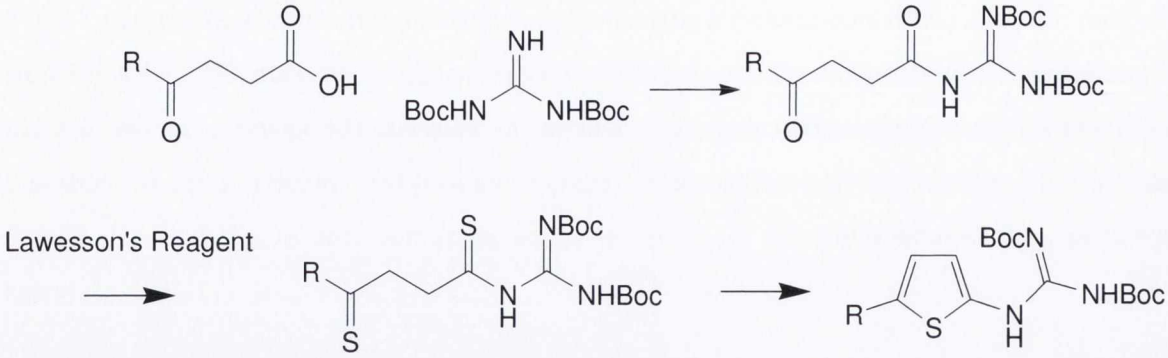
5.1.5 Conclusions

In light of the disappointing results while exploring the possibility of synthesising thiophene guanidines using palladium cross coupling reactions, it was envisaged that perhaps using an alternative protecting group or another ligand would allow the guanidine moiety to coordinate better to the metal centre. However, such studies were deemed to be too time demanding at this stage of the project, so it was decided that the development of conditions under which guanidine could be successfully cross coupled to an aromatic halide would be best carried out as a separate project.

5.2 The Paal-Knorr Reaction

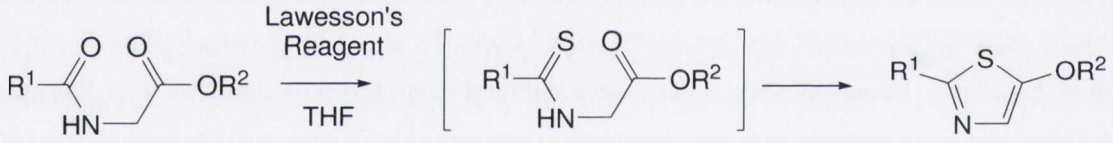
5.2.1 Introduction

Due to the difficulties encountered in attempting to synthesise thiophene amines for subsequent guanidylation, a novel synthesis of thiophene guanidines from acyclic guanidines was devised. Since the conversion of 1,4-dicarbonyl compounds to thiophenes using Lawesson's reagent has been described, and the amide coupling of *mono*-Boc-guanidine to aliphatic acids has been successfully carried out in the Rozas group, it was decided to attempt the synthetic strategy depicted in Scheme 5.4 in order to avoid the formation of the unstable thiophene amine intermediates. It is also of note that modification of the last step of this synthetic pathway could allow for the formation of furan and pyrrole analogues.



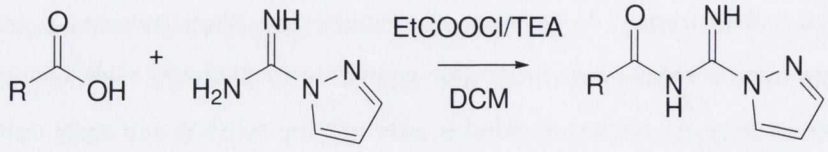
Scheme 5.4

Amides are known to convert to thioamides under Lawesson's Reagent conditions,[178] and in particular, these thioamides can cyclise to form aromatic materials in the manner described by Grubb *et al.* in which a thioamide with an ester in the gamma position is aromatised to a thiazole (Scheme 5.5).[179] It is of note that the ester does not convert to a thioester with Lawesson's Reagent. Similarly Sosnicki and co-workers converted an amide to a thioamide using Lawesson's Reagent.[180]



Scheme 5.5

The formation of amidine type amides has been described in the literature, with ethylchloroformate having been used to couple an amidine to an acid by Oh *et al.* (Scheme 5.6).[181] In a similar fashion aromatic acids were coupled to 2-aminotetrazole by Makino and co-workers.[182]

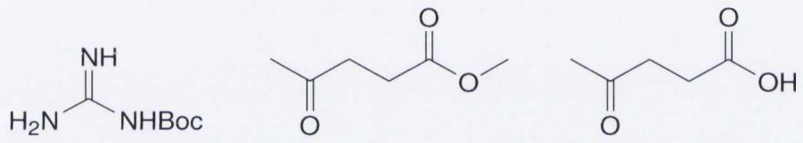


Scheme 5.6

Since methyl levulinate, a cheap and readily available ester of levulinic acid (Figure 5.5) is commercially available, it was chosen as a starting point for the synthetic strategy.

5.2.2 Attempted Guanidylation of Methyl Levulinate

DiBocGua is commercially available; however *mono*-Boc guanidine is not, so the first stage in the reaction sequence was to form this *mono*-Boc derivative. A procedure had already been developed in Rozas' group for this preparation. Thus, 1 eq. of di-*tert*-butyl dicarbonate was reacted with 1.25 eq. of Gua HCl in the presence of 2.5 eq. of NaOH. The reaction was carried out in a mixture of water and dioxane (1:2). This resulted in the isolation of *N*-*tert*-butoxycarbonyl guanidine (**154**, Figure 5.5) in 61% yield after work up. Peaks observed in ^{1}H NMR in DMSO at 6.78 and 1.34 ppm were in good agreement with the literature (Lit.[183] 6.82 and 1.34 ppm) and HRMS was consistent with the structure.



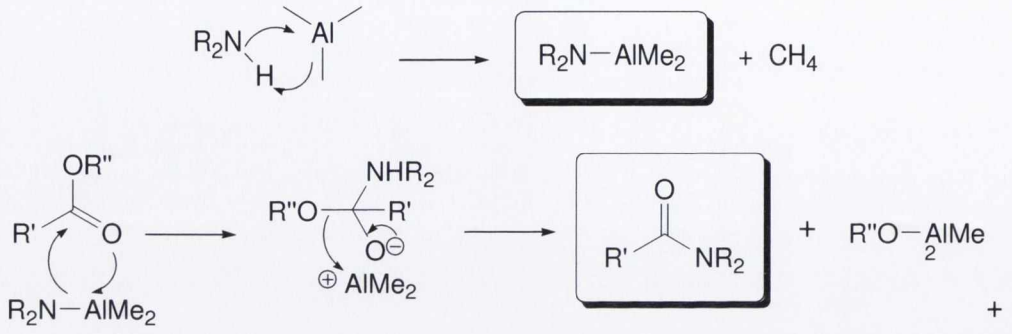
154 **155** **156**

Figure 5.5 – Structures of *N*-*tert*-butoxycarbonyl guanidine, methyl levulinate and levulinic acid

Since the methyl ester of levulinic acid (**155**, Figure 5.5) was commercially available, it was decided to first attempt direct coupling of the ester with **154**. To test if the conversion could happen easily, methyl levulinate was mixed with **154**, and *N*,*N*-diisopropylethylamine (DIPEA) in DCM and left overnight. No reaction was observed under these conditions. The experiment was repeated using NaH under similar conditions, but again no product was formed.

A final method chosen for the amide coupling of a guanidine source and **155** was the use of trimethylaluminium (Al_2Me_6) as it has been documented in the literature.[184-187] Thus, Basha *et al.*[184] report that the active species in the reaction is a dimethylaluminium amide formed when the amide and Al_2Me_6 react. The mechanism, presumed to occur as shown in Scheme 5.7, consists of an abstraction of a proton from the amine by one of the methyl groups adjacent to Al resulting in the

dimethylaluminium amide and methane. Then this intermediate is nucleophilic enough to attack even an unactivated ester such as an alkyl ester forming the amide.



Scheme 5.7

The initial addition of Al_2Me_6 was carried out at 0 ℃, and the reaction was heated at 80 ℃ subsequently and was monitored by LRMS, but after 48 h no product was observed.

5.2.3 Attempted Guanidylation of Levulinic Acid

Next, compound **155** was hydrolysed to levulinic acid (**156**, Figure 5.5) using refluxing KOH. This gave a quantitative yield and the ^{1}H NMR signals in $CDCl_3$, at 2.75, 2.61 and 2.20 ppm, matched reported in the literature (Lit. [188] 273, 259, 217 ppm).

5.2.3.1 Use of BOP Reagent

Next **156** was mixed with DiBocGua, which was readily available in the laboratory at this time, and DIPEA in the presence of benzotriazole-1-yloxy-*tris*-(dimethylamino)phosphonium hexafluorophosphate (BOP) using the conditions of Michel Brunel *et al.*,[189] which had worked for other carboxylic acids in the Rozas group.

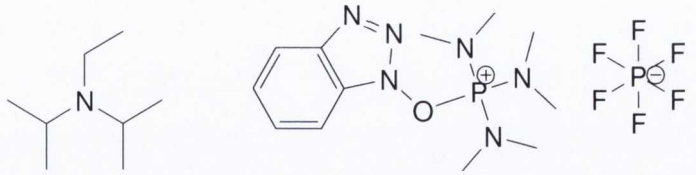
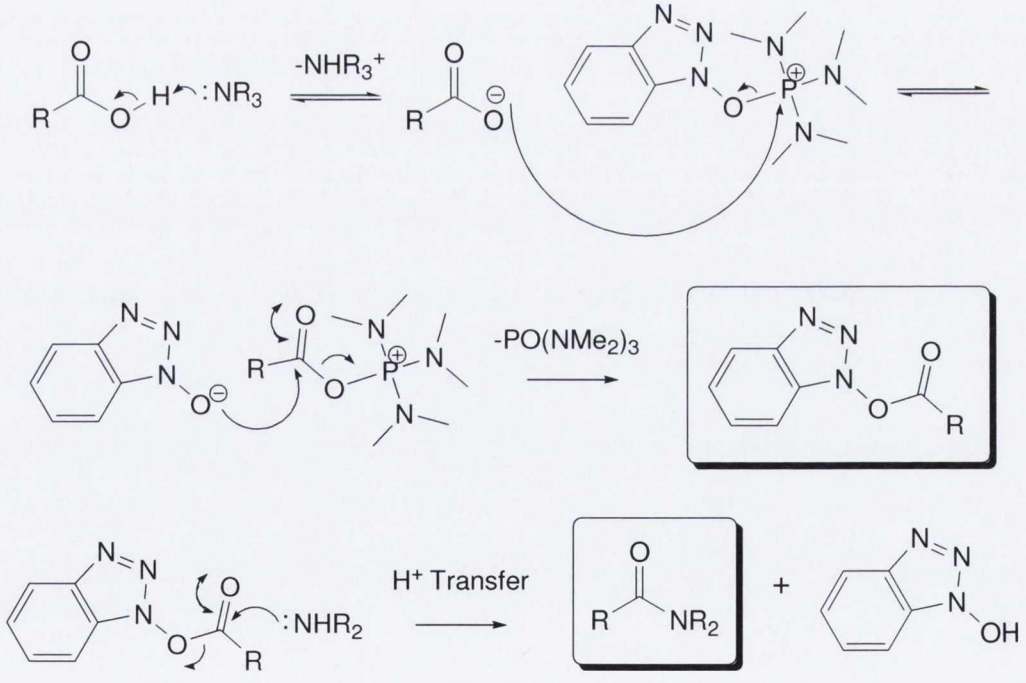


Figure 5.6 – Structures of *N,N*-diisopropylethylamine (left) and benzotriazole-1-yloxy-*tris*-(dimethylamino)phosphonium hexafluorophosphate (right)

The mechanism for the BOP mediated coupling of carboxylic acids with amines is depicted in Scheme 5.8. Firstly, the non nucleophilic base used (in this case DIPEA) deprotonates the acid. The carboxylate anion can then attack the positively charged phosphorous in BOP, due to the strong affinity of oxygen and phosphorous. This occurs concomitantly with the cleavage of the O-P bond in BOP. The anion can then attack the newly formed ester, with the elimination of hexamethylphosphoramide (HMPA). This forms an activated ester, which can be readily attacked by the relevant amine resulting in an amide and benzotriazol-1-ol.



Scheme 5.8

Unfortunately, these conditions did not afford the desired product. A similar reaction was attempted using **154** instead of DiBocGua, which initially gave some promising ^{1}H NMR spectroscopic results and HRMS data. However, multiple attempts to purify or isolate any desired product by column chromatography failed. It was hoped that by reacting the crude material with Lawesson's Reagent (Figure 5.7), perhaps some of the desired thiophene (Scheme 5.4) could be isolated.

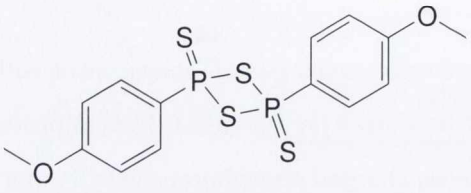
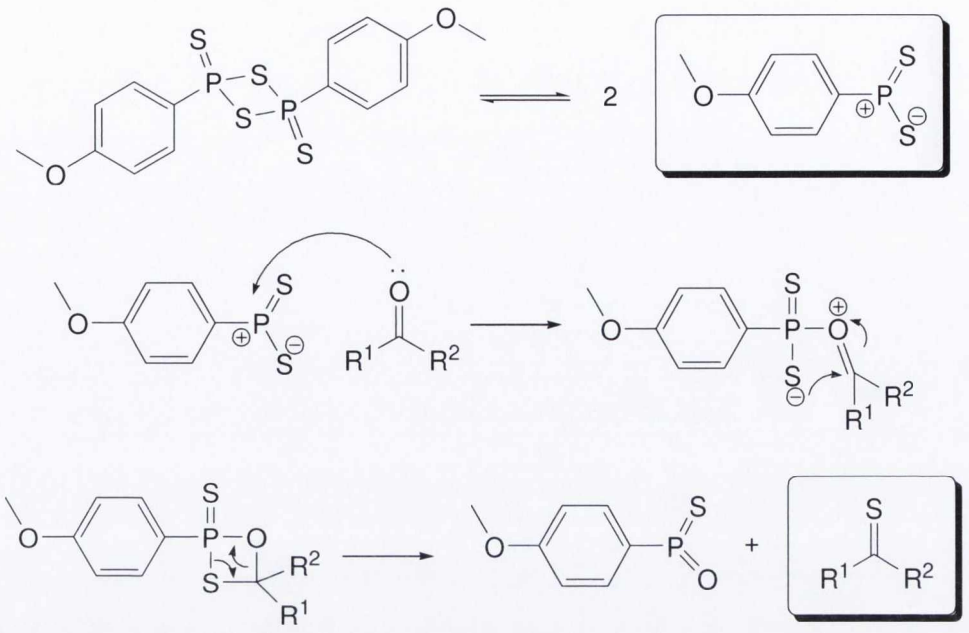


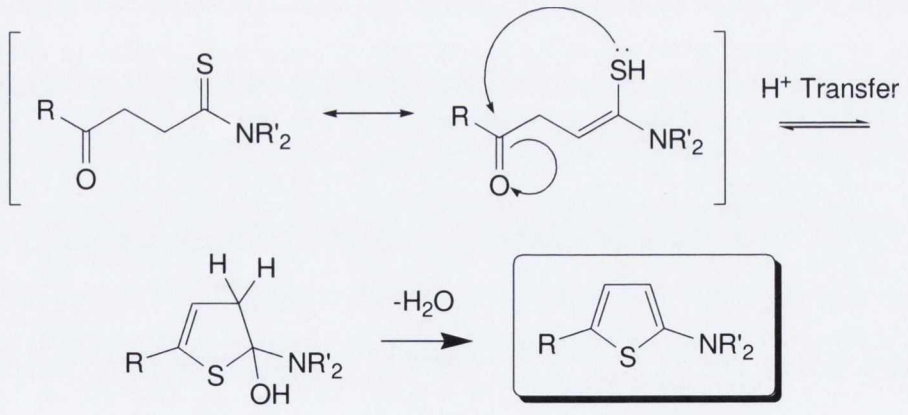
Figure 5.7 – Structure of Lawesson's Reagent

The mechanism by which Lawesson's Reagent works is similar to the Wittig reaction in that a four-membered heterocycle is broken in a cycloreversion step due to the formation of a thermodynamically strong P=O bond. The mechanism proposed (Scheme 5.9) involves the initial dissociation of dimeric Lawesson's reagent into two zwitterionic species containing a phosphonium ion. Attack by the oxygen of the carbonyl group onto the phosphonium ion, positions the carbonyl carbon close to the negatively charged sulfur slowing the latter to attack the former. This sets up the heterocycle, which subsequently breaks forming the thiocarbonyl compound.



Scheme 5.9

Next, is the Paal-Knorr stage of the reaction (Scheme 5.10). The thiocarbonyl compound exists in a tautomeric equilibrium, and once formed, the sulfur atom of the thiol can attack the other carbonyl (or thiocarbonyl) to form a dihydrothiophene after proton transfer. Elimination of H_2O (or SH_2) leads to the requisite thiophene.



Scheme 5.10

Due to the difficulties encountered in isolating any product from the reaction of *N-tert*-butoxycarbonyl guanidine (**154**) and levulinic acid (**156**) using BOP reagent, it was decided to attempt the next reaction of the sequence on the crude material in the hopes that any desired product would be more easily isolated from the reaction mixture. The conditions of Yin *et al.*,[190] involving heating Lawesson's reagent, were employed on the crude material. Yet again none of the desired product or any identifiable intermediate was observed by ^{1}H NMR or HRMS.

Yang *et al.*[191] reported an amide coupling reaction involving the use of silica gel, so the procedure was carried out on **154** and **156**. Also different variations were tried involving the addition of DIPEA, and DIPEA and BOP to the silica gel reaction pot. All of these attempts to form the amide failed.

A reaction using DIPEA and BOP in DMF was also carried out, and a new spot was observed by TLC; however, attempts to isolate this new material from the DMF resulted in failure, probably due to the high polarity of this product.

5.2.3.2 Use of Other Reagents

Compound **156** was successfully coupled to an aromatic amine by Beemelmanns *et al.* using thionyl chloride ($SOCl_2$),[192,193] hence this coupling attempted next. The procedure involves initially reacting **156** with excess $SOCl_2$, to form the acid chloride, which after removal of the excess $SOCl_2$, is treated with the desired amine.

In the case of **154** and **156**, no product was observed. The use of Vilsmeier-Hack conditions, in which DMF catalyses the reaction, were not attempted since DMF was perceived to be too polar to remove from the product.

In a final attempt to form the desired amide, ethylchloroformate was used in a similar two-pot manner to $SOCl_2$, using the method of Oh and co-workers.[181] The mechanism involves the formation of a carboxylic anhydride which can be attacked by an amine to form the desired amide. Again the results were disappointing with only hydrolysed ethylchloroformate observed.

5.3 Conclusion

Implementation of the Buchwald-Hartwig reaction using a variety of conditions was attempted in order to circumvent the need to use unstable thiophene amines. In particular, 2- and 3-

chlorothiophene and 2- and 3-bromothiophene were used as the aromatic halides, DiBocGua and Gua HCl were chosen as guanidine sources, bases K_2CO_3, KO^tBu and NaH were used along with ligands BINAP (in toluene) and DCHPB (in dioxane). Disappointingly, none of the conditions employed afforded any of the desired thiophene guanidines.

Next, a synthetic strategy based on the Paal-Knorr thiophene reaction was devised, a method which could be altered to yield furans and pyrroles additionally. The first step in the reaction sequence was to couple an acid (or and ester) to a guanidine source. One guanidine source, DiBocGua was already at hand but *mono*-Boc guanidine (**154**) was successfully synthesised using an in-house procedure.

Firstly, attempts were made to couple the methyl ester of levulinic acid (**155**) to **154** using the base DIPEA, NaH and Al_2Me_6. However, despite best attempts, the coupling reaction did not yield the desired product.

Secondly, coupling using levulinic acid (**156**) was attempted. This required first hydrolysing the ester of **155**, which was carried out successfully. BOP was the first coupling reagent tested for the coupling of DiBocGua with **156**, which gave a hit by HRMS, but multiple attempts to isolate the required amide failed. Thus, the crude material was tried in the next stage of the reaction sequence, the Paal-Knoor reaction; however, these conditions did not afford the requisite product.

The next candidates for coupling reagents chosen were silica gel and silica gel plus BOP, both of which did not yield the amide product. A reaction using BOP and DIPEA in DMF, gave a new spot by TLC; however, this spot was highly polar and this product could not be isolated from the solvent.

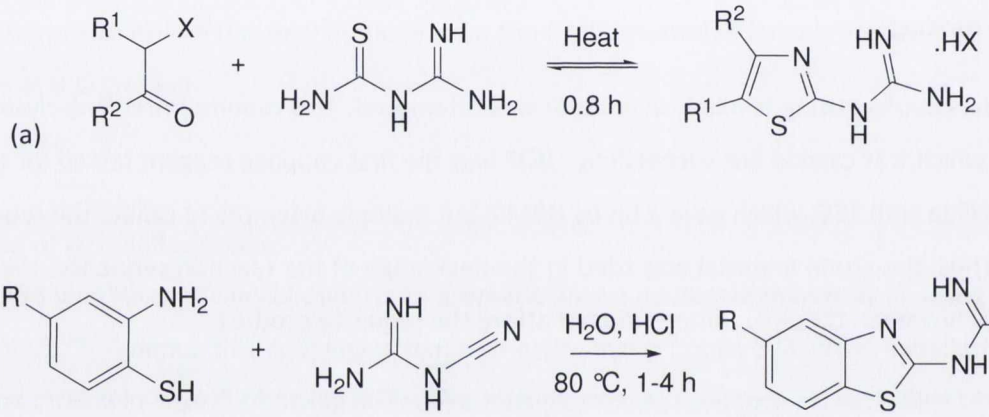
The final conditions tried involved 2-step coupling reaction, namely use of $SOCl_2$ or ethylchloroformate. The intermediates formed are the acid chloride or the ethyl anhydride of **156** respectively. Unfortunately, neither reagent afforded the requisite amide.

Due to time constraints, and the fact that the decarboxylation and subsequent guanidylation of Gewald thiophenes had resulted in the desired products, the Paal-Knorr based reaction sequence was not taken any further in this work.

6 Guanidine-like Thiazoles

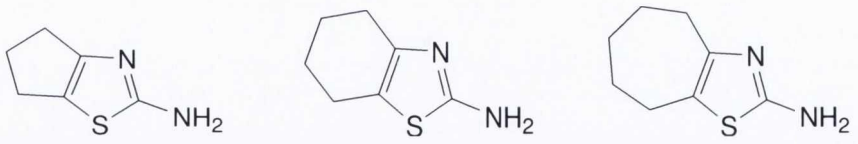
6.1 Premise

2-Aminothiazoles are stable, unlike their thiophene counterparts, and it is because of this fact that it was decided to synthesise 2-aminothiazole analogues of the lead molecules discussed in Section 1.9. Synthetic routes for the formation of thiazole and benzothiazole guanidines have been previously described, with thiazole guanidines having been prepared from α-haloketones and 2-imino-4-thiobiuret via the method of Beyer (Scheme 6.1 (a)),[116] and benzothiazole guanidines were prepared from 2-aminothiophenols and cyanoguanidine via the method of Dolzhenko (Scheme 6.1 (b)).[117]



Scheme 6.1

Since few of the desired α-chloroketones and 2-aminothiophenols are commercially available, it was decided to attempt guanidylation of 2-aminothiazoles using the Kim and Qian method.[112] However since cycloalkylthiazol-2-ylamines such as cyclopenta-fused thiazolylguanidine **157**, cyclohexa-fused thiazolylguanidine **158** and cyclohexa-fused thiazolylguanidine **159** (All Figure 6.1) are not commericially available it was decided to synthesise these by the method of Beyer.[116]



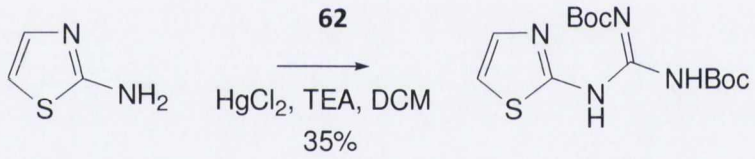
157 **158** **159**

Figure 6.1 – Structures of 5,6-dihydro-4*H*-cyclopentathiazol-2-ylamine (**157**), 4,5,6,7-tetrahydro-benzothiazol-2-ylamine (**158**) and 5,6,7,8-tetrahydro-4*H*-cycloheptathiazol-2-ylamine (**159**)

6.2 Thiazole Guanidines from Aminothiazoles

6.2.1 Application of the Kim and Qian Method

The Kim and Qian procedure[112] using *S*-methylthiopseudourea was applied to a number of commercially available 2-aminothiazoles and worked to some satisfaction on 2-aminothiazole itself giving a yield of 35% of thiazolylguanidine **160** (Scheme 6.2).

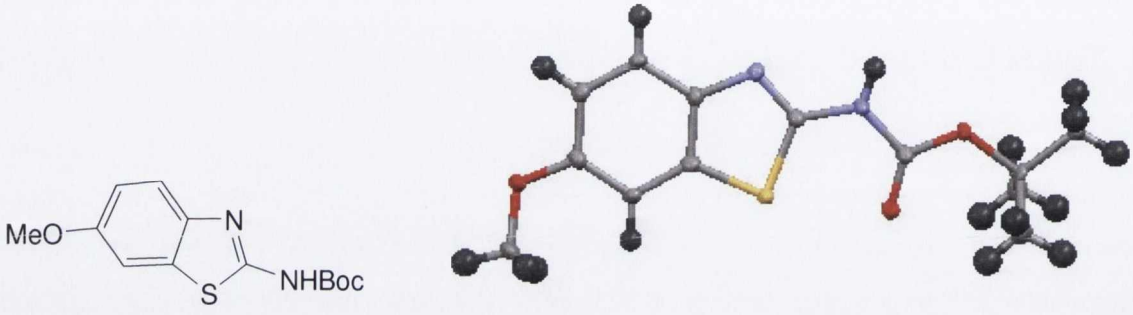


160

Scheme 6.2

However, attempts to guanidylate 4-methyl-2-aminothiazole, 5-methyl-2-aminothiazole and 6-methoxy-2-aminobenzothiazole using the Kim and Qian procedure merely resulted in the Boc protection of the amines as the main products along with a side product. In particular, *tert*-butyl *N*-(5-methyl-thiazol-2-yl)carbamate (**161**, Figure 6.2) and *tert*-butyl *N*-(6-methoxy-2-thiazol-2-yl)carbamate (**162**, Figure 6.2) were synthesised and isolated, with ^{1}H and ^{13}C NMR, MS and X-ray crystallographic data confirming their formation.

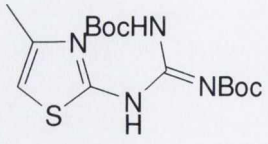
161



162

Figure 6.2 – Crystal structures of *tert*-butyl *N*-(5-methyl-thiazol-2-yl)carbamate (**161**) and *tert*-butyl *N*-(6-methoxy-2-thiazol-2-yl)carbamate (**162**)

In the guanidylation reaction of 4-methyl-2-aminothiazole, the presence of traces of [2,3-di(*tert*-butoxycarbonyl)]-1-(4-methylthiazol-2-yl)guanidine (**163**, Figure 6.3) was observed by ^{1}H NMR spectroscopy and MS. However, it could either not be cleanly isolated, or exists as a number of isomers. In particular, it is postulated that the increased basicity of the ring nitrogen, in comparison to the 2-aminothiazole analogue **160**, results in the intramolecular proton exchange depicted in Scheme 6.3



163

Figure 6.3 – Structure of [2,3-di(*tert*-butoxycarbonyl)]-1-(4-methylthiazol-2-yl)guanidine

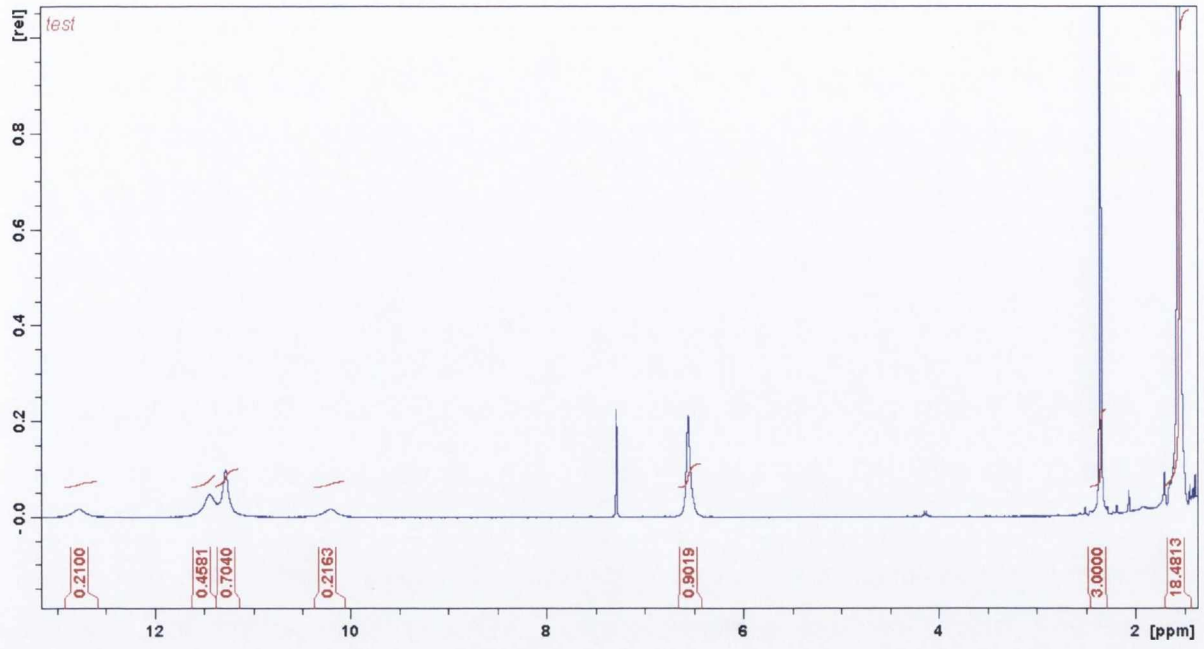
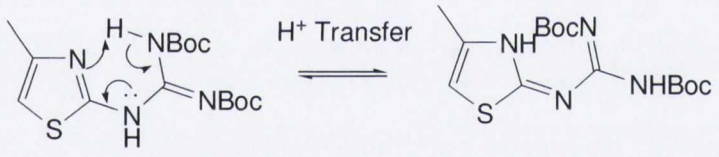


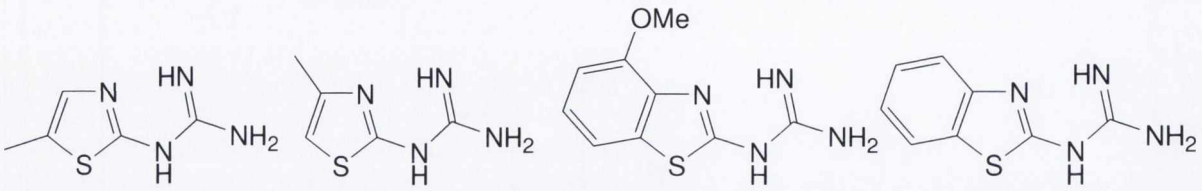
Figure 6.4 – ^{1}H NMR spectrum of **163** showing four separate NH signals at 11.76, 11.44, 11.28 and 10.20 ppm.



Scheme 6.3

Then, we set out to find conditions under which guanidylation of the 2-aminothiazoles was possible, trying various guanidine precursors such as **62**, 1,3-*bis*(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, thiourea and *S*-methylisothiourea hemisulfate were used with solvents such as DCM, methanol and DMF. Initial results using $HgCl_2$ with thiourea or *S*-methylisothiourea hemisulfate in refluxing methanol were promising with hits via HRMS at *m*/z 157 having been observed for 1-(5-methylthiazol-2-yl)guanidine (**164**, Figure 6.5) as well as plausible ^{1}H NMR data having been obtained from the crude material. In particular, the HRMS peak for **157** was substantially stronger than the starting material signal being at least 9 times more intense. Although this is not confirmation that **164** was indeed the major product of the reaction, it was considered a positive result.

On foot of this result, preparation and purification of 1-(4-methylthiazol-2-yl)guanidine (**165**, Figure 6.5), 1-(4-methoxylbenzothiazol-2-yl)guanidine (**166**, Figure 6.5) and 1-(benzothiazol-2-yl)guanidine (**167**, Figure 6.5) was attempted.



164 **165** **166** **167**

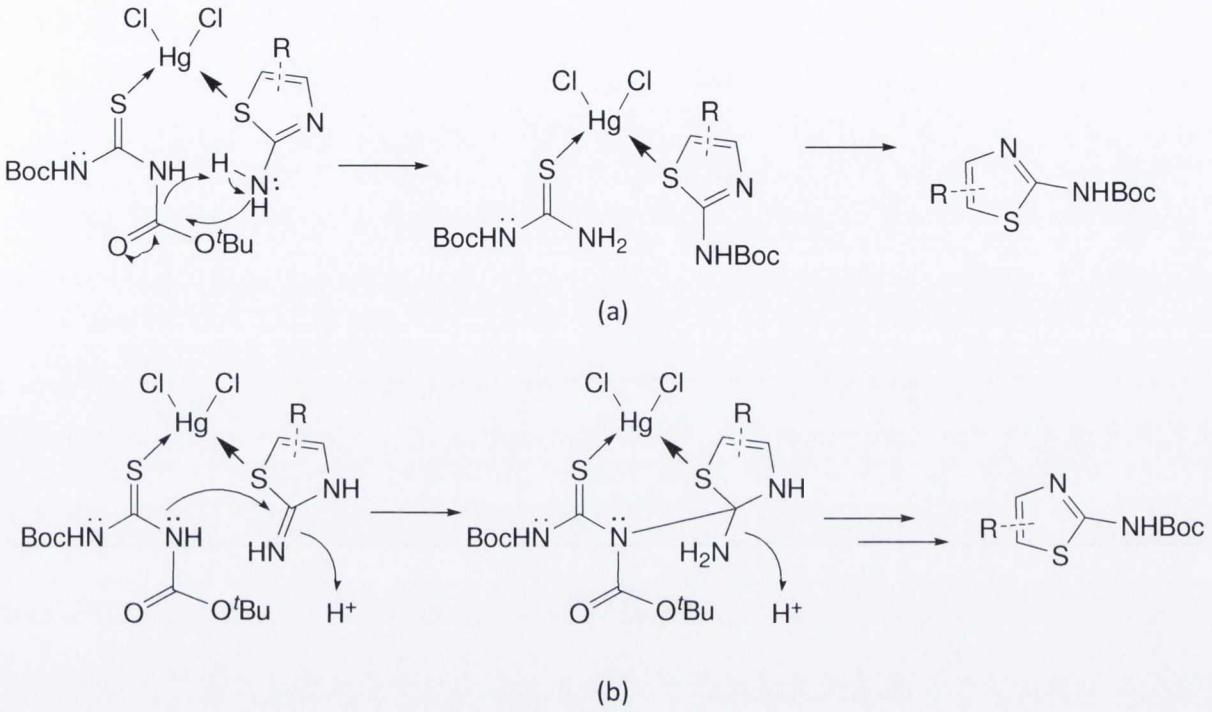
Figure 6.5 – Desired thiazole and benzothiazole guanidines

However, purification proved difficult as the crude materials were sticky and highly amorphous and in addition to this the HRMS intensities of **165-167** compared with their respective starting material were all lower than in the previous example, with **166** in particular displaying the opposite relative intensities than **164** (i.e. smaller product peak compared to the starting material peak). This then brought up the question as to whether the desired guanidines had indeed been formed as the major components of the coupling reactions. Therefore each sample and its parent amine were analysed separately by ^{1}H NMR in DMSO-δ_6 in order to see the exchangeable NH protons more clearly. Disappointingly, all four materials gave the exact same spectra to their parent amine counterparts, in addition to one extra peak at 162.8 ppm, which though not thiourea is perhaps a related simple hydrocarbon. Further to this, the crude melting points were all lower than those of the relevant amine, with the melting point of the crude material, which was proposed to be **167**, at 90 – 105 °C being substantially lower than the literature melting point of 172 – 173 °C.[117]

6.2.2 Use of Other Guanidylating Agents

Due to the problems encountered when using $HgCl_2$ as a coupling reagent it was postulated that perhaps the thiazole nucleus, being less aromatic than the thiophene (for which no side products are observed) can coordinate somewhat to the mercury centre, thus forming a complex such as that depicted in Scheme 6.4 (a), which aligns the amine of the thiazole with a carbamate group allowing perhaps for the exchange of the carbamate group or perhaps in a scenario in which the carbamate

nitrogen can act as nucleophile (Scheme 6.4 (b)) and the aminothiazole the electrophile due to the basicity of the ring nitrogen.



Scheme 6.4

To test this theory, it was decided to attempt the guanidylation of 5-aminothiazole using both Mukaiyama's reagent and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (Figure 6.6)

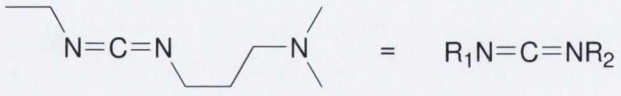


Figure 6.6 – Structure of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

When EDCI was used on 5-methyl-2-aminothiazole, the *mono*-Boc protected thiazole **161**, was almost the only component decected upon spectral examination; however, when Mukaiyama's reagent was used, the main component was the di-Boc guanidine **168** (Scheme 6.5), which formed in 67% yield. However, when this procedure was carried out on 4-methyl-2-aminothiazole, **163** was only observed

in a yield of 6%, with a yields for **160** and thiazolylguanidine **169** (Scheme 6.5) under the same conditions at 52% and 20% respectively.

160, $R^1 = R^2 = H$ (52%)

163, $R^1 = Me$, $R^2 = H$ (6%)

168, $R^1 = H$, $R^2 = Me$ (67%)

169, $R^1 = R^2 = Me$ (20%)

Scheme 6.5

It is of note that there seems to be a pattern in relation to the quantities of desired product versus other side-products. A methyl group in the 5-position improves the quantity of desired product obtained, whereas one in the 4-position dramatically reduces the quantity of the desired product, resulting in more by-products. When a methyl group is present in both positions, the detrimental effect of the methyl group in the 4-position appears to be stronger than the enhancing effect of the methyl group in the 5-position, with the yield being closer to that of the desired product when the methyl group is in the 4-position (**163**).

When Mukaiyama's Reagent was used, the same products were isolated at the end of the reaction, as occurred when $HgCl_2$ was used. This time, however, the final type of product, which has a very similar ^1H NMR spectrum to the *mono*-Boc thiazole family and a ^{13}C spectrum with one extra carbon, was determined to be a [3-(*tert*-butoxycarbonyl)]-1-(thiazol-2-yl)thiourea (Figure 6.7) based on these NMR spectra and HRMS.

No reaction occurred when Mukaiyama's Reagent was used with *S*-methylthiopseudourea as guanidine source. In addition, experiments carried out with no coupling reagent also failed to give any products.

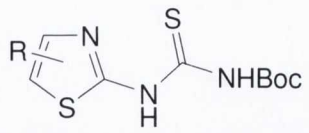


Figure 6.7 – General structure of a [3-(*tert*-butoxycarbonyl)]-1-(thiazol-2-yl)thiourea

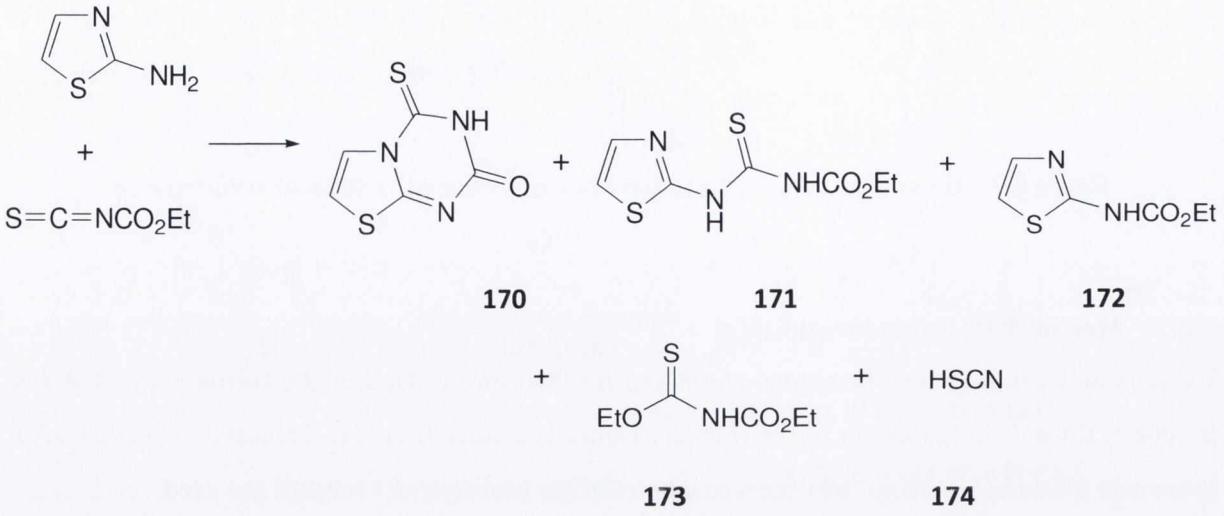
6.2.3 Mechanistic Considerations

These results propose new questions about the mechanism involved in the formation of the side products, since if complexation to the mercury centre is involved, as was discussed in Section 6.2.2, these side products would not be observed when EDCI or Mukaiyama's reagent are used.

In order to propose a mechanism, it is important to look at the situations in which these side products were observed. The fact that *S*-methylthiopseudourea can lead to side products when $HgCl_2$ is used as coupling reagent, but not when Mukaiyama's reagent is used leads to the conclusion that perhaps the methyl group on the S atom blocks the pathway for this reaction with Mukaiyama's Reagent but not with $HgCl_2$.

The desired *N*,*N*-di-Boc guanidines must certainly form via the normal Kim and Qian and Mukaiyama's Reagent pathways. However, when considering the other products, another question which must be considered is whether the *mono*-Boc thiazole or the [3-(*tert*-butoxycarbonyl)]-1-(thiazol-2-yl)thiourea forms first or indeed whether they are competing reactions.

A literature survey on the basicity of 4-aminothiazole resulted in an interesting paper on this topic. Nagano *et al.*[194] published an article in 1972, on the reactivity of 2-aminothiazoles with ethoxycarbonyl isothiocyanate, in which [3-(tert-butoxycarbonyl)]-1-(thiazol-2-yl)thioureas (**171**, Scheme 6.6) were observed as a side product along with mono-Boc thioureas (**172**, Scheme 6.6), thiazolo[3,2-*a*]-*s*-triazine-4-thio-2-ones (**170**, Scheme 6.6), alkyl-N-alkoxycarbonylthiocarbamates (**173**, Scheme 6.6) and thiocyanic acid (**174**, Scheme 6.6).

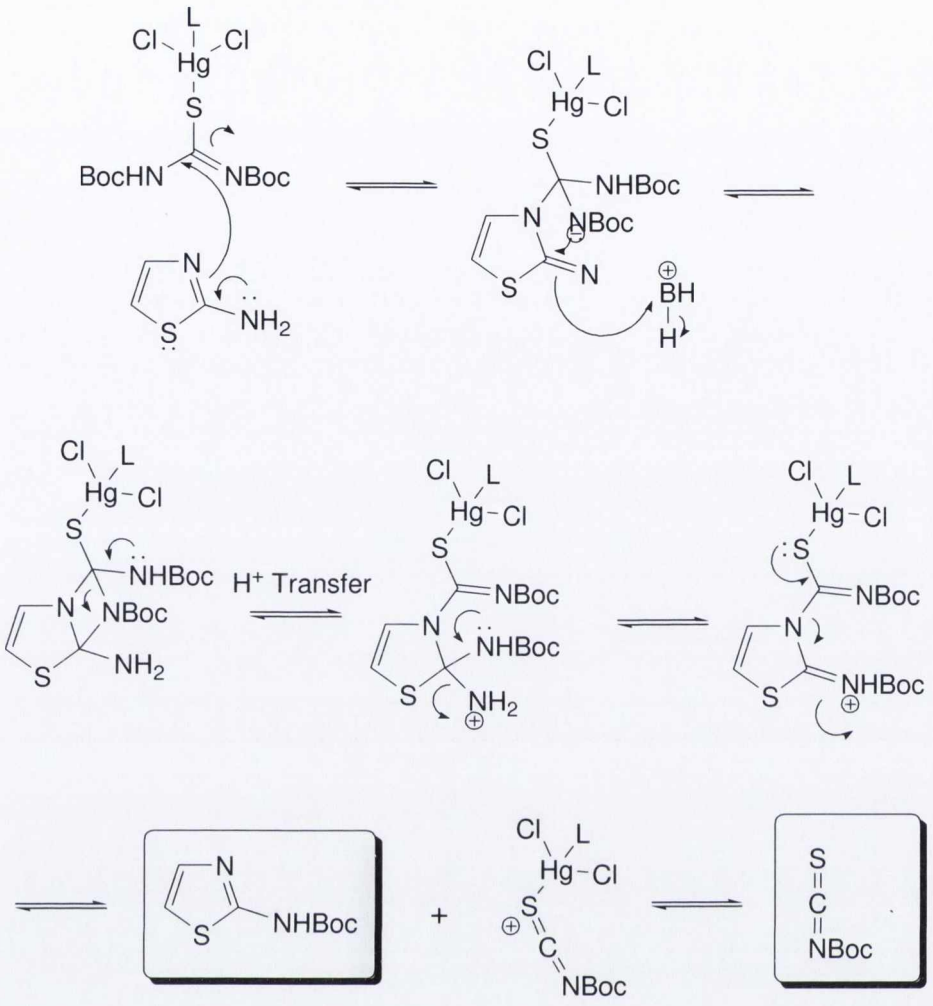


Scheme 6.6

While compounds of types **170**, **173** and **174** were not observed under the conditions used in this work, it is still possible that they could have been formed. The number of products noted was based on the number of spots observed by TLC and the products were isolated by column chromatography. Since compounds of type **173** and **174** have no chromophores, they would not be expected to show up as products using TLC with UV detection. Further to this, materials of type **170** would be highly polar, and thus may not have been distinguishable from the starting material spot under the highly non-polar TLC conditions (3:1 Hexane:EtOAc). Since the starting material was not collected after the reaction, it is possible that the compound A could have been present. Therefore, the part of Nagano's work which has most relevance to this work is the isolation of compounds of type **171** and **172**.

Nagano observed, in all cases, that **171** was the product found in the highest yield, with **170** occurring as the next largest component when the thiazole is unsubstituted or has a simple alkyl moiety in the 5 position, but not at all in other cases. Compounds of type **172** were isolated in quantities of less than 10% in all cases, which is in stark contrast with the results observed by us in which these materials were often those present in the greatest quantities. This suggests that perhaps the *mono*-Boc protected thiazoles, **172**, form first under the experimental conditions reported in this text, and that perhaps *tert*-butoxycarbonyl isothiocyanate is formed as a side product and then can react forming all other side products found.

Based on these observations a mechanism for the $HgCl_2$ catalysed formation of mono-Boc thiazoles is proposed in Scheme 6.7.



Scheme 6.7

Since the reaction proceeds to a greater extent for aminothiazoles with higher bascitity, it seems likely that an initial complex involving the basic ring nitrogen bound to a thiourea-mercury intermediate is formed. The tetrahedral intermediate created is probably well positioned for an anionic carbamate nitrogen to attack the imine group of the pseudothiazole. This would generate a highly strained four membered ring, which can be broken easily by attack from the other carbamate nitrogen, with hydrogen transfer leading to a tetrahedral intermediate, which can either result in the elimination of ammonia gas or a free carbamate group to give either the *mono*-Boc thiazole or the thiazole starting material. The thiazole can then reform aromaticity eliminating the isothiocyanate – mercury complex, which can then dissociate due to the presence of two electron deficient, formally positively charged atoms adjacent to each other *i.e.* the sulfur and mercury atoms.

Interesting points in this hypothetical mechanism are that if a negatively charged carbamate intermediate is involved in attack at the imine forming a highly strained four membered ring, the use of a deuterated thiourea analogue would not necessarily lend support to confirm this mechanism. However, this would be an important future experiment to carry out because if the N in the *mono*-Boc protected aminothiazole is deuterated, it would lend support to this mechanism. ^{15}N thiourea is currently prohibitively expensive therefore whether future experiments using it will be carried out remains to be seen.

In an analogous manner, Mukaiyama's reagent and EDCI can also form complexes which can participate in this type of mechanism. Both proposed intermediates are depicted in Figure 6.8 along with the presumed complex for mercury chloride and thiopseudourea.

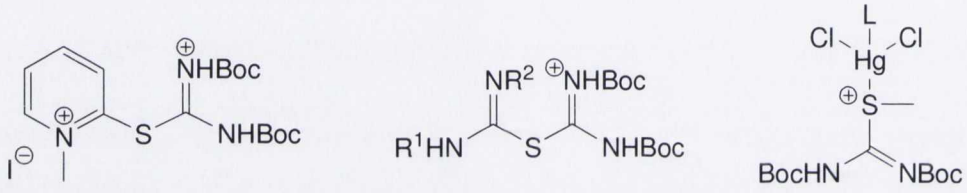
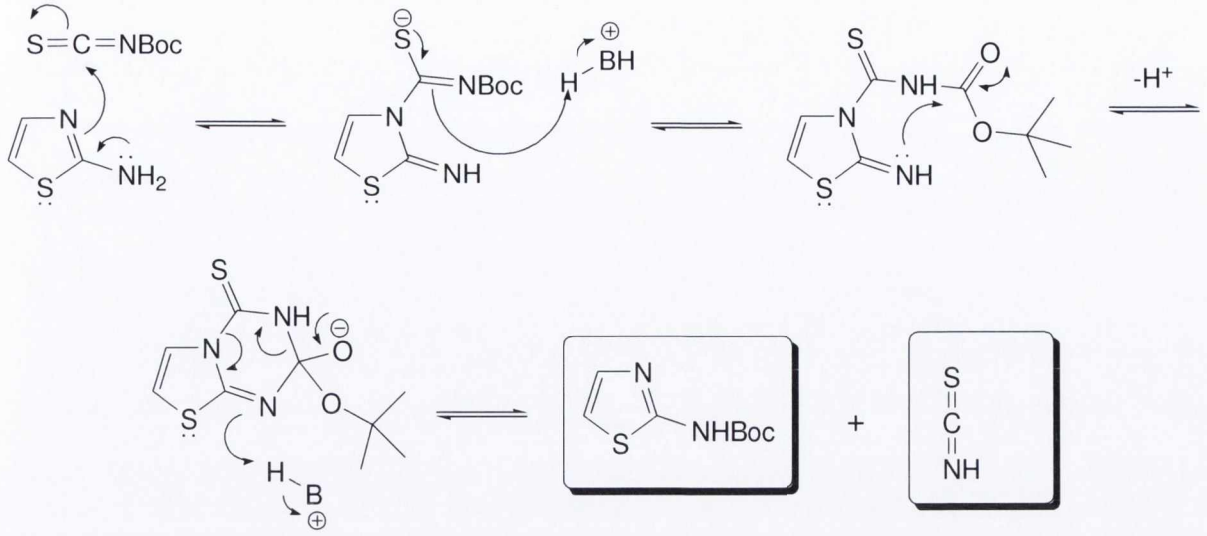


Figure 6.8 – Structures of speculative Mukaiyama's reagent (left) and EDCI (centre) - thiourea conjugate intermediates and mercury chloride – thiopseudourea intermediate

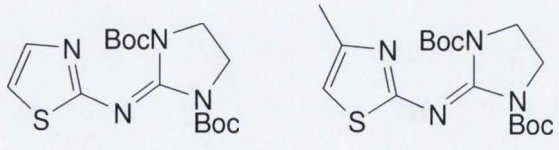
It is then presumed that the isothiocyanate formed can react at the amine forming the thiazole thiourea or could also react with the basic ring nitrogen. The imine formed could then intramolecularly attack the carbamate group resulting in the small portion of mono-Boc protected thiazole found in the presence of isothiocyanates alone. This speculative mechanism is shown in scheme Scheme 6.8.



Scheme 6.8

6.2.4 2-Iminoimidazolidylation of 2-Aminothiazoles

2-Iminoimidazolidylation was attempted using the Dardonville and Rozas method on 2-aminothiazole and 4-methyl-2-aminothiazole and resulted in the synthesis of [1,3-di-*tert*-butoxycarbonyl]-2-(thiazol-2-ylimino)imidazolidine (**175**, Figure 6.9) and [1,3-di-*tert*-butoxycarbonyl]-2-(4-methylthiazol-2-ylimino)imidazolidine (**176**, Figure 6.9). Compound **176** was isolated cleanly in 25% yield after alumina column chromatography followed by recrystallisation, however, **175** could not be isolated cleanly after alumina column chromatography and a number of attempts at recrystallisation. A crystal structure of **176** confirms the structure. Due to the problems encountered in Section 6.2.5, no further 2-iminoimidazolidylation was attempted.



175 **176**

Figure 6.9 – Structures of [1,3-di-*tert*-butoxycarbonyl]-2-(thiazol-2-ylimino)imidazolidine (**175**) and [1,3-di-*tert*-butoxycarbonyl]-2-(4-methylthiazol-2-ylimino)imidazolidine (**176**)

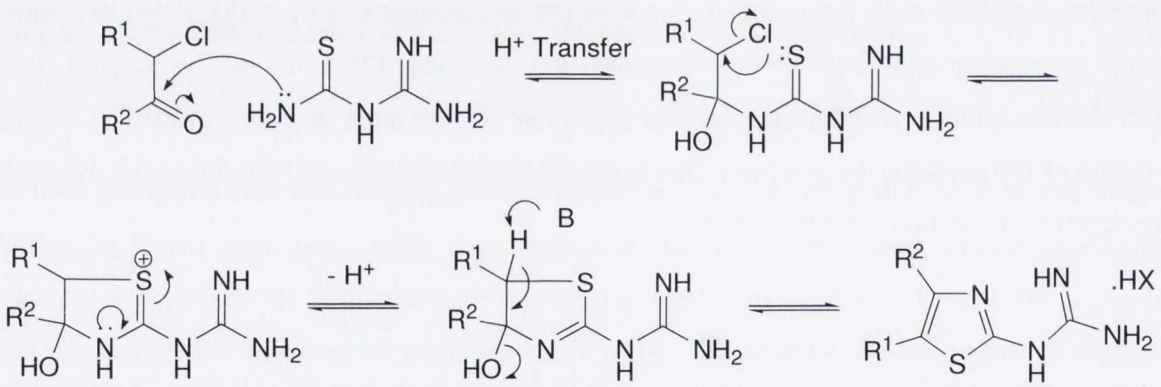
6.2.5 Deprotection of Thiazole Guanidines

Deprotection of **160** using TFA and DCM via the method of Kim and Qian[112] or HCl and dioxane produced a compound with the same ^{1}H NMR spectrum as the salt of the free amine. A ^{13}C NMR spectrum did not confirm the presence of four carbon signals, with only three signals present, therefore eliminating the possibility of guanidine **160** merely having the same ^{1}H NMR spectrum as its parent amine. Due to this disappointing result other methods of producing thiazole guanidines were examined, in particular the method of Beyer and Hantschel.[116]

6.3 Thiazole Guanidines from Acyclic Materials

6.3.1 The Beyer and Hantschel Method

The Beyer and Hantschel method of preparing thiazole guanidines dates from 1962 and has been applied to the synthesis of a number of simple thiazole guanidines. It involves the union of an α-haloketone with 2-imino-4-thiobiuret, as shown in Scheme 6.1 (a). The method is based on the Hantzsch thiazole synthesis and the mechanism proceeds as shown in Scheme 6.9.



Scheme 6.9

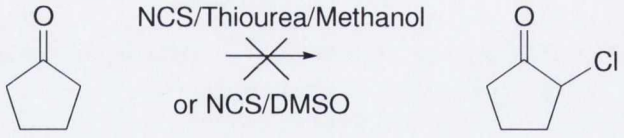
Firstly, the "hard" nitrogen nucleophile of 2-imino-4-thiobiuret attacks the "hard" carbonyl electrophile forming a tetrahedral carbonyl addition intermediate. This places the sulfur atom in an ideal position to attack the carbon adjacent to the chlorine, with chlorine acting as a leaving group. This is based on the classic S_N2 mechanism. The positive charge on the sulfur is quenched when the lone pair on the ring nitrogen feeds in, with resulting loss of a proton. Finally, any base present should

be sufficient to dehydrate the ring as in doing so aromaticity is formed and thus this reaction is highly favourable.

Due to the lack of availability of many commercially available α-haloketones, it was decided to look for a general method for preparing these precursors. In particular, since the hydrochloride salts are desired, α-chloroketones were sought.

6.3.2 α-Chloroketones

Mei *et al.* reported the synthesis of simple α-chloroketones in high yields using *N*-chlorosuccinimide (NCS) in methanol with thiourea acting as catalyst.[195] Since thiourea is both cheap and available in our lab, this method initially seemed promising. However, when carried out on cyclopentanone, ^{1}H NMR spectroscopic results on the crude material were inconclusive so MS was carried out on the isolated products but failed to show the presence of α-chlorocyclopentanone (Scheme 6.10). It was postulated that any α-chloroketone produced in this reaction could subsequently react with the thiourea to form a small quantity of thiazole via the Hantzsch thiazole synthesis. To examine if this was indeed the case, it was decided to use one equivalent of 2-imino-4-thiobiuret in the reaction to see if the thiazole guanidines could be formed directly. However, this failed to provide the thiazole guanidine since the ketones used in the paper were mostly highly activated, with ester groups β to the ketone. Hence, it was decided to look for a more suitable method for the preparation of simple ketones.

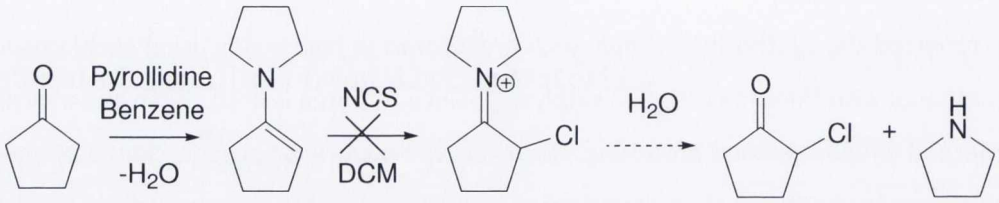


Scheme 6.10

A 2007 paper by Sreedhar *et al.* reported the generation of α-chlorocyclopentanone using simply NCS in DMSO in 87% yield after 15 min.[196] This method was applied to pentanone resulting in only starting material present after this period of time (Scheme 6.10).

Considering that this method failed to yield the desired product, we tried to generate an enolate intermediate. Since the use of enolate intermediates to generate α-chlorocyclopentanone has not

been reported, the method of Stork *et al.*[197] for forming a pyrrolidine enolate, from cyclopentanone, in the presence of benzene was chosen as a starting point as it had been used previously in the Rozas group. Thus formation of the pyrrolidine enolate under Dean-Stark conditions followed by treatment with NCS, which could be subsequently hydrolysed to form the α-chloroketone was attempted (Scheme 6.11). Unfortunately, this method of preparing the alphachloroketone failed to yield any of the desired product.



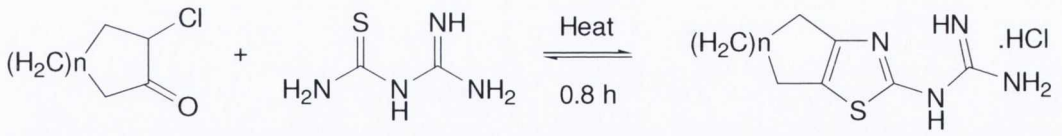
Scheme 6.11

A further paper by Meshram *et al.* reports the synthesis of α-chlorocyclopentanone using Amberlyst-15 and NCS in EtOAc over 30 min in a yield of 81%.[198] Since Amberlyst-15 was already present in our lab, this method was attempted, however no product was detected upon purification after 30 min, nor when the reaction was left overnight.

Due to the poor results obtained when trying to generate α-chlorocyclopentanone using a variety of methods and to time constraints, it was decided to buy both this reagent and α-chlorocyciohexanone for use in the subsequent reaction.

6.3.3 Synthesis of Thiazole Guanidine Hydrochloride Salts

The method of Beyer and Hantschel was then applied to both cyclopentanone and cyclohexanone with the desired products of these reactions being guanidine hydrochlorides **60** and **61** (both Scheme 6.12).



60, n = 1

61, n = 2

Scheme 6.12

Interestingly, both **60** and **61** are reported in a patent by Actelion Pharmaceuticals;[199] however, no mention is made of their synthesis. Compounds synthesized by the Beyer method are reported to be high yielding; nevertheless, the purity of the simple thiazole guanidines prepared by this method was tested using elemental analysis rather than high performance liquid chromatography (HPLC). Though the values obtained by Beyer were reasonable, a mixture of the starting materials would have the same proportion of atoms, therefore this is not an optimal measure of purity in the age of HPLC.

While the crude yields of both **60** and **61** both proved high at approximately 90%, purification was difficult, with yields of 15% and 28% respectively on samples tested with HPLC. The samples had been purified by reverse phase column chromatography.

6.4 Conclusions

After studies on the viability of the application of the Kim and Qian method to the synthesis of thiazole guanidines, using a variety of thiourea sources, it was determined that the desired products formed only in low yields with side products such as *mono*-Boc thiazoles and [3-(*tert*-butoxycarbonyl)]-1-(thiazol-2-yl)thiourea forming preferentially. Use of Mukaiyama's Reagent and EDCI produced similar results.

Use of no coupling reagent resulted in no reaction taking place; hence proving that the coupling reagent is necessary for the side-product forming reaction. Thus, a mechanism is proposed with accounts for the formation of an intermediate formed from the coupling reagent and the thiourea source. This is then attacked by the aminothiazole, and through a series of steps forms both side products.

Preliminary attempts at 2-iminoimidazolidylation were somewhat successful, with **176** having been isolated, however **175** could not be purified after multiple attempts.

The deprotection of the **160** resulted in cleavage of the guanidine moiety, resulting in the application of the Kim and Qian method of guanidylation followed by deprotection being abandoned.

Attempts to synthesise α-chlorocyclopentanone using a number of methods involving NCS, proved unsuccessful, with no product isolated via any of the methods examined.

Finally, **60** and **61** were synthesised using the method of Beyer and Hantschel in order to test them as α_2-AR ligands.

7 Biological Results

7.1 Introduction to Assays

The pharmacological assays were carried out in collaboration with the Department of Pharmacology of the Faculty of Medicine at the University of the Basque Country (Bilbao, Spain). Neural membranes were prepared from the PFC of human brains obtained at autopsy in the Instituto Vasco de Medicina Legal (Bilbao, Spain).

7.1.1 [^{3}H]RX821002 Binding Assays

The α_2-AR binding affinities of all compounds were measured by competition assay with the selective radioligand [^{3}H]RX821002 in human PFC, which was used at a constant concentration of 2 nM. Specific [^{3}H]RX821002 binding was measured in neural membranes, which were incubated with [^{3}H]RX821002 for 30 min at 25 °C in the absence or presence of the competing compounds in ten concentrations (10^{-12} to 10^{-3} M). After incubation, the wells were filtered under vacuum, after which the membranes remain on filter paper, which can be examined by scintillation spectrometry. High radioactivity counts in a given well demonstrated the presence of high concentrations of [^{3}H]RX821002 , and therefore, indicated that the radioligand was not displaced significantly by the test compound, and vice versa. Therefore, with increasing concentrations of test compound, less radioactivity was detected. The nonspecific binding was determined in the presence of adrenaline (10^{-5} M) and was subtracted from the total binding resulting in the specific binding. Via this method, the specific binding was determined, and was plotted as a function of the compound concentration and the affinities obtained were expressed as the minus logarithm of the binding constants, p*K*i.

7.1.2 [^{35}S]GTPγS Binding Assays

A direct evaluation of G-protein activity can be made by determining the G nucleotide exchange using radiolabeled GTP analogues such as [^{35}S]GTPγS in human PFC. This assay allows for the direct evaluation of G-protein activity by determining the GTP exchange using [^{35}S]GTPγS. Thus, upon interaction of an agonist with the α_2-AR, a molecule of GDP dissociates from the α subunit of the coupled G-protein; then, a molecule of GTP binds to the α subunit. Then, the α and βγ subunits dissociate and move to their effector molecules, which are involved in passing on the signal; finally the α subunit of the G-protein, which has GTPase activity, hydrolyses the GTP to GDP and becomes

inactive again (see Figure 7.1). Hence, if a radiolabelled GTP molecule is present, the activation of the α_2-AR will result in the intake of such a radiolabelled molecule, and this uptake can be measured. In particular, agonists increase the nucleotide binding, inverse agonists reduce nucleotide binding and antagonists don't affect binding.

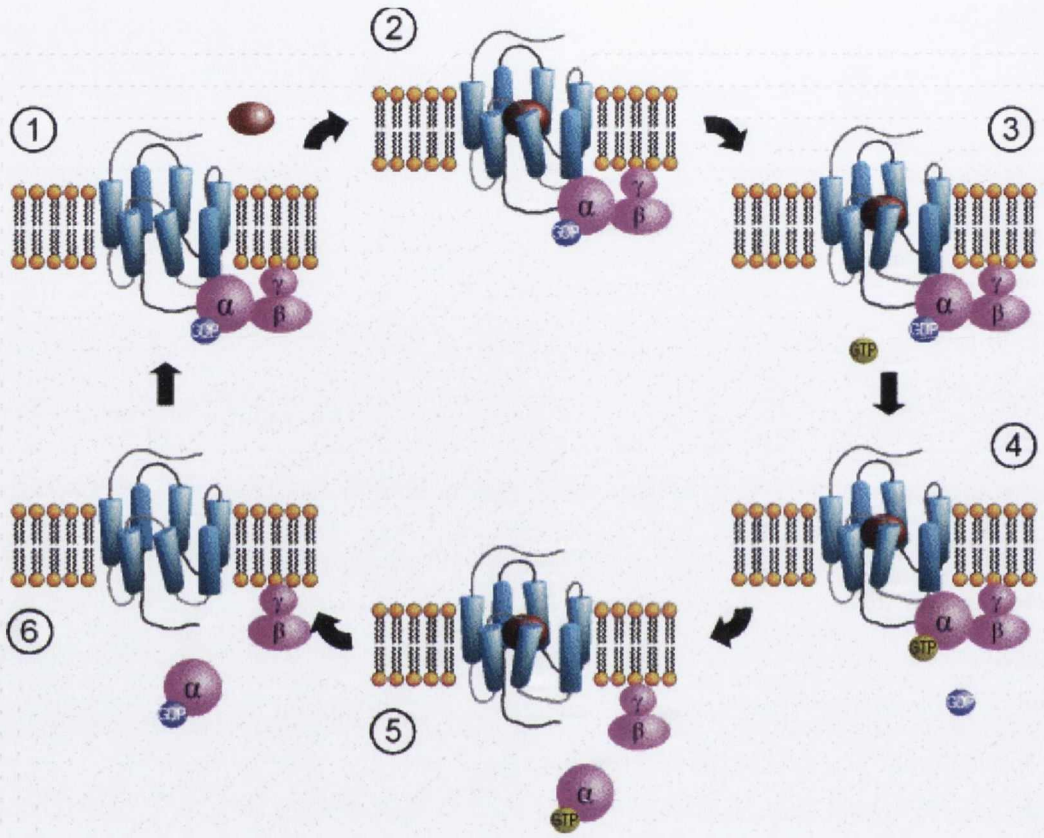


Figure 7.1 – Mechanism of action of the α_2-AR[200]

Assays were incubated at 30 ℃ for 120 min with shaking. Ten concentrations (10^{-12} to 10^{-3} M) of the different compounds were added to the assay in order to evaluate the effect of the compounds on [^{35}S]GTPγS binding. Similarly, the plates were subjected to vacuum filtration and the radioactivity of the filter was measured by scintillation spectrometry.

The EC_{50} values of the compounds and percentage efficacy relative to the well-known α_2-AR agonist UK14304 were measured. Typical potency values are 2-3 log units lower than the affinity values obtained in radioligand receptor binding experiments since assays were performed in low-affinity receptor conditions for agonists (in the presence of G nucleotides and sodium).

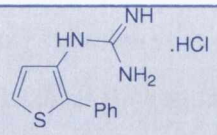
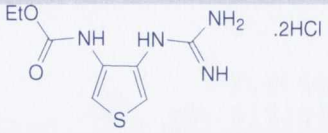
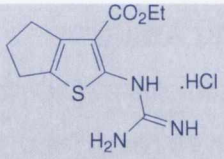
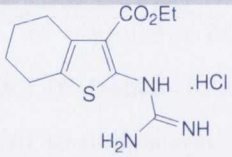
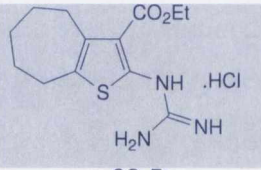
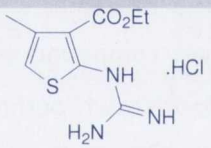
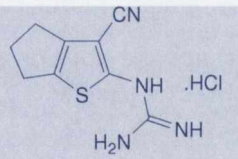
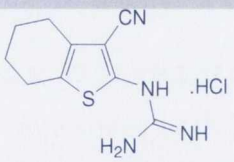
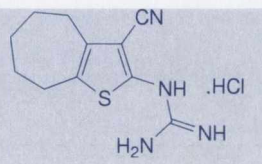
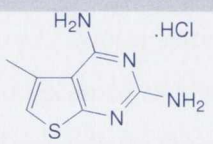
All compounds that showed an antagonist activity in the [^{35}S]GTPγS binding assays, *i.e.* those that did not stimulate binding of [^{35}S]GTPγS by their own, were also assayed at a constant concentration (10^{-5} M) for [^{35}S]GTPγS binding in the presence of increasing concentrations of the α_2-AR agonist UK14304 (10^{-13}-10^{-4} M). If the concentration-response curve for UK14304 in these assays is shifted to the right, the antagonist effect of these derivatives against the α_2-ARs is confirmed. These experiments were also performed in order to investigate the potential of the compounds to specifically modify the EC_{50} or the E_{max} values in the UK14304 stimulation curve.

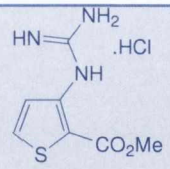
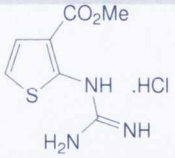
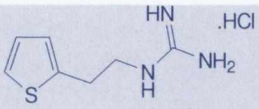
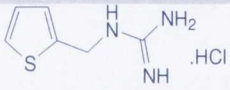
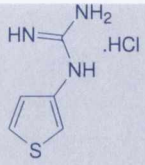
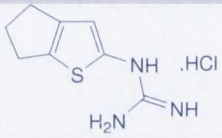
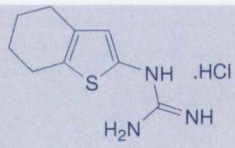
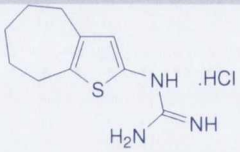
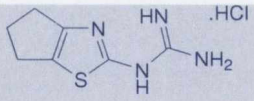
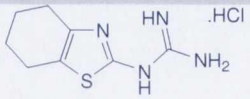
7.2 [^{3}H]RX821002 Binding Affinity Results and Analysis

The results of the [^{3}H]RX821002 binding affinity experiments for some of the thiazole and thiophene derivatives prepared are listed in Table 7.1. Firstly thiophene **70** had a reasonable pK_i of close to 6, which is important considering that all compounds tested previously for α_2-AR affinity had a substituent on the aromatic ring in the *para* orientation with respect to the guanidinium cation, whereas the substituent in **70** is in the *ortho* position. The implication of this is that perhaps the active site of the α_2-AR is not long and narrow, but is instead more compact, allowing for a compound such as **70** to fit well into it. This is a serendipitous result as **70** was only designed as a model compound for testing reduction and guanidylation procedures.

On the contrary, compound **87**, which also was not a target compound initially and contains a carbamate group *ortho* to the guanidinium cation, gave a poor result, perhaps indicating that the electron density of the substituent (electron rich aromatic *vs.* electron poor carbamate groups), the steric nature of the group (bulky *vs.* long and narrow) or perhaps the position on the thiophene ring has a role to play in the difference in binding affinities.

Table 7.1 – Binding affinity for the human α_2-ARs expressed as pK_i calculated from [^{3}H]RX821002 (≈2nM) competition binding curves

Compound	Structure	pK_i	2 Position	3 Position	4,5 Positions
70		5.89	Ph	Gua	H, H
87		4.93	H	Gua	$NHCO_2Et$, H
116		3.93	Gua	CO_2Et	$-(CH_2)_3-$
117		4.58	Gua	CO_2Et	$-(CH_2)_4-$
118		<2	Gua	CO_2Et	$-(CH_2)_5-$
119		4.18	Gua	CO_2Et	Me, H
129		5.65	Gua	CN	$-(CH_2)_3-$
130		6.13	Gua	CN	$-(CH_2)_4-$
131		5.95	Gua	CN	$-(CH_2)_5-$
134		4.17	2,4-diaminopyrimidine		Me, H

140		<2	CO_2Me	Gua	H, H
141		<2	Gua	CO_2Me	H, H
142		5.72	$(CH_2)_2$Gua	H	H, H
143		5.41	CH_2Gua	H	H, H
147		5.71	H	Gua	H, H
152		7.06	Gua	H	$-(CH_2)_3-$
151		7.50	Gua	H	$-(CH_2)_4-$
153		6.81	Gua	H	$-(CH_2)_5-$
60		6.15	Gua	Thiazole	$-(CH_2)_3-$
61		5.92	Gua	Thiazole	$-(CH_2)_4-$

The family of (3-ethoxycarbonylthiophen-2-yl)guanidines *i.e.* compounds **116**-**119** also contain a substituent *ortho* to the guanidine moiety in position 2, along with alkyl groups in positions 4 and 5. However, all the pK_i values of this family are lower than those of compounds **70** and **87**. Interestingly, the optimal ring size for the cycloalkyl derivatives is six (compound **117**), with the five membered derivative **116** having marginally poorer affinity, and with seven membered derivative **118** showing

no affinity under the assay conditions. Compound **119**, which has a methyl group in the 4-position, has the second highest affinity of the series, with a pK_i less than half a unit lower than that of **117**. The non-linear binding curves of compounds **116** and **117** are illustrated in Figure 7.2.

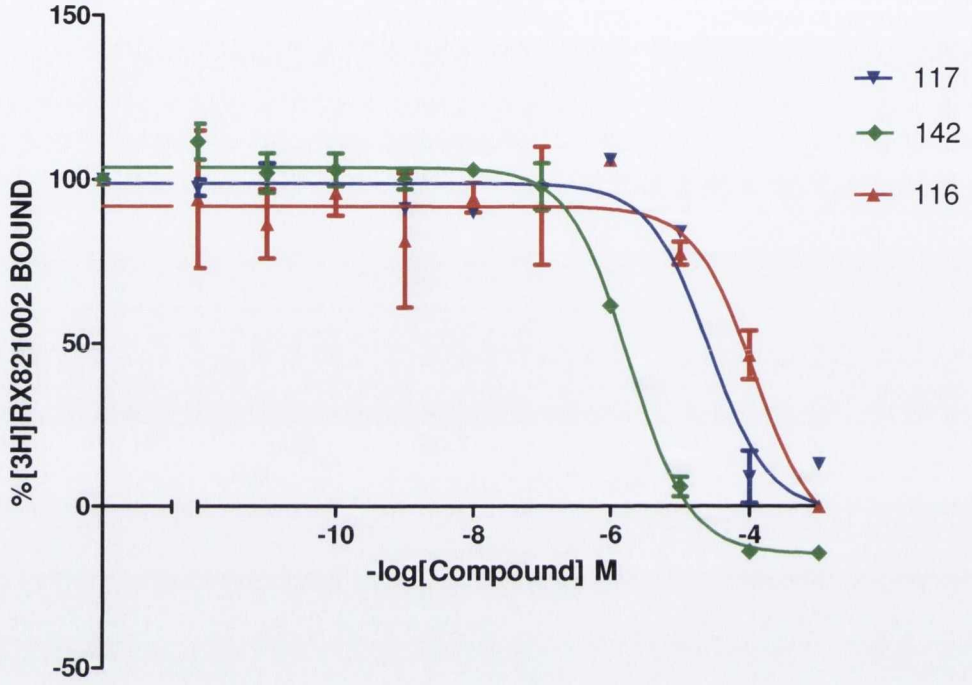


Figure 7.2 – Representative non-linear binding curves for selected compounds

Replacement of the ethyl ester moieties of **116** and **118** with a cyano group, as in molecules **129**-**131**, results in an improvement in affinity. In the case of **129** and **130**, the pK_i values are more than 1.5 units larger than the ester analogues **116** and **117**, whereas the difference is even larger between seven membered analogues **131** and **118**, with the pK_i of the latter close to 6 while the former does not bind to the receptor under the assay conditions. This is very interesting as it indicates that perhaps **118**, with both a bulky ethyl ester group in the 3-position and a large seven membered ring in 4- and 5-positions, is simply too large to fit the active site of the receptor, whereas **131**, with the smaller cyano group in the 3-position can actually fit the receptor well.

Compound **134**, which lacks a cyano group and is proposed to be a 2,4-diaminopyrimidine, displays alternated behaviour in comparison to the binding activity of its ester counterpart **119**. In particular, the pK_i values of **119** and **134** are almost identical. In other families such as the (3-

ethoxycarbonylthiophen-2-yl)guanidines [as well as the (thiophene-2-yl)guanidines discussed later] the six membered derivative displays a much greater affinity to the α2-AR than the seven membered one, whereas interestingly, the difference between pK_i values for **130** and **131** is small.

Isomeric compounds **140** and **141**, which both contain a guanidine substituent and a methyl ester, display no affinity for the α_2-AR under the experimental conditions. Both compounds possibly possess the wrong sterics and electronics to fit well into the active site. The pK_i of **140**, which has a methyl ester in the 2-position, is much lower than that of **70**, which has a phenyl group in the same position, even though both molecules are otherwise identical. Compound **147**, which has only a proton in the 2- position but is otherwise identical to both **70** and **140**, displays a much higher affinity to the α_2-AR than **140**, but a slightly lower affinity than **70**. This tells us that having a substituent in the 2-position is only favourable if the substituent is highly electron rich, indicating that perhaps a dipole-dipole or charge-dipole interaction is in play at this position.

Compounds **140** and **141** also have a much lower affinity for the α_2-AR than the (3-ethoxycarbonylthiophen-2-yl)guanidines **116** and **117**, which are somewhat related, having ester groups. However, it seems that having an alkyl substituent in position 4 is necessary for affinity at the α_2-AR when an ester moiety is also present in the 3-position.

Related thiophene alkyl guanidines, **142** and **143,** display fair pK_i values demonstrating that there is room in the active site to accommodate a linker between the guanidine and the thiophene nucleus. It is of note that **142**, which has a larger alkyl chain length than **143**, displays better binding to the α_2-AR. However **147**, which is a thiophene guanidine with no substituents and is as such comparable to **142** and **143**, has an almost identical pK_i to **142**, the derivative with the larger alkyl linker and better pK_i. Therefore the trend for the effect of the linker between thiophene and guanidine is not consistent. The non-linear binding curve for **142** is shown in Figure 7.2.

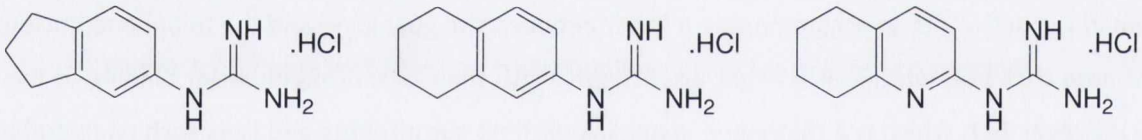
(Thiophene-2-yl)guanidines **152** to **153**, which are analogous to (3-ethoxycarbonylthiophen-2-yl)guanidines **116** to **118**, except that they do not have an ethyl ester group in the 3-position, all have better affinities towards the α_2-AR. Specifically, the three members of this family have the highest pK_i values towards the receptor of all the molecules tested in this work. A similar trend is observed to that in the (3-ethoxycarbonylthiophen-2-yl)guanidine series, in that the six membered ring derivative (**151**) has the highest pK_i value, with the five membered ring analogue second (**152**) and the seven membered ring compound last (**153**). Since this family represents the compounds with the highest pK_i values towards the α_2-AR, all of which are in the vicinity of 7, these molecules are the most promising

lead compounds of this work. This is satisfying since the synthesis and pharmacological evaluation of these molecules was one of the primary objectives of this body of work.

7.2.1 Comparison of [^{3}H]RX821002 Binding Affinity Results with Related Non-thiophene Containing Molecules

Thiazole compounds **60** and **61**, which both have good pK_i values (see Table 7.1), display the opposite affinity pattern to their thiophene counterparts, with five membered ring analogue **60** giving a higher pK_i than six membered ring derivative **61**, and therefore bind better to the receptor. Perhaps the ring nitrogen is involved in a dipole-dipole or hydrogen bonding interaction, altering the orientation of the ligand in the active site accounting for this difference. This is the only family of compounds where this occurs.

Figure 7.3 shows the structures of the related cycloalkyl derivatives, *N*-indan-5-yl (**177**), *N*-(5,6,7,8-tetrahydronaphthalen-2-yl) (**54**) and *N*-(5,6,7,8-tetrahydroquinolin-2-yl) guanidine hydrochloride (**178**), for which the pK_i data is already known. Table 7.2 summarises the pK_i data for these molecules.



177 **54** **178**

Figure 7.3 – Structures of compounds *N*-indan-5-ylguanidine hydrochloride (**177**), *N*-(5,6,7,8-tetrahydronaphthalen-2-yl)guanidine hydrochloride (**54**) and *N*-(5,6,7,8-tetrahydroquinolin-2-yl)guanidine hydrochloride (**178**)

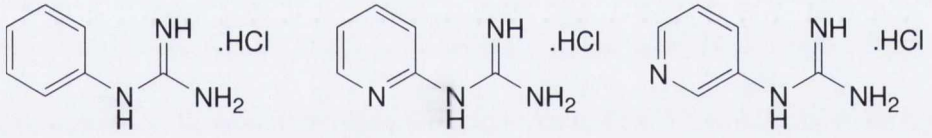
It is evident from examining the data that for both the five and six membered ring derivatives, the relevant thiophene guanidine has a higher pK_i than the thiazole (already described), the phenyl derivative and also the 2-pyridinyl derivative. In particular the thiophene derivatives have pK_i values of just under 0.5 units greater than their phenyl counterparts. This difference is more striking when examining the 2-pyridinyl derivative **178**, which has a pK_i of over 1 unit less than **151**, the thienyl

analogue. These differences could be due to the higher electron density on each ring atom across the thiophene nucleus. The pK_i values for the thiophene analogues are also higher than the thiazole derivatives.

Table 7.2 – Comparison of pK_i data for selected cycloalkyl derivatives

Compound	pK_i	Aromatic Ring	Substituent
152	7.06	Thienyl	- 4,5-$(CH_2)_3$-
177	6.51[104]	Phenyl	-4,5-$(CH_2)_3$-
60	6.15	Thiazolyl	-4,5-$(CH_2)_3$-
151	7.50	Thienyl	-4,5-$(CH_2)_4$-
54	7.11[104]	Phenyl	-4,5-$(CH_2)_4$-
178	6.33[201]	Pyridinyl	-5,6-$(CH_2)_4$-
61	5.92	Thiazolyl	-4,5-$(CH_2)_4$-

Figure 7.4 depicts a series of aromatic guanidines with no other substituents. These are the hydrochloride salts of 1-[2,3-di(*tert*-butoxycarbonyl)guanidino]benzene (**179**), 2-[2,3-di(*tert*-butoxycarbonyl)guanidino]pyridine (**180**) and 3-[2,3-di(*tert*-butoxycarbonyl)guanidino]pyridine (**181**).



179 **180** **181**

Figure 7.4 – Structures of compounds 1-[2,3-di(*tert*-butoxycarbonyl)guanidino]benzene hydrochloride (**179**) 2-[2,3-di(*tert*-butoxycarbonyl)guanidino]pyridine hydrochloride (**180**) and 3-[2,3-di(*tert*-butoxycarbonyl)guanidino]pyridine hydrochloride (**181**)

The pK_i data for the derivatives depicted in Figure 7.4, along with that of **147**, is shown in Table 7.3. When the pK_i data for **179** to **181** is compared with analogue **147**, the thiophene derivative shows less favourable results. Phenyl derivative **179** has a slightly higher pK_i value than the 3-thienyl derivative **147**. In contrast, **147** and 2-pyridinyl derivative **180** have more or less comparable pK_i values. This is

despite the fact that the thiophene has its guanidine substituent in the 3-position compared with the pyridine which has its substituent in the 2-position. For comparison, **181**, the 3-pyridinyl guanidine derivative has a much lower pK_i value than 3-thienyl compound **147** with a difference of more than 2 log units.

Table 7.3 - Comparison of pK_i data for selected un-substituted derivatives

Compound	pK_i	Aromatic Ring	Other Substituents
147	5.71	3-Thienyl	H
179	6.19	Phenyl	H
180	5.74	2-Pyridinyl	H
181	3.34	3-Pyridinyl	H

It is of note that while the cycloalkyl thiophene derivatives **152** and **151** give notably higher pK_i values than related bioisosteres. **147**, Which is lacking in substituents, does not result in more favourable affinity in comparison to its congeners. Perhaps this discrepancy in the relative affinities across the thiophene series compared with other aromatics is due to the fact that those thiophenes displaying increased affinity are thiophen-2-yl guanidines whereas **147**, which does not give increased affinity in comparison to **179** to **181**, is a thiophen-3-yl guanidine. Another possible reason for the discrepancy is the relatively small size of **147** in comparison to its analogues. While this is also true of **152** and **151**, the difference in size is less pronounced.

7.3 GTPγS Activity Results

Activity at the α_2-AR was examined for compounds with a $pK_i > 6$, along with the thiopheneethyl guanidine deriviative **142**. Thiazole **61** was also tested for comparison with similar compounds. A summary of the activity of each compound selected is presented in Table 7.4.

Table 7.4 - [^{35}S]GTPγS Binding Activity of Selected Compounds

Compound	pK_i	Activity
60	6.15	Antagonist
61	5.92	Antagonist
130	6.13	Antagonist
142	5.72	Antagonist
152	7.06	Antagonist
151	7.50	Antagonist
153	6.81	Agonist

Interestingly, all compounds tested, except **153**, displayed antagonistic activity in the [^{35}S]GTPγS assays. It is fortunate that most of the compounds tested for give antagonistic activity, which is perhaps due to the smaller size and orientation of the thiophene nucleus. Compound **153**, which, containing a seven membered alkyl ring, is sterically the most bulky molecule, is the only one that possesses agonistic activity, which is contrary to what is often expected, namely that larger molecules result in antagonistic activity.

Table 7.5 - EC_{50} values obtained from the concentration-response curves for UK14304 (10^{-13} - 10^{-4} M) stimulation of [^{35}S]GTPγS binding in the absence or presence of the different compounds (10^{-5} M).

Experiment	EC_{50} (μM) ±sem	Emax (%)±sem
UK14304	0.4 ±0.01	128 ±2
UK14304 + 130	10.3 ±0.52	138 ±6
UK14304 + 142	0.8 ±0.06	118 ±4
UK14304 + 152	0.5 ±0.04	112 ±2
UK14304 + 151	0.8 ±0.09	111 ±4
UK14304 + 60	1.3 ±0.06	126 ±3
UK14304 + 61	11.4 ±3.40	105 ±4

EC_{50} data for compounds which showed antagonistic activity are shown in Table 7.5, with each compound showing a rightward shift in the concentration response curves for UK14304 (**182**, Figure 7.5) stimulation of [^{35}S]GTPγS binding (Figure 7.6).

182

Figure 7.5 – Structure of UK14304

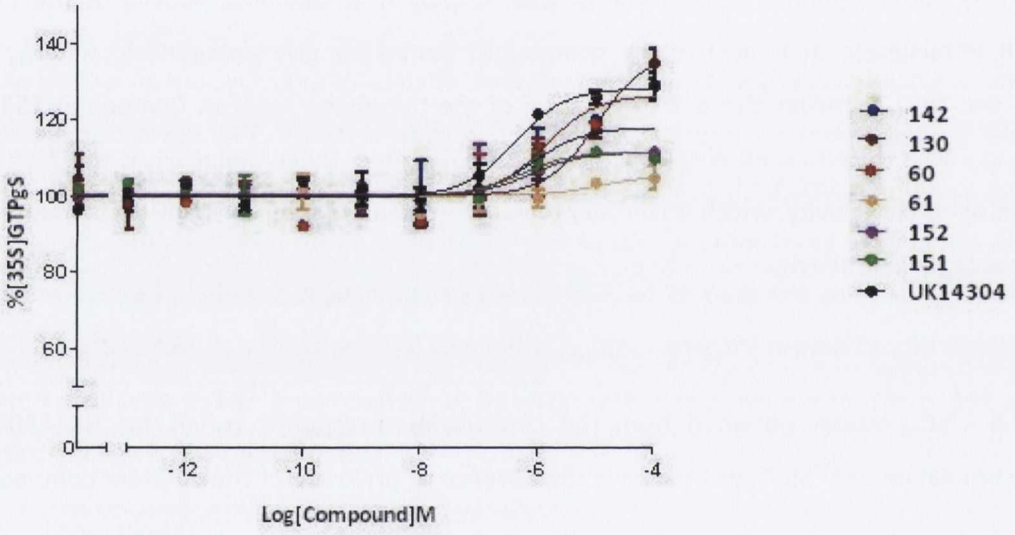


Figure 7.6 - Concentration-response curves for UK14304 (10^{-13} - 10^{-4} M) stimulation of [^{35}S]GTPγS binding in the absence or presence of the different compounds (10^{-5} M).

Table 7.6 shows the activity data at the α_2-AR for two six membered cycloalkyl derivatives. It is interesting that no cyclohexane fused analogue gives agonistic activity in [^{35}S]GTPγS binding assays, and only pyridinyl derivative **178** gives inverse agonistic activity.

Table 7.6 – Comparison of [^{35}S]GTPγS binding activity of other aromatic derivatives

Compound	Activity	Aromatic Ring	Substituent
54	Antagonist[104]	Phenyl	-4,5-$(CH_2)_4$-
178	Inverse Agnoist[201]	Pyridinyl	-5,6-$(CH_2)_4$-

7.4 Conclusions

Compounds **118**, **140** and **141** gave disappointing binding affinities for the human α_2-ARs as they failed to elicit any response in [^{3}H]RX821002 (≈ 2 nM) competition binding curves. Compounds **87**, **116**, **117**, **119** and **134** bound poorly to the α_2-AR, having pK_i values in the region of 4 units. Medium pk_i values were obtained for compounds **70**, **129, 131**, **142**, **143**, **147** and **61**, all of which have pK_i values in the region of 5 units. Good binding affinities of $pK_i > 6$ units were found for **130**, **153**, **60**. And finally, compounds **152** and **151** had very good binding affinities of $pK_i > 7$.

Some trends were observed in the binding affinities of certain compounds, most notably in the compounds with cycloalkyl substituents. Six membered cycloalkyl rings conferred the best affinities in all cases for the thiophene series, however, for the thiazoles the five membered analogue proved a better binder. For the six membered cycloalkyl derivatives the thiophene having an ethyl ester in the 3- position (**117**) resulted in the poorest affinity, with the thiazole derivative (**61**) coming next. This is closely followed by the thiophene with a cyano group in the 3- position (**130**) derivative, and the thiophene with no substituent in the 3- postion (**151**) gave superior binding affinity. Similar results were observed with the seven membered ring analogues when available.

The thiophen-2-ylguanidines with cycloalkyl substituents alone (**152** and **151**) gave improved binding affinities in comparison to their thiazolyl, phenyl, and in the case of **151**, 2-pyridinyl derivatives. However in the case of the thiophen-3-ylguanidine **147**, more modest results were observed in comparison to derivatives containing other aromatic rings.

Fortunately almost all compounds tested for activity proved to be antagonists. These were **130**, **142**, **152**, **151**, **60** and **61**, with only compound **153** showing agonistic activity. In particular, compounds **152** and **151**, which have very good binding affinities ($pK_i > 7$), were found to be antagonists, making these compounds of interest for future investigations.

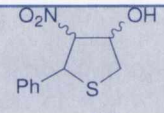
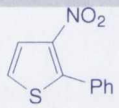
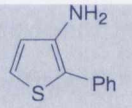
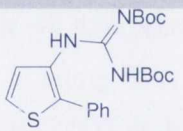
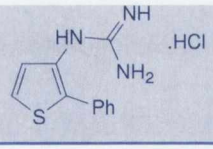
8 Conclusions and Future Work

8.1 Conclusions

The first objective of this project was to investigate methods for the preparation of guanidine salts for the thiophene nucleus. To explore the applicability of the Kim and Qian method[112] to the thiophene series, compounds **62** and **63** were first successfully synthesised in good yields of 87 and 91%.

Compound **70** was successfully synthesised in an overall yield of 30% across five steps with the yields for each step shown in Table 8.1.

Table 8.1 – Yields obtained in the synthesis of **70**

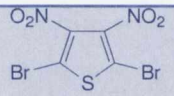
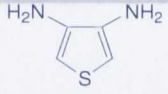
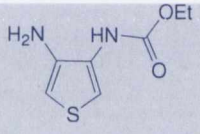
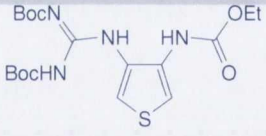
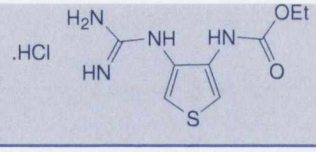
Compound	Structure	Yield (%)
68		99
67		60
66		86
69		59
70		99

Thus, the applicability of the Kim and Qian method[112] to the thiophene series has been proven in this body of work, as well as the methods superiority over the use of Mukaiyama's reagent[137] for these compounds. Similarly the use of TFA and Amberlite IRA-400 in the Cl^- form to deprotect the Boc protected guanidines formed via the Kim and Qian method and their conversion to hydrochloride salts proved successful. However due to the contamination of the Amberlite resin procured for this project and the complications in removing the contaminant, alternative methods of deprotection were sought. Of these both methods involving the use of $SnCl_4$[163] as well as direct deprotection with

HCl in anhydrous dioxane proved successful, with the latter proving to give better purity and not involving the addition of a heavy metal to the potential ligands in the last synthetic step.

Attempts to synthesise compounds **77** and **78** using intermediate **75** failed. New strategies were devised involving compounds **79**, **80**, **83** and **85** as intermediates instead, however problems arose the purification of related hydrochloride salt **86**, resulting in the cessation of this line of research. However, compound **87**, which has some structural similarity to **52** was synthesised in an overall yield of 6% across five steps with the yield of each step shown in Table 8.2.

Table 8.2 – Yields obtained in the synthesis of **87**

Compound	Structure	Yield (%)
71		69
72		41
81		37
85		58
87		92

Use of EAS, and specifically nitration followed by reduction, to prepare aminothiophenes lead to disappointing results, with the reduction of thiophenes without a group *ortho* to the nitro group proving unsuccessful for example in **75**, **92**, and **95**. NAS, though seen as a possible method for the preparation of desirable aminothiophenes, was not attempted due to difficulties encountered in reducing nitro groups.

The Gewald synthesis of 2-aminothiophenes proved to be a highly successful method for producing stable compounds, which could then be guanidylated and converted to hydrochloride salts for

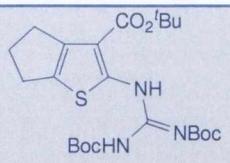
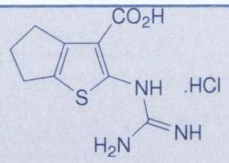
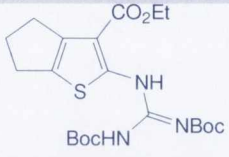
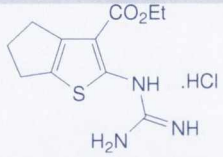
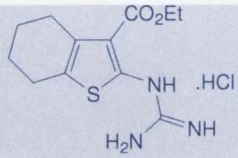
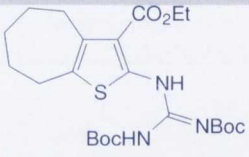
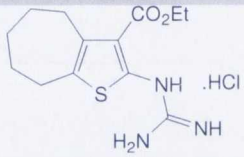
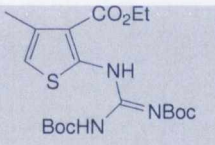
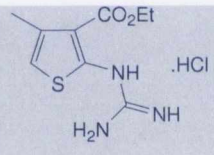
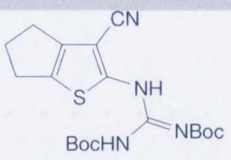
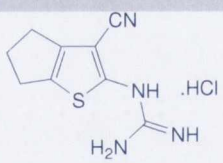
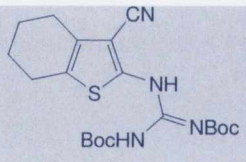
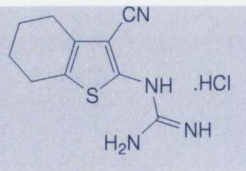
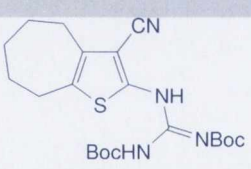
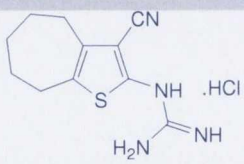
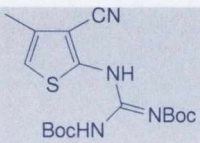
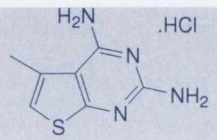
testing. In particular compounds **103**, **106-108**, **112**, **113** and **120-123** were prepared with the yields, though usually adequate, varying from 2-79%; these are documented in Table 8.3.

Table 8.3 – Yields obtained of compounds prepared via the Gewald synthesis

Compound	Substituent			Yield (%)
	3-	4-	5-	
103	$CO_2{}^tBu$	$-(CH_2)_3-$		18
106	CO_2Et	$-(CH_2)_3-$		26
107	CO_2Et	$-(CH_2)_4-$		79
108	CO_2Et	$-(CH_2)_5-$		61
112	CO_2Et	Me	H	19
113	CO_2Et	dihydro-naphtho[2,1-b]		3
120	CN	$-(CH_2)_3-$		46
121	CN	$-(CH_2)_4-$		51
122	CN	$-(CH_2)_5-$		43
72	CN	Me	H	11

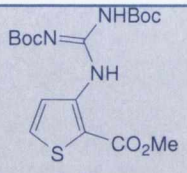
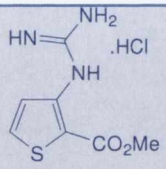
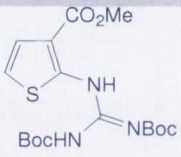
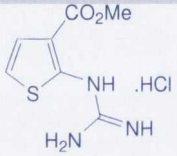
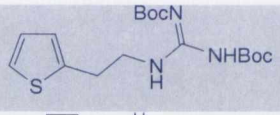
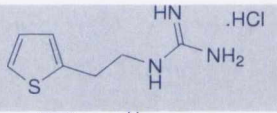
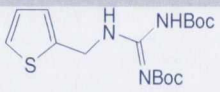
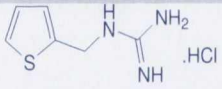
From these Gewald intermediates, compounds **105**, **109-111**, **114** and **125-128** were prepared in yields ranging from 20-68%. Hydrochloride salts of these derivatives were synthesised resulting in the isolation of compounds **115-119**, **129-131** and **134** in yields ranging from 39-93%. Details of both di-Boc guanidine and hydrochloride salt yields are noted in Table 8.4 as well as the overall yields of each salt over 3-4 steps. A number of these hydrochloride salts with an ethyl ester or cyano group (including tautomers of such) in the 3-position were tested in the α_2-AR, giving some giving good results *i.e.* **116-119**, **129-131**, and **134**.

Table 8.4 – Yields of di-Boc guanidines and hydrochloride salts prepared from Gewald thiophenes

Di-Boc Guanidine	Yield (%)	Structure	Salt	Yield (%)	Structure	Overall Yield (%)
105	41		**115**	84		6
109	36		**116**	90		8
110	52		**117**	93		38
111	49		**118**	71		21
114	68		**119**	93		12
125	20		**129**	39		6
126	36		**130**	93		17
127	61		**131**	58		15
128	29		**134**	43		1

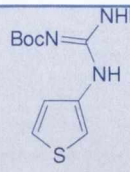
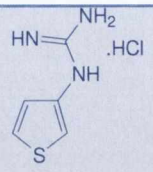
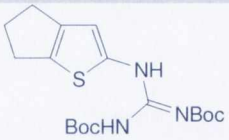
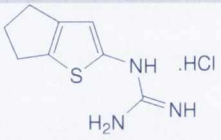
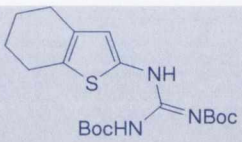
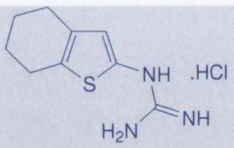
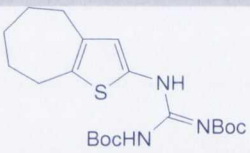
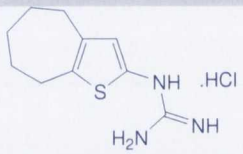
A number of di-Boc protected guanidines (**136-139**) and their hydrochloride salts (**140-143**) were also prepared from commercially available amines. The yields over two steps are recorder in Table 8.5 along with the overall yield.

Table 8.5 – Yields of guanidines prepared from commercial amines

Di-Boc Guanidine	Yield (%)	Structure	Salt	Yield (%)	Structure	Overall Yield (%)
136	57		**140**	99		56
137	37		**141**	90		33
138	80		**142**	95		76
139	94		**143**	54		51

Also after multiple attempts to decarboxylate aminothiophenes containing an ester moiety, finally a method was found, leading to four target guanidine-like molecules being synthesised and tested for affinity and activity towards the α_2-AR: Compounds **147** and **152-153**. Yields for these molecules are documented in Table 8.6 including overall yields across 2-5 steps. Direct guanidylation of the oxalate salt of 3-aminothiophene led to **146**, a dimer of **145**.

Table 8.6 – Yields of thienyl guanidines prepared via decarboxylation

Di-Boc Guanidine	Yield (%)	Structure	Salt	Yield (%)	Structure	Overall Yield (%)
145	5		**147**	88		4
148	4		**152**	60		<1
149	23		**151**	79		14
150	35		**153**	99		21

Some work was carried out into the synthesis of thiophene guanidines via indirect methods, not involving the formation of aminothiophenes. These works included the use of Buchwald-Hartwig cross coupling chemistry, as well as the Paal-Knorr reaction. Implementation of the Buchwald-Hartwig reaction using a variety of conditions was attempted in order to circumvent the need to use unstable thiophene amines. In particular, 2- and 3-chlorothiophene and 2- and 3-bromothiophene were used as the aromatic halides, DiBocGua and Gua HCl were chosen as guanidine sources, bases K_2CO_3, KO^tBu and NaH were used along with ligands BINAP (in toluene) and DCHPB (in dioxane). Disappointingly, none of the conditions employed afforded any of the desired thiophene guanidines and further investigations were not completed thoroughly due to time constraints, with the application of Buchwald-Hartwig chemistry to the preparation of aromatic guanidines already being investigated under another project.

The Paal-Knorr strategy involved first coupling a guanidine source to an acid (or an ester). One guanidine source, DiBocGua was already at hand but *mono*-Boc guanidine (**154**) was successfully synthesised using an in-house procedure. Firstly, attempts were made to couple the methyl ester of levulinic acid (**155**) to **154** using the bases DIPEA, NaH or Al_2Me_6. However, despite best attempts, the coupling reaction did not yield the desired product. Coupling was then attempted using levulinic acid

(**156**) and hydrolysis of ester **155** to form **156** was carried out successfully. Use of BOP for the coupling of DiBocGua with **156** resulted in a hit by HRMS, but multiple attempts to isolate the required amide failed and it appeared that the requisite product was only present in trace amounts. Then, the crude material was carried forward to the next stage of the reaction sequence, the Paal-Knoor reaction. These conditions, however, did not afford the desired product. Other coupling conditions such as use of: silica gel, silica gel plus BOP, BOP plus DIPEA in DMF also failed to yield the desired product. 2-step coupling conditions, namely use of $SOCl_2$ or ethylchloroformate, were also attempted but neither reagent afforded the requisite amide. Therefore, this reaction sequence did not yield the desired amide, the product of the first step, and therefore no thiophene guanidines were produced via this method.

The applicability of the Kim and Qian method of guanidylation to the thiazole series, using a variety of guanidine sources, gave disappointing results, with numerous side products having been observed. The desired products formed only in low yields with the side products, *mono*-Boc thiazoles and [3-(*tert*-butoxycarbonyl)]-1-(thiazol-2-yl)thiourea, forming preferentially. After a number of experiments to try to generate thiazole guanidine thiazoles using other methodologies, the use of Mukaiyama's reagent proved more successful, though still gave similar side products, albeit in lower yields.

When no coupling reagent was used, no reaction took place proving that the coupling reagent is necessary for the side-product forming reaction. A mechanism is proposed, which accounts for the formation of an intermediate formed from the coupling reagent and the thiourea source. This is then attacked by the aminothiazole, and through a series of steps forms both side products.

Disappointingly, when the guanidylated compounds were deprotected using either TFA in DCM or HCl in dioxane, the guanidine moiety was cleaved off, leaving only the salt of the corresponding amine. Due to this discovery, investigations into the guanidylation of thiazole guanidines were ceased.

However, since methods for the synthesis of thiazole guanidines had been previously described by Beyer and Hantschel,[116] it was decided to employ this method. Attempts to prepare some α-chloroketones using a variety of methodologies involving NCS failed, hence due to time constraints, two molecules of this type were purchased for use in the subsequent reaction. Synthesis of the two corresponding thiazole guanidines (Compounds **60** and **61**) proved successful and these molecules were then tested for affinity and activity towards the α_2-AR.

Twenty molecules prepared in this work were tested for α_2-AR binding affinity against the [^{3}H]RX821002 radioligand in human PFC. In particular compounds **118**, **140** and **141** did not bind to

the receptor under the assay conditions, compounds **87**, **116**, **117**, **119** and **134** bound poorly to the receptor, compounds **70**, **129**, **131**, **142**, **143**, **147** and **61** had medium binding affinities, compounds **130**, **153** and **60** had good binding affinities and compounds **152** and **151** had very good binding affinities.

Some trends were observed in the binding affinities of certain compounds, most notably in the compounds with cycloalkyl substituents. Six membered cycloalkyl rings resulted in the best affinities in all cases for the thiophene series, but in the thiazole series, the five membered analogue was the superior binder. For the six membered cycloalkyl derivatives the binding affinities in ascending order are: the thiophene having an ethyl ester in the 3- position (**117**), the thiazole derivative (**61**), the derivative with a cyano group in the 3- position (**130**) derivative, and the thiophene with no substituent in the 3- postion (**151**). Similar results were observed with the seven membered ring analogues when available.

The thiophen-2-ylguanidines, which have only cycloalkyl substituents such as **152** and **151** bound to the α_2-AR better than their thiazolyl, phenyl, and in the case of **151**, 2-pyridinyl derivatives. However in the case of the thiophen-3-ylguanidine **147**, more modest results were observed in comparison to derivatives containing other aromatic rings.

Seven compounds displaying medium-very good affinities were then tested for α_2-AR activity using [^{35}S]GTPγS also in human PFC. Fortunately, six of the molecules tested proved to be antagonists, including the two molecules with very good binding affinities: **152** and **151** ($pK_i > 7$).

8.2 Future Work

Given the relative synthetic ease with which **70** was prepared, along with its moderately good α_2-AR binding affinity, it would be of interest to synthesise and evaluate the binding affinities of analogues of **70** prepared by the same methodology. In particular, the method of O'Connor and Southern[127] allows for the formation of a variety of aromatic, heteroaromatic and alkyl 3-nitrothiophenes.

It could also be interesting to synthesise and test **132**, though since this readily converts to **134**, they would be of little biological use. It would also be of interest to synthesise the 2,4-diaminopyrimindine based thiophene series in order to examine what the difference in binding affinity is between them ands the 3-cyano thiophenes.

Since guanidine-like thiophenes with a linker, such as **138**, gave good pK_i values, substitution of the thiophene ring could afford other related target molecules for testing and would cast further light on the effect that a linker has on binding affinity.

Since lead molecule **151** had a very good $pK_i > 7$, it can now be used as a lead compound and modifications could be made perhaps by adding substituents to the cyclohexane ring. It would also be of pharmacological interest to synthesise larger quantities of **151** for *in vivo* testing.

Work is already underway on the possible use of the Buchwald-Hartwig reaction for the preparation of aromatic guanidines and this work will be continued in the future.

The application of the Paal-Knorr based reaction sequence to form guanidine-like thiophenes though bypassing the aminothiophene intermediates had only just begun, so extensive investigations of this route are necessary in order to determine its viability. This pathway should be further examined considering the low overall yields obtained for the simple thiophene guanidines prepared through decarboxylation of the Gewald aminothiophenes. This could potentially unlock an easier method to produce compounds such as **151** in better overall yields. In particular, the use of other coupling reagents as well as the modification of the conditions used, involving acid anhydride and acid chloride intermediates as these reagents have not been examined extensively in this work and have been known to afford amides with some structural similarities.

It may be interesting to begin investigations into the mechanism involved in the side reactions of the guanidylation of thiazole. This could be done using thiourea based reagents involving radiolabelled isotopes, such as deuterated thiourea. This reagent could be Boc protected using the standard procedure and used in reaction under differing conditions, which could shed some light on mechanistic details.

Another possible future area to be worked on is the preparation of a larger body of guanidine-like thiazoles including 2-iminoimidazolidines, via methods based on those of Beyer and Hantschel.[116] In order to do this, it may be necessary to revisit the synthesis of α-chloroketones via methods not covered in this work, such as the use of chlorine gas in water.

9 Experimental Section

9.1 Chemistry

General

All commercial chemicals were obtained from Sigma-Aldrich, Fluka or Acros and were used without further purification unless stated. Deuterated solvents for NMR use were purchased from Apollo. THF, DCM and MeOH were distilled from calcium hydride and TEA over type 4 molecular sieves. Manual chromatographic columns were run using silica gel 60 (230-400 mesh ASTM) or aluminium oxide (activated, Neutral Brockman I STD grade 150 mesh). Biotage chromatographic columns were run using a Biotage SP4 using either a KP-Sil 10g SNAP Cartridge or a KP-Sil 50 g SNAP Cartridge at a collection wavelength of 254 nm and a monitor wavelength of 290 nm. Reverse phase pencil columns were performed using a depth of 2.5-3.0 cm of reverse phase silica in a Pasteur pipette, using either the stated number column volumes (CV) of the stated solvents, or until all material had eluted as detected by TLC. TLC was performed using Merck Kieselgel 60 F_{254} silica gel plates or Polygram Alox N/UV_{254} aluminium oxide plates using 3:1 hexane/EtOAc unless otherwise stated. Visualisation was performed by UV light (254 nm).

Proton NMR spectra were recorded on either a Bruker DPX-400n or AV-400 spectrometer operating at 400.13 MHz for ^{1}H NMR, 100.6 MHz for ^{13}C NMR and 376.5 MHz for ^{19}F, or a DPX-600 spectrometer operating at 600.13 MHz for ^{1}H NMR 150.6 MHz for ^{13}C NMR and 60.8 MHz for ^{15}N NMR. NMR data were processed using Bruker Topspin 2.1 software. Electrospray mass spectra were recorded on a MassLynx V 4.0 and HRMS spectra were recorded on a Waters (Micromass) LCT-Tof mass spectrometer operating in the positive ion electrospray mode. The source was operated at 100 °C with a cone voltage of 30 V. The instrument was operated at a resolution of 5000 fwhm. Spectra were recorded over a range m/z 100 to m/z 1000. The MS was controlled and data acquired and mass measured with MassLynx 4.0 software. HPLC grade methanol was used as carrier solvent. Melting points were determined using an Electrothermal IA9000 digital melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Spectrum 100 FT-IR spectrometer equipped with Universal ATR sampling accessory. The X-ray crystallography data for crystal samples were collected on a Rigaku Saturn 724 CCD Diffractometer. Elemental analysis was carried out at the Microanalysis Laboratory, School of Chemistry and Chemical Biology, University College Dublin.

HLPC purity analysis was carried out using a Varian ProStar system equipped with a Varian Prostar 335 diode array detector and a manual injector (20 µl). UV detection was performed at 245 nm and

peak purity was confirmed using a purity channel. The stationary phase consisted of an ACE 5 C18-AR column (150x4.6 mm), and the mobile phase used the following gradient system, eluting at 1.0 mL/min: aqueous formate buffer (30 mM, pH 3.0) for 10 minutes, linear ramp to 85% methanol buffered with the same system over 25 minutes and held at 85% buffered methanol for 10 minutes.

Preparation of Boc-protected Amidine Precursors (Method A)

To a solution of thiourea or imidazoline-2-thione (30 or 40 mmol; 1 eq.) in dry THF (600 mL) at 0 °C, under argon atmosphere NaH (60% in mineral oil) (4.5 eq.) was added. The mixture was kept at 0 °C for 5 min and was allowed to warm up to r.t. over 10 min. The mixture was again cooled to 0 °C before di-*tert*-butyl dicarbonate (2.2 eq.) was added. After 30 min the ice bath was removed and the reaction was stirred at room temperature overnight.

The reaction was quenched carefully with saturated $NaHCO_3$ solution (100 mL), was poured into water (300 mL), and the aqueous layer extracted using EtOAc (3 x 150 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The crude powder was triturated with cool hexane (100 mL) and cooled in a fridge. The powder was then collected by vacuum filtration and washed with cool hexane (3x50 mL) giving a white powder which produced one spot by TLC (hexane/EtOAc 2:1).

Nitration using Potassium Nitrate and Sulfuric Acid (Method B)

The thiophene derivative (1 eq.) was added slowly to H_2SO_4 (1-2 mL/mmol) at 0 °C. Then, KNO_3 (1.0 or 2.1 eq.) was added slowly and the mixture stirred at 0 °C for 30 min and at r.t. for at least 2 h. The mixture was poured onto ice (~4-8 g/mmol). Upon melting of the ice, the precipitate was collected by vacuum filtration, and washed giving a crude product which was further purified.

Preparation of 2-Amino-3-substituted Thiophenes via the Gewald Reaction (Method C)

A mixture of ketone (1.0 eq.), sulfur powder (1.0 eq.) and *t*-butyl cyanoacetate, ethyl cyanoacetate or malononitrile (1.0 eq.) in EtOH (2 mL/mmol) was prepared before morpholine (1.0 eq.) was added

dropwise, ensuring that the reaction did not heat up above 60 °C during the addition. The mixture was then heated at 40 °C and stirred for 4-20 h.

Synthesis of the Boc-Protected Guanidine and 2-Iminoimidazolidine Derivatives using $HgCl_2$ (Method D)

A solution of amine (1 eq.), **62**, **63** or *S*-methylthiopseudourea (1 eq.), TEA (~3.5 eq.) in dry DCM (10-20 mL/mmol) was prepared at 0 °C and stirred for 20 min. $HgCl_2$ (1.2 or 1.5 eq.) was then added and the solution stirred at 0 °C for a further 40 min and was then stirred for 2 days until the reaction had reached completion (TLC). * The reaction was carried out under argon or nitrogen in the absence of light. The reaction mixture was filtered through a pad of celite in a sintered glass funnel, and washed with copious amounts of EtOAc. The filtrate was then washed with brine, dried over $MgSO_4$ and the solvents removed, before further purification.

Synthesis of the Boc-Protected Guanidine Derivatives using Mukaiyama's Reagent (Method E)

To a solution of the 2-aminothiazole (3 mmol; 1 eq.) in DCM (25 mL), **62** (994.86 mg; 3.6 mmol; 1.2 eq.), TEA (668 mg; 920 µL; 6.6 mmol; 2.2 eq.) and Mukaiyama's reagent (919.76 mg; 3.6 mmol; 1.2 eq.) were added and the reaction was allowed to stir at r.t. overnight. The solvents were removed and the product dissolved in ether (25 mL) washed with water (25 mL) and the aqueous layer extracted with ether (2 x 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents removed giving a crude product, which was purified by column chromatography on a Biotage (hexane/EtOAc 3 CV/% gradient).

Synthesis of the Boc-Protected Guanidine Derivatives involving Decarboxylation (Method F)

The relevant ester was refluxed in 2M NaOH (10 mL) or (5 mL) with ethanol (5 mL) for the period of time specified. The solution was cooled, acidified to pH 3 with concentrated HCl and the thick precipitate was filtered off. It was pressed dry as possible before being dissolved in acetone (12.5 or 6.5 mL). The solution was dried ($MgSO_4$), filtered and the solvent evaporated at 20 °C. The resulting thick paste was treated with 2-propanol (3 or 1.5 mL) and anhydrous oxalic acid dihydrate (1 or 0.6 g)

at 38 °C for 45 min. The mixture was cooled, diluted with ether and the solid filtered off and washed with ether and dried.

The salt was dissolved in water, basified with conc. ammonia. The mixture was extracted with DCM (3 x 5 mL), the combined extracts were dried ($MgSO_4$) and the solvents removed.

The material was redissolved in DCM (10 mL) before an appropriate quantity of each of the following were added sequentially, based on the mass of the crude dicationic salt: **62** (2.4 eq.), TEA (8.0 eq) and $HgCl_2$ (3.0 eq.). The solution was stirred for 2 days, was filtered through a pad of celite and washed with EtOAc. The filtrate was washed with brine before being dried over $MgSO_4$ and the solvents removed. The resulting material was purified on using column chromatography on a Biotage (hexane/EtOAc 1%/CV over 20 CV).

Preparation of the Hydrochloride Salts using TFA/DCM (Method G)

To the relevant di-Boc-protected guanidine precursor (1.0 eq.) was added a 50% (v/v) solution of TFA in DCM (10 mL). The mixture was stirred for 4 h at r.t., and the solvent was then removed under vacuum yielding the trifluoroacetate salt. This was dissolved in water (10 mL) and IRA400 Amberlyte Resin in its chloride form (1.0 g/eq.) was added. The mixture was stirred at r.t. for 24 h. The resin was then removed by filtration, the aqueous solution was washed with DCM, and the water evaporated, yielding the pure hydrochloride salt. Absence of the trifluoroacetate anion was checked for by ^{19}F NMR.

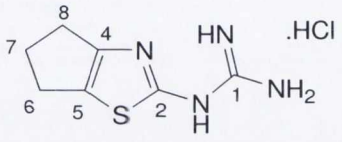
Preparation of the Hydrochloride Salts using HCl Dioxane (Method H)

The di-Boc-protected guanidine precursor (1 eq.) was dissolved in 4 M HCl in dioxane (25 eq.). The reaction was stirred at 60 °C over 6 h before the solvent was removed. The sample was purified through a 3 cm reverse phase pencil column (3 CV water, 2 CV 9:1 water:acetonitrile, 2 CV 1:1 water:acetonitrile, 2 CV acetonitrile).

Direct Preparation of the Thiazole Hydrochloride Salts (Method I)

2-Imino-4-thiobiuret (236.3 mg; 2 mmol; 1 eq.) and the α-chlorocycloketone (2 mmol; 1 eq.) were heated to 130 °C for approx 50 min. After cooling, the mixture was filtered and washed with water and HCl (32%; 1 mL). The filtrate was taken and evaporated down in vacuo.

***N*-[5,6-Dihydro-4*H*-cyclopenta-[1,3]-thiazol-2-yl]guanidine hydrochloride (60)**



Synthesised using Method I from α-chlorocyclopentanone (212 µL; 237.1 mg; 2 mmol; 1 eq.). The product was filtered through a plug of reverse phase silica and washed with water until no product remained (TLC) giving an off white solid. Yield: 65.2 mg (15.0%)

mp decomposes > 190 °C

^{1}H NMR (400 MHz, D_2O) δ 2.82 (t, 2H, *J* = 7.2 Hz, H_6), 2.68 (t, 2H, *J* = 7.2 Hz, H_8), 2.42-2.35 (app. quint., 2H, *J* = 7.2 Hz, H_7) ppm

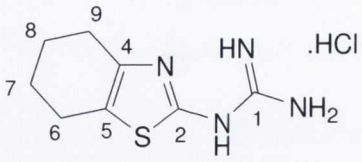
^{13}C NMR (100 MHz, D_2O) δ 156.2 (C_1), 152.9 (C_2), 134.1 (C_4), 128.2 (C_5), 27.1 (C_6), 26.3 (C_8), 26.3 (C_7) ppm

IR ν_{max} vmax 3227 (NH), 3105 (NH), 2866 (NH), 2486, 1688, 1598 (C=N), 1556, 1504, 1466, 1369, 1312, 1204, 1174, 977, 855, 710 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_7H_{10}N_4S$ $[M + H]^+$ 183.0704, found 183.0701

Purity by HPLC 98.0% (t_R 24.43 min)

_N_-[4,5,6,7-Tetrahydrobenzo-[1,3]-thiazol-2-yl]guanidine hydrochloride (61)



Synthesised using Method I from α-chlorocyclohexanone (228 μL; 265.2 mg; 2 mmol; 1 eq.). The product was filtered through a plug of reverse phase silica until no product remained (TLC) giving a white solid. Yield: 128.4 mg (28%)

mp 215-217 °C

^{1}H NMR (400 MHz, D_2O) δ 2.53 (t, 2H, J = 4.1 Hz, H_6), 2.46 (t, 2H, J = 4.1 Hz, H_9), 1.69-1.66 (m, 4H, $H_{7,8}$) ppm

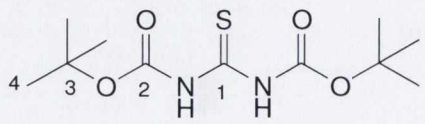
^{13}C NMR (100 MHz, D_2O) δ 157.5 (C_1), 154.6 (C_2), 145.2 (C_4), 123.9 (C_5), 25.5 (C_6), 22.5 (C_9), 22.3 (C_8), 22.0 (C_7) ppm

IR ν_{max} 3461, 3303 (NH), 3011 (NH), 2931 (NH), 1686 (C=N), 1607 (C=O), 1563 (C=O), 1499, 1473, 1141, 1204, 993, 709 cm^{-1}

HRMS (ESI) m/z calc. for $C_8H_{12}N_4S$ $[M + H]^+$ 197.0861, found 197.0854

Purity by HPLC 97.0% (t_R 26.77 min)

_N,N'_-Di(*tert*-butoxycarbonyl)thiourea (62)[115]



Synthesised from thiourea (3.045 g; 40 mmol), NaH (60% in mineral oil) (7.200 g; 180 mmol) and di-*tert*-butyldicarbonate (19.200 g; 88 mmol) using Method A giving a white powder. Yield: 9.640 g (87%)

mp 126-129 °C

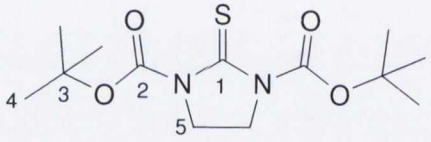
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, H_4) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4 (C_2), 149.9 (C_1), 83.6 (C_3), 27.5 (C_4) ppm

IR ν_{max} 3170 (NH), 2885, 2936, 1769 (C=O), 1718, 1554, 1504, 1225, 1129, 768 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_{11}H_{20}O_4N_2S$ $[M + Na]^+$ 299.1036, found 299.1042

_N,N'_-Di(*tert*-butoxycarbonyl)imidazolidine-2-thione (63)[115]

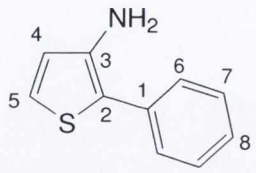


Synthesised from imidazoline-2-thione (7.550 g; 30 mmol), NaH (60% in mineral oil) (5.400 g; 135 mmol) and di-*tert*-butyldicarbonate (14.400 g; 66 mmol) via Method A giving a yellow powder. Yield: 8.260 g (91%)

mp 115-117 (Lit.[115] 117-119 °C)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 4H, C_5), 1.57 (s, 18H, C_4) ppm (Lit.[115] 3.84, 1.46)

3-Amino-2-phenylthiophene (66)[134]



Compound **67** (50.0 mg; 0.243 mmol; 1 eq.) in 35% HCl (5 mL) was cooled to 0 °C before tin powder (87.0 mg; 0.732 mmol; 3 eq.) was added slowly and the mixture left at r.t. overnight. After this time, the starting material had not dissolved so a minimal amount of EtOH was added to dissolve it, and a further portion of tin (72.0 mg) was added at 0 °C. The mixture was left stirring at r.t. for 4 h and was then extracted with DCM (2x5 mL). The aqueous layer was basified to pH 13 using 4 M NaOH, and extracted using DCM (3x30 mL), dried over Na_2SO_4 and the solvents removed giving a yellow oil. Yield: 28.3 mg (66%)

Or

An aqueous solution (5 mL) containing $Na_2S.9H_2O$ (280.0 mg; 1.17 mmol; 5 eq.) and NaOH (110.0 mg; 2.75 mmol; 12 eq.) was added to a stirred suspension of **67** (50.0 mg; 0.24 mmol; 1 eq.) in EtOH and the mixture was stirred under reflux for 6 h and allowed to stand overnight. The EtOH and most of the water were removed under reduced pressure, which resulted in an orange solution with a cream precipitate on standing. This mixture was dissolved in DCM dried over Na_2SO_4 and the solvents removed giving a yellow oil. Yield: 36.8 mg (86%)

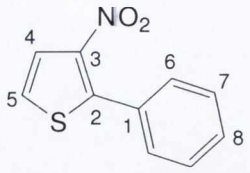
bp 165±0.5 °C at 12.5±2.5 mbar

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, 2H, *J* = 7.5 Hz, H_6), 7.44 (t, 2H, *J* = 7.5 Hz, H_7), 7.29 (t, 1H, *J* = 7.5 Hz, -H_8), 7.15 (d, 1H, *J* = 5.3 Hz, H_5), 6.70 (d, 1H, *J* = 5.3 Hz, H_4), 3.48 (brs, 2H, NH_2) ppm (Lit.[134] 7.52, 7.42, 7.22-7.29, 7.12, 6.66, 3.82)

^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4 (C_2), 134.4 (C_1), 129.1 (C_7), 127.7 (C_6), 126.5 (C_8), 123.6 (C_5), 122.1 (C_4), 116.8 (C_3)

Attempt to further purify product using acid extraction resulted in decomposition.

3-Nitro-2-phenylthiophene (67)[127]



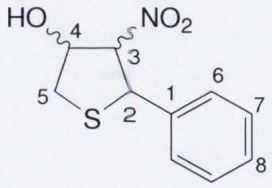
Compound **68** (1.000 g; 4.45 mmol; 1 eq.), silica (8.000 g; 8 eq. w/w) and DDQ (4.060 g; 17.84 mmol; 4 eq.) were heated at reflux in chloroform for 24 h. Then, KOH (500.0 mg/mmol) and activated charcoal (500.0 mg/mmol) were added and the mixture stirred for 1 h before being washed through a plug of silica. The residue was recrystallised from EtOH:water 1:1 giving golden crystals. Yield: 551.4 mg (60%)

mp 100-101 °C (Lit.[129] 101.5-102.5 °C)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, 1H, *J* = 5.5 Hz, H_4), 7.53-7.47 (m, 5H, H_{6-8}), 7.30 (d, 1H, *J* = 5.5 Hz, H_5) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 145.1 (C_2), 142.5 (C_3), 130.1 (C_1), 129.2 (C_6), 129.2 (C_8), 128.0 (C_7), 124.6 (C_4), 123.5 (C_5) ppm

4-Nitro-5-phenyl-tetrahydrothiophen-3-ol (68)[127]



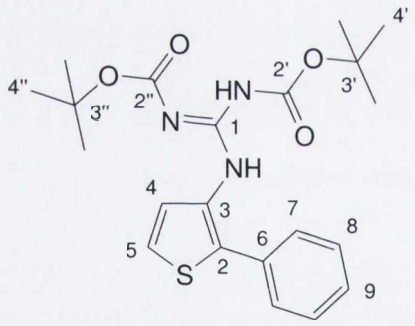
To a stirred solution of *trans*-β-nitrostyrene (800.0 mg; 5.36 mmol; 1 eq.) in DCM (15 mL) was added TEA (150 µL; 109.0 mg; 1.08 mmol; 0.2 eq.) and 2,5-dihydroxy-1,4-dithiane (612.0 mg; 4.02 mmol; 0.75 eq.) under argon. The reaction was stirred at r.t. overnight. The mixture was diluted with DCM

(54 mL) and filtered to remove the solids. The filtrate was washed with saturated ammonium chloride solution (54 mL) and the aqueous layer extracted with DCM (2x54 mL). The combined organic layers were washed with water (54 mL) and brine (54 mL), dried over $MgSO_4$ and the solvents removed under reduced pressure, giving a colourless oil, which solidified overnight to form a white solid and appears as 2 close spots by TLC. The ratio of isomers present is 3:1. Yield: 1.180 g (99%)

R_f (hexane/EtOAc 4:1) 0.25, 0.16 (Lit.[127] 0.24, 0.16)

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51-7.54 (m, 2H, $H_{7\ min}$), 7.47-7.49 (m, 2H, $H_{7\ maj}$), 7.30-7.38 (m, 3H, $H_{6,8\ maj}$), 7.28-7.36 (m, 3H, $H_{6,8\ min}$), 6.31 (d, 1H, J = 6.1 Hz, $OH_{\ maj}$), 6.23 (d, 1H, J = 4.8 Hz, $OH_{\ min}$), 5.61 (dd, 1H, J = 4.0, 10.5 Hz, $H_{4\ min}$), 5.18 (dd, 1H, J = 9.9, 10.0 Hz, $H_{4\ maj}$), 5.13 (d, 1H, J = 10.5 Hz, $H_{5\ min}$), 4.92-4.96 (m, 1H, $H_{3\ min}$), 4.89 (d, 1H, J = 10.0 Hz, $H_{5\ maj}$), 4.74 - 4.81 (m, 1H, $H_{3\ maj}$), 3.54 (dd, 1H, J = 4.0, 11.3 Hz, $H_{2\ min}$), 3.20 (dd, 1H, J = 7.5, 9.9 Hz, $H_{2\ maj}$), 3.10 (dd, 1H, J = 9.7, 9.9 Hz, $H_{2\ maj}$), 2.83 (d, 1H, J = 11.3 Hz, $H_{2\ min}$) ppm (lit.[127] maj = 7.48, 7.35, 6.30, 5.18, 4.89, 4.78, 3.20, 3.10 and min = 7.53, 7.31, 6.23, 5.61, 5.14, 4.94, 3.54, 2.84)

[2,3-Di(*tert*-butoxycarbonyl)]-1-(2-phenylthien-3-yl)guanidine (69)



To a solution of **66** (35.1 mg; 0.2 mmol; 1 eq.) in DCM (1.5 mL) were added **62** (66.4 mg; 0.24 mmol; 1.2 eq.), TEA (60 µL; 44.6 mg; 0.44 mmol; 2.2 eq.) and Mukaiyama's reagent (61.4 mg; 0.24 mmol; 1.2 eq.), and the reaction was allowed to stir at r.t. overnight. The reaction was monitored by TLC (6 close spots) and upon completion, the solvents were removed and the product dissolved in ether (3 mL) washed with water (3 mL) and the aqueous layer extracted with ether (2 x 1 mL). The combined organic layers were dried over Na_2SO_4 and the solvents removed giving a crude product which was

purified by column chromatography (hexane/EtOAc 3:1) giving an off white powder. Yield: 7.9 mg (10%)

Or

Synthesised from **7** (147.4 mg; 0.84 mmol; 1 eq.), **62** (278.9 mg; 1.01 mmol; 1.2 eq.), TEA (468 µL; 340.5 mg; 3.36 mmol; 4 eq.) and $HgCl_2$ (342.5 mg; 1.26 mmol; 1.5 eq.) using Method D. The crude product was purified using column chromatography (hexane/EtOAc 3:1) giving a colourless oil, which solidified over time. Yield: 205.5 mg (59%)

mp 101 °C

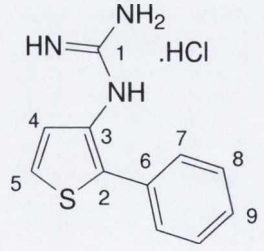
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 10.42 (s, 1H, N<u>H</u>C_3), 7.77 (d, 1H, J = 5.5 Hz, H_5), 7.51-7.54 (m, 2H, H_8), 7.42-7.46 (m, 2H, H_7), 7.34-7.38 (m, 1H, H_9), 7.26 (d, 1H, J = 5.5 Hz, H_4), 1.53 (s, 9H, $H_{4''}$), 1.50 (s, 9H, $H_{4'}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5 (C_1), 153.8 ($C_{2'}$), 153.0 ($C_{2''}$), 132.4 (C_6), 130.3 (C_2), 130.2 (C_3), 128.9 (C_7), 128.8 (C_8), 127.9 (C_9), 126.0 (C_5), 123.1 (C_4), 83.6 ($C_{3'}$), 79.6 ($C_{3''}$), 28.2 ($C_{4''}$), 28.0 ($C_{4'}$) ppm

IR ν_{max} 3265 (NH), 3113 (NH), 2976, 2930, 1718, 1614 (2xC=O), 1389, 1324, 1250, 1155, 1112, 1088, 1055, 757 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_{21}H_{27}O_4N_3S$ $[M + H]^+$ 418.1801, found 418.1887

_N_-(2-Phenylthien-3-yl)guanidine hydrochloride (70)



Synthesised using Method G from **69** (205.5 mg; 0.49 mmol; 1 eq.) giving a colourless gel. Yield: 14.7 mg (12 %)

Or

Synthesised using Method H from **69** (50.0 mg; 0.119 mmol; 1 eq.). Yield: 29.9 mg (99%)

^{1}H NMR (400 MHz, D_2O) δ 7.44 (d, 2H, *J* = 5.7 Hz, H_5), 7.39-7.32 (m, 4H, H_{7-9}), 6.94 (d, 1H, *J*= 5.7 Hz, H_4) ppm

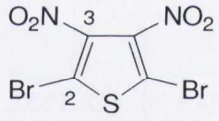
^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0 (C_1), 138.3 (C_6), 131.1 (C_2), 128.7 (C_7), 128.1 (C_8), 127.3 (C_9), 126.7 (C_5), 126.4 (C_3), 124.8 (C_4) ppm

IR ν_{max} 3147 (NH), 2433, 1659 (C=N), 1594, 1541, 1492, 1445, 1389, 1117, 1076, 838, 757, 691 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{11}H_{11}N_3S$ $[M + H]^+$ 218.0752, found 218.0758

cLogP: 2.81

2,5-Dibromo-3,4-dinitrothiophene (71)[132]



Sulfuric acid (20 µL) and fuming nitric acid (8 µL) were cooled at 0 °C before 2,5-dibromothiophene (50 µl; 107.7 mg; 0.44 mmol; 1 eq.) was added maintaining a temperature of 20-30 °C. The mixture was allowed to react for 3 h, and was then poured onto 50 g ice. Upon melting of the ice, the white precipitate was collected by vacuum filtration giving 83.1 mg of crude product which was recrystallised from methanol giving a pale green solid. Yield: 11.4 mg (8%)

Or

Synthesised from 2,5-dibromothiophene (4.0 mL; 8.560 g; 35.52 mmol; 1 eq.), H_2SO_4 (60 mL) and KNO_3 (7.600 g; 71.04 mmol; 2.1 eq.) using Method B, and recrystallised from MeOH giving orange/brown crystals. All repeats of this reaction were purified in this way and the leftover filtrate dried and further purified by recrystallisation or column chromatography (hexane/EtOAc 7:1) to give

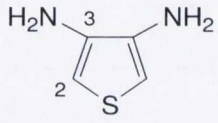
brown crystals/powder. Yield: 7.130 g (61%) or 69% average overall, over multiple reactions after column chromatography.

mp 136-138 °C (Lit.[132] 135-137 °C)

^{13}C NMR (100 MHz, DMSO-d_6) δ 139.7 (C_3), 116.6 (C_2) ppm

IR ν_{max} 1538 (NO_2), 1498, 1389, 1344, 1315 (NO_2), 1082 (C-Br), 937, 899, 749, 735 cm^{-1} (lit.[132] 1546, 1501, 1405, 1390, 1345, 1317, 1082, 937, 900, 749, 736)

3,4-Diaminothiophene (72)[132]



Compound **71** (6.000 g; 18 mmol; 1 eq.) was added to conc. HCl (250 mL) and cooled to 0 °C. Tin powder (40.000 g; 32 mmol; 1.8 eq.) was added, maintaining a temperature below 25 °C. The reaction was kept at 25 °C for 18 h and was then placed in a freezer overnight. The grey salt precipitate was recovered by vacuum filtration and was washed with diethyl ether followed by acetonitrile until the washings were colourless. The free amine was liberated prior to use by dissolving the salt in water basifying to pH 10-12 using 1 M NaOH and extracted using diethyl ether giving an off white solid. Yield: 787.7 mg (38%)

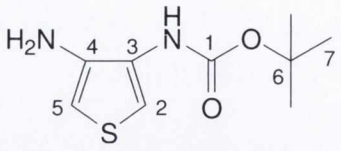
mp 88-96 °C (lit.[132] 95.5-96.9 °C)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.20 (s, 2H, H_2), 3.32 (brs, 4H, NH_2) ppm (Lit.[132] 6.16, 3.36)

^{13}C NMR (100 MHz, DMSO-d_6) δ 138.5 (C_3), 95.8 (C_2) ppm

IR ν_{max} 3355 (NH_2), 3282, 3092 (NH_2), 1606, 1476, 1443, 1134, 752, 702 cm^{-1}

tert-Butyl _N_-(4-aminothien-3-yl)carbamate (73)[140]



Compound **72** (1.667 g; 14.6 mmol; 1 eq.) was dissolved in dry THF (50 mL). Di-*tert*-butyldicarbonate (3.340 g; 15.33 mmol; 1.05 eq.) was dissolved in dry THF (300 mL) and was added to the solution of **72** dropwise over 2 h. The solution was stirred for a further 12 h before the solvents were removed. The residue was dissolved in minimal DCM, extracted with 1 N HCl, washed with DCM and basified to pH 10-12 using 1 M NaOH. The aqueous layer was then extracted in ether and dried over $MgSO_4$ giving a crude product. This was dissolved in minimal DCM and hexane was added until the dark impurity had precipitated out. The mixture was filtered and most of the solvents were removed, before the remaining mixture was placed in a freezer for 24 h. The mixture was then cold filtered and the precipitate washed with hexane to give an orange powder. Yield: 1.280 g (41%)

mp 113-115 °C (Lit.[140] 115 °C)

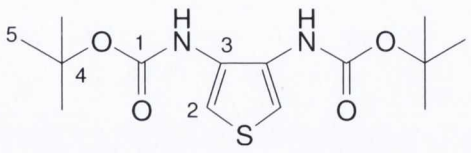
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (app. brs, 1H, H_2), 6.68 (app. brs, 1H, H_5), 6.32 (s, 1H, NH), 3.41 (s, 2H, NH_2), 1.53 (s, 9H, H_7) ppm (Lit.[140] 7.10, 6.50, 6.30, 3.25, 1.49)

IR ν_{max} 3360 (NH_2), 3118 (NH_2), 2961, 1685, 1523, 1282, 1243, 1153, 1051, 855 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_9H_{14}O_2N_2S$ $[M + H]^+$ 215.0854, found 215.0864

Isolation of product was also carried out by column chromatography (3:1 hexane/EtOAc) but usually resulted in poorer yields.

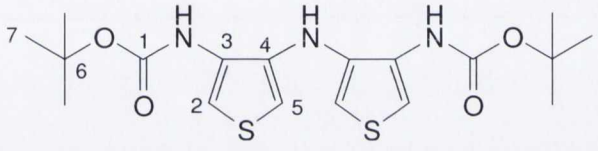
The corresponding 3,4-dicarbamate, *O-tert*-Butyl-*N*-(4-*tert*-butoxycarbonylaminothien-3-yl)carbamate (**76**),[140] was also formed and was collected from the first DCM layer giving a brown solid. Yield: 1.800 g (39%)



mp 159 °C (Lit.[140] 160 °C)

^{1}H NMR (400 MHz, CDCl3) δ 7.17 (app. brs, 2H, H_2), 6.84 (s, 2H, NH), 1.53 (s, 18H, H_{35}) ppm (Lit.[140] 7.12, 6.77, 1.49)

O-tert-Butyl-[4-(4-_tert_-butoxycarbonylaminothien-3-yl)-aminothien-3-yl]carbamate (74)[140]



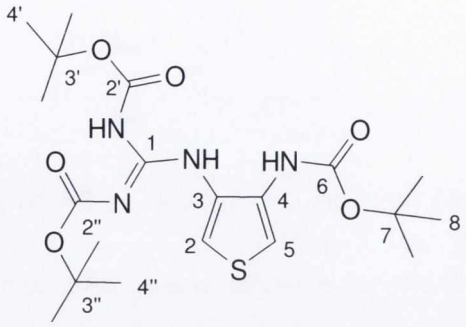
Compound **73** (1.000 g; 5.04 mmol; 1 eq.) in AcOH (12.5 mL) was heated under reflux for 1 h. The AcOH was removed under reduced pressure and the residue was dissolved in DCM, washed with 1 N HCl and brine, dried over Na_2SO_4 and the solvents removed. The crude product was purified by column chromatography (hexane/EtOAc 3:1) giving a brown powder. Yield: 596.9 mg (62%)

mp 90 °C, decomposes 96-98 °C (Lit.[140] 98 °C)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (app. brs, 2H, H_2), 6.70 (brs, 2H, 2xNH), 6.53 (d, 2H, J = 3.5 Hz, H_5), 5.32 (brs, 1H, NH), 1.53 (s, 18H, H_7) ppm (Lit.[140] 7.19, 6.80, 6.46, 5.70, 1.48)

IR ν_{max} 3314 (NH_2 and NH), 2977 (NH_2), 1691, 1523, 1366, 1241, 1150, 1047, 1019, 859, 767 cm^{-1}

[2,3-Di(*tert*-butoxycarbonyl)]-1-(4-*tert*-butoxycarbonylaminothien-3-yl)guanidine (79)



Synthesised from **73** (350.0 mg; 1.63 mmol; 1 eq.), **62** (541.7 mg; 1.96 mmol; 1.2 eq.), TEA (800 µL; 578.0 mg; 5.72 mmol; 3.5 eq.) and $HgCl_2$ (532.0 mg; 1.96 mmol; 1.2 eq.) using Method D*. The product was purified by column chromatography (hexane/EtOAc; 3:1) giving a white powder. Yield: 453.0 mg (61%)

mp 200-203 °C

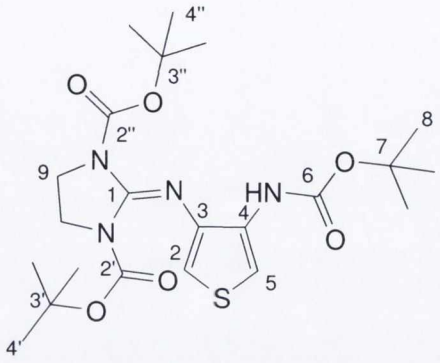
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.57 (brs, 1H, N<u>H</u>$C_{2'}$), 10.25 (brs, 1H, N<u>H</u>C_3), 8.25 (brs, 1H, N<u>H</u>C_4), 7.39 (s, 1H, H_2), 7.12 (s, 1H, H_5), 1.54 (s, 9H, $H_{4''}$), 1.52 (s, 9H, $H_{4'}$), 1.50 (s, 9H, H_8) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7 (C_1), 153.8 (C_6), 152.6 ($C_{2'}$), 153.1 ($C_{2''}$), 131.0 (C_3), 127.8 (C_4), 114.8 (C_5), 112.0 (C_2), 84.1 ($C_{3'}$), 80.1 ($C_{3''}$), 79.9 (C_7), 28.4 (C_8), 28.1 ($C_{4''}$), 28.0 ($C_{4'}$) ppm

IR ν_{max} 3370 (NH), 3165 (NH), 2980 (NH), 1720, 1649 (C=O), 1619 (C=O), 1597 (C=O), 1496, 1450, 1392, 1365, 1319, 1275, 1234, 1148, 1096, 1058, 1029, 862, 799, 772, 662 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{20}H_{32}N_4O_6S$ $[M + Na]^+$ 457.2121, found 457.2118

[1,3-Di(*tert*-butoxycarbonyl)]-2-(4-*tert*-butoxylcarbonylaminothiophene-3-ylimino)imidazolidine (80)

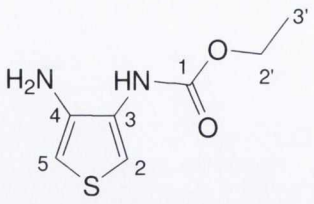


Synthesised from **73** (50.0 mg; 0.233 mmol; 1 eq.), **63** (84.7 mg; 0.28 mmol; 1.2 eq.), TEA (115 µL; 82.6 mg; 0.816 mmol; 3.5 eq.) and $HgCl_2$ (76.0 mg; 0.28 mmol; 1.2 eq.) using Method D*. 168.8 mg of crude product was obtained. Purification was attempted by silica column chromatography (3:2 hexane/EtOAc) (TLC systems tried EtOAc, DCM, hexane:EtOAc 3:1, 3:2, 4:1 on silica and 3:1 and 3:2 on alumina) and by fractional recrystallisation from ether/hexane, but the product was not separable from **63** but was obtained in a 2:1 ratio. 10.6 mg of a yellow solid was obtained calculated as 8.1 mg intended product (7%).

mp decompose 80 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (app. brs, 1H, H_2), 6.52 (app. brs, 1H, H_5), 3.76 (s, 4H, H_9), 1.60 (s, 9H, $H_{4'}$), 1.52 (s, 9H, $H_{4''}$), 1.42 (s ,9H, H_8) ppm

Ethyl *N*-(4-aminothien-3-yl)carbamate (81)[140]



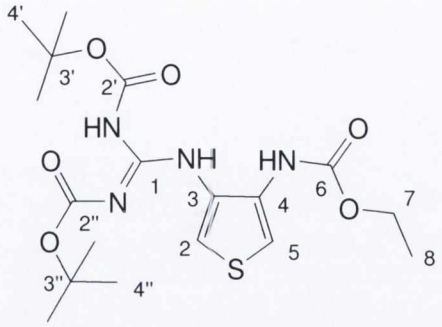
Ethyl chloroformate (611 µL; 696.0 mg; 6.4 mmol; 1 eq.) was added to a suspension of 3,4-diaminothiophene dihydrochloride (1.200 g; 6.4 mmol; 1 eq.) in dry DCM (65 mL) under argon. A solution of TEA (3.13 mL; 2.274 g; 1.6 mmol; 1 eq.) in dry DCM (20 mL) was added dropwise and the mixture was stirred for 4 h. The solvents were then removed and the crude material was dissolved in DCM, extracted in 1N HCl, and the aqueous layer washed with DCM. The aqueous layer was then basified to pH 9 using $NaHCO_3$, extracted in DCM, dried over Na_2SO_4 and the solvents removed giving a brown oil. Yield: 439.3 mg (37%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (app. brs, 1H, H_2), 6.85 (brs, 1H, NH), 6.24 (d, 1H, J = 3.5 Hz, H_5), 4.15 (q, 2H, J = 7.1 Hz, $H_{2'}$), 3.37 (brs, 2H, NH_2), 1.23 (t, 3H, J = 7.1 Hz, $H_{3'}$) ppm (Lit.[140] 7.10, 7.00, 6.24, 4.18, 3.40, 1.26)

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1 (C_1), 136.8 (C_4), 128.5 (C_3), 110.4 (C_5), 104.1 (C_2), 61.3 ($C_{2'}$), 14.4 ($C_{3'}$)ppm (Lit.[140] 154.3, 137.1, 127.8, 110.6, 102.3, 61.1, 14.1)

HRMS (ESI) *m/z* calc. for $C_7H_{10}N_2O_2S$ $[M+H]^+$ 187.0541, found 187.0538

[2,3-Di(*tert*-butoxycarbonyl)]-1-(4-ethoxycarbonylaminothien-3-yl)guanidine (82)



Synthesised from **81** (96.5 mg; 0.52 mmol; 1 eq.), **62** (171.8 mg; 0.62 mmol; 1.2 eq), TEA (290 µL; 209.8 mg; 2.07 mmol; 4 eq.) and $HgCl_2$ (168.8 mg; 0.62 mmol; 1.2 eq.) using Method D giving a white powder. Purified by column chromatography (hexane/EtOAc 3:1). Yield: 192.0 mg (86%)

mp decomposes 200 °C

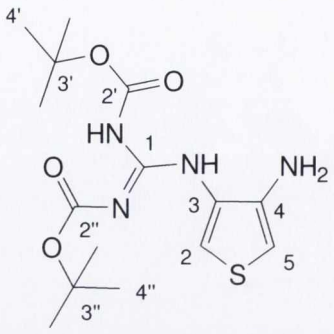
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.54 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 10.25 (s, 1H, N<u>H</u>C_3), 8.70 (brs, 1H, N<u>H</u>CO_2Et), 7.42 (app. brs, 1H, H_2), 7.09 (app. brs, 1H, H_5), 4.21 (q, 2H, J = 7.1 Hz, H_7), 1.54 (s, 9H, $H_{4''}$), 1.50 (s, 9H, $H_{4'}$), 1.31 (t, 3H, J = 7.1 Hz, H_8) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6 (C_1), 154.5 (C_6), 153.7 ($C_{2'}$), 153.1 ($C_{2''}$), 130.9 (C_3), 127.7 (C_4), 114.9 (C_5), 112.1 (C_2), 84.2 ($C_{3'}$), 80.0 ($C_{3''}$), 61.1 (C_7), 28.1 ($C_{4''}$), 28.0 ($C_{4'}$), 14.6 (C_8) ppm

IR ν_{max} 3357 (NH), 3160 (NH), 2978 (NH), 1732, 1721, 1643 (C=O), 1623 (C=O), 1602 (C=O), 1393, 1368, 1309, 1276, 1233, 1139, 1091, 1056, 797, 766, 752 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{18}H_{28}N_4O_6S$ $[M + H]^+$ 429.1808, found 429.1787

[2,3-Di(*tert*-butoxycarbonyl)]-1-(4-aminothien-3-yl) guanidine (85)



Synthesised from **72**.HCl (100.0 mg; 0.53 mmol; 1.0 eq.), **5** (147.7 mg; 0.534 mmol; 1.0 eq.), TEA (450 µL; 325.0 mg; 3.2 mmol; 6.0 eq.) and $HgCl_2$ (94.8 mg; 0.349 mmol; 1.5 eq.) using Method D. Purified by Biotage column chromatography (hexane/EtOAc; 4 CV/% gradient) giving a pale yellow solid which turned brown over time. Yield: 93.3 mg (49%)

mp 128-130 °C

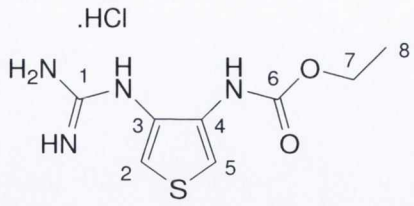
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.55 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 10.30 (s, 1H, N<u>H</u>C_3), 7.43 (d, 1H, ^4J = 3.5 Hz, H_2), 6.30 (d, 1H, ^4J = 3.5 Hz, H_5), 3.78 (2H, brs, NH_2), 1.53 (s, 9H, $H_{4''}$), 1.49 (s, 9H, $H_{4'}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9 (C_1), 152.8 ($C_{2'}$), 152.7 ($C_{2''}$), 137.9 (C_3), 127.0 (C_4), 113.7 (C_5), 103.1 (C_2), 83.3 ($C_{3'}$), 79.2 ($C_{3''}$), 27.7 ($C_{4''}$), 27.6 ($C_{4'}$) ppm

IR ν_{max} 3405 (NH_2), 3321 (NH_2), 3265 (NH), 2975 (NH), 1713, 1618(2xC=O), 1578, 1618, 1578, 1406, 1368, 1328, 1251, 1139, 1060, 776 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{15}H_{24}N_4O_4S$ $[M + Na]^+$ 379.1416, found 379.1416

_N_-(4-Ethoxycarbonylaminothien-3-yl)guanidine hydrochloride (87)



Synthesised using Method G from **82** (100.0 mg; 0.23 mmol; 1 eq.) giving a white solid. Yield: 42.9 mg (58%)

Or

Synthesised using Method H from **82** (100 mg; 0.23 mmol; 1 eq.) Yield: 56.9 mg (92%)

mp decomposes > 150 °C

^{1}H NMR (400 MHz, D_2O) δ 7.35 (brs, 1H, H_2), 7.29 (brs, 1H, H_5), 4.06 (q, 2H, J = 1.8 Hz, H_7), 1.15 (t, 3H, J = 1.8 Hz, H_8) ppm

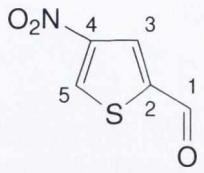
^{13}C NMR (100 MHz, D_2O) δ ppm 156.5 (C_1), 156.1 (C_6), 130.8 (C_2), 124.7 (C_3), 122.8 (C_5), 113.4 (C_4), 62.0 (C_7), 13.2 (C_8) ppm

IR ν_{max} 3392 (NH), 3186 (NH), 3101 (NH), 2944 (NH), 2477, 2259, 1644 (C=O), 1600, 1551 (C=N), 1499, 1373, 1253, 1064, 766 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_8H_{12}N_4O_2S$ $[M + H]^+$ 229.0759, found 229.0755

$C_8H_{13}ClN_4O_2S \cdot 1.3D_2O$ requires C, 33.05; H, 4.51; N, 19.27%. Found: C, 33.44; H, 4.6; N, 19.49%

4-Nitro-2-thiophenecarboxaldehyde (91)[143]



Synthesised, using Method B, from **90** (1 mL; 1.223 g; 10.9 mmol; 1 eq.), H_2SO_4 (10 mL) and KNO_3 (1.100 g; 10.9 mmol; 1 eq.) and poured over 40 g ice, and recrystallised twice from ether/hexane 1:1 giving yellow needles or purified by silica column (2:1 hexane/EtOAc). Yield: 950.0 mg (55%)

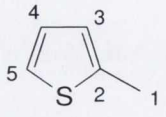
mp 54-56 °C (Lit.[143] 55-56 °C)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.98 (d, 1H, ^4J = 1.4 Hz, H_1), 8.66 (dd, 1H, ^4J = 1.4 Hz, 1.4 Hz, H_3), 8.30 (d, 1H, ^4J = 1.4 Hz, H_5) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6 (C_1), 148.3 (C_4), 143.2 (C_2), 134.0 (C_3), 129.0 (C_5) ppm

IR ν_{max} 3120, 3098, 2869, 1669 (C=O), 1531, 1505 (NO_2), 1402, 1363, 1334 (NO_2), 1220, 1166, 1080, 882, 867, 786, 734, 706 cm^{-1}

2-Methylthiophene (93)[151,202]



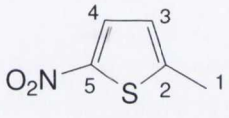
Compound **90** (1 mL; 1.223 g; 10.9 mmol; 1 eq.), hydrazine hydrate (1 mL) and ethylene glycol (10 mL) were heated until the excess water and hydrazine had been distilled off at 130-160 °C. The mixture was then cooled to below 60 °C before KOH (1.000 g) was added and the mixture heated under reflux until nitrogen evolution ceased. The product was then collected by distillation, and the distillate was

taken up in EtOAc and the aqueous layer separated. The organic layer was then dried over Na_2SO_4 and the solvent removed giving a colourless liquid. Yield: 592.6 mg (55%)

bp 112 °C (Lit.[151] 112-113 °C)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (dd, 1H, J = 5.2, ^4J = 1.2 Hz, H_5), 6.98 (dd, 1H, J = 5.2, 3.4 Hz, H_4), 6.86-6.84 (m, 1H, H_3), 2.59 (d, 3H, ^4J = 1.0 Hz, H_1) ppm (Lit.[202] 7.07, 6.89, 6.75, 2.50)

2-Methyl-5-nitrothiophene (94)[153,203]

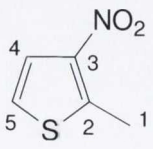


To a solution of **93** (1 mL; 1.014 g; 10.3 mmol; 1 eq.) in AcOH (1.9 mL) at -20 °C under nitrogen, was added slowly a solution of fuming HNO_3 (430 µL; 649.1 mg; 10.3 mmol; 1 eq.) in AcOAc (1.5 mL). After the addition was completed, the mixture was poured over ice, neutralised with $NaHCO_3$, extracted with ether, dried over $MgSO_4$ and the solvents removed. Steam distillation of the residue was carried out, and the distillate extracted with EtOAc, dried and the solvent removed. Vacuum distillation was attempted with products distilling at 60 °C and 110 °C at 20 mbar. No pure products were isolated, so the fractions were combined giving a yellow liquid as a 3:1 mixture of regioisomers to be used in the next step. Yield: 613.1 mg (42%)

bp 110 °C at 20 mbar (Lit.[153] 104 °C at 15 mm Hg)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, 1H, J = 4.2 Hz, H_4), 6.77 (d, 1H, J = 4.2 Hz, H_3), 2.57 (s, 3H, H_1) ppm (Lit.[204] 7.76, 6.76, 2.55)

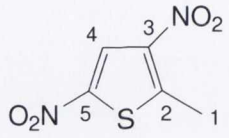
The minor isomer 2-methyl-3-nitrothiophene (**48**) was also collected:



bp 60 °C at 20 mbar

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, 1H, J = 5.7 Hz, H_4), 7.05 (d, 1H, J = 5.7 Hz, H_3), 2.81 (s, 3H, H_1) ppm

2-Methyl-3,5-dinitrothiophene (99)[153]



Synthesised, following Method B, from **93** (1 mL; 1.019 g; 10.34 mmol; 1 eq.), H_2SO_4 (15 mL) and KNO_3 (1.05 g; 10.34 mmol; 1 eq.) using 40 g ice. The crude product was recrystallised from 2-propanol giving golden crystals. Yield: 220.5 mg (23%)

mp 98 °C (Lit.[154] 98-99 °C)

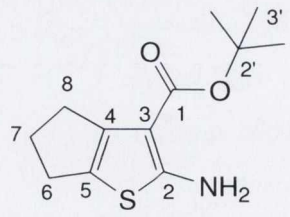
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H, H_4), 2.91 (s, 3H, H_1) ppm

^{13}C NMR (100 MHz, $CDCl_3$) 149.4 (C_3), 145.4 (C_5), 142.3 (C_2), 124.4 (C_4), 16.3 (C_1) ppm

IR ν_{max} 3107, 1550, 1508 (NO_2), 1498 (NO_2), 1318 (2xNO_2), 1103, 1040, 888, 817, 772, 730, 674 cm^{-1} (Lit.[153] 3111, 1555, 1511, 1329, 1103, 1040, 889, 818, 731, 675)

HRMS (ESI) *m/z* calc. for $C_5H_4N_3O_4S$ $[M - H]^-$ 186.9814, found 186.9809

***tert*-Butyl-2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (103)**[205]



Synthesised from cyclopentanone (168.0 mg; 2.0 mmol; 1.0 eq.), via method C for 4 h. The solvent was removed under reduced pressure and the crude product was purified by silica column chromatography (hexane/EtOAc 10:1) and recrystallisation from hexane/EtOAc giving trace amounts of an the desired product as an orange powder.

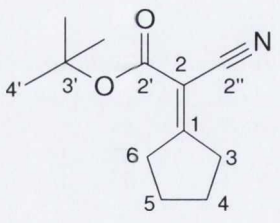
Or

To a mixture of **104** (50.0 mg, 0.24 mmol, 1.0 eq.), sulfur powder (7.7 mg, 0.24 mmol, 1.0 eq.) and EtOH (300 µL) at 60 °C was added dropwise morpholine (21.0 mg, 0.24 mmol, 1.0 eq.). The mixture was stirred at 60 °C for 4h and at r.t. for 14 h. The mixture was diluted with 0.4 M AcOH (1.44 mL), extracted with ether, dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification via silica column chromatography (hexane/EtOAc 10:1) yielded a yellow oil. Yield: 10.5 mg (18%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (brs, 2H, NH_2), 2.82 (t, 2H, J = 7.0 Hz, H_6), 2.73 (t, 2H, J = 7.0 Hz, H_8), 2.32 (quint., 2H, J = 7.0 Hz, H_7), 1.56 (s, 9H, $H_{3'}$) ppm (Lit.[205] 2.78, 2.69, 2.27, 1.51)

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6 (C_1), 165.3 (C_2), 142.9 (C_4), 121.3 (C_5), 104.9 (C_3), 79.9 ($C_{2'}$), 31.0 (C_6), 28.9 (C_8), 28.5 ($C_{3'}$), 27.2 (C_7) ppm (Lit.[205] 165.32, 142.84, 121.01, 104.40, 79.78, 30.94, 28.84, 28.50, 27.14)

***tert*-Butyl-(1-cyclopentylidene)-2-cyanoacetate (104).[205]**



Synthesised from cyclopentanone (168 mg; 2.0 mmol; 1.0 eq.), via method C for 4 h affording a yellow powder. The solvent was removed under reduced pressure and the crude product was purified by silica column chromatography (hexane/EtOAc 10:1) giving an orange powder. Yield: 58.1 mg (14%)

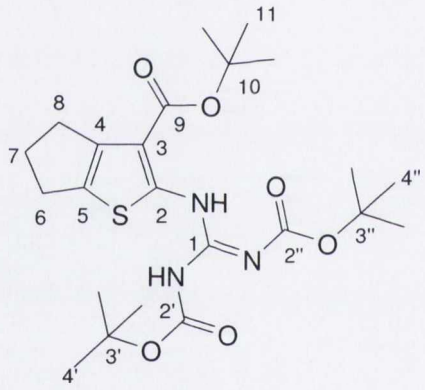
mp 76-78 °C (Lit.[206] 87-88 °C)

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.99 (t, 2H, *J* = 7.6 Hz, H_6), 2.81 (t, 2H, *J* = 6.8 Hz, H_3), 1.85 (m, 4H, $H_{4,5}$), 1.55 (s, 9H, $H_{4'}$) ppm (Lit.[205] 2.92-2.95, 2.74-2.77, 1.73-1.85, 1.50)

^{13}C NMR (100 MHz, $CDCl_3$) δ 185.6 (C_1), 161.1 ($C_{2'}$), 115.9 ($C_{2''}$), 102.40 (C_2), 82.6 ($C_{3'}$), 37.7 (C_6), 35.2 (C_3), 28.0 ($C_{4'}$), 26.6 (C_5), 25.1 (C_4) ppm (Lit.[205] 185.68, 161.09, 115.88, 102.35, 82.61, 37.67, 35.18, 28.02, 26.59, 25.06)

IR ν_{max} 3323, 2971, 2221 (CN), 1718 (C=O), 1662, 1612, 1475, 1457, 1396, 1373, 1365, 1315, 1285, 1259, 1240, 1214, 1151, 1094, 1030, 1000, 949, 841, 795, 777 cm^{-1}

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-*tert*-butoxycarbonyl-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)guanidine (105)



Synthesised from **103** (218.0 mg; 0.91 mmol; 1.0 eq.), **62** (276.0 mg; 1.00 mmol; 1.1 eq.), TEA (443 µl; 322.0 mg; 3.18 mmol; 3.5 eq.) and $HgCl_2$ (272.0 mg; 1.0 mmol; 1.1 eq.) using Method D. The product was purified by silica column chromatography (hexane/ EtOAc; 5:1) and was obtained as a white powder. Yield: 179.0 mg (41%)

mp 196 °C

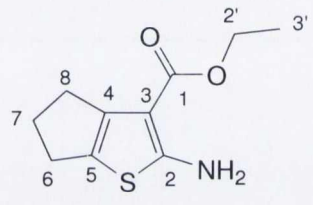
^{1}H NMR (600 MHz, $CDCl_3$) δ 12.63 (brs, 1H, N<u>H</u>C_2), 11.36 (brs, 1H, N<u>H</u>$CO_2{}^tBu$), 2.90 (t, 2H, *J* = 7.2 Hz, H_6), 2.86 (t, 2H, *J* = 7.0 Hz, H_8), 2.31-2.38 (m, 2H, H_7), 1.60 (s, 9H, C_{11}), 1.58 (s, 9H, $H_{4'}$), 1.56 (s, 9H, $H_{4'}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7 (C_9), 162.5 (C_1), 151.6 ($C_{2'}$), 150.7 ($C_{2''}$), 148.8 (C_2), 141.7 (C_4), 134.5 (C_5), 111.7 (C_3), 83.5 ($C_{3'}$), 81.0 (C_{10}), 79.9 ($C_{3''}$), 30.9 (C_6), 29.0 (C_8), 28.5 (C_{11}), 28.2 ($C_{4''}$), 28.2 ($C_{4'}$), 27.7 (C_7) ppm

IR ν_{max} 3070 (NH), 2978 (NH), 1737 (C=O), 1680 (C=O), 1643 (C=O), 1610, 1567, 1389, 1367, 1303, 1222, 1148, 1116, 1060, 1027, 807, 763, 713 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_{23}H_{35}N_3O_6S$ $[M+H]^+$ 482.2325, found 482.2330, $[M+Na]^+$ 504.2144, found 504.2149

Ethyl-2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (106)[159]

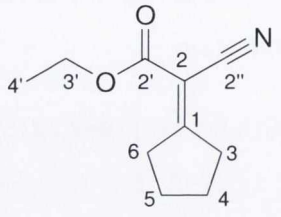


Synthesised from cyclopentanone (3.4 mL 3.230 g, 0.04 mol, 1.0 eq.), sulfur powder (1.230 g, 0.04 mol, 1.0 eq.), ethyl cyanoacetate (4 mL; 4.350 g; 0.04 mol; 1.0 eq.), morpholine (3.4 mL) and EtOH (8 mL) via method C over 4 h. The solvents were removed and the oily residue was purified as much as possible by column chromatography (Biotage: hexane/EtOAc; 0-10% over 30 CV) giving an orange powder, which was about ¾ the intended product by ^{1}H NMR. Yield: 1.027 g (approximately 800 mg intended product; 10%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84 (brs, 2H, NH_2), 4.21 (q, 2H, *J* = 7.0 Hz, $H_{2'}$), 2.74 (t, 2H, *J* = 6.6 Hz, H_6), 2.64 (t, 2H, *J* = 6.8 Hz, H_8), 2.27-2.20 (m, 2H, H_7), 1.28 (t, 3H, *J* = 7.0 Hz, $H_{3'}$) ppm (Lit.[159] 5.83, 4.25, 2.85-2.80, 2.74-2.69, 2.36-2.26, 1.33)

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 (C_1), 165.7 (C_2), 142.7 (C_4), 121.3 (C_5), 103.0 (C_3), 59.4 ($C_{2'}$), 30.8 (C_6), 28.9 (C_8), 27.2 (C_7), 14.4 ($C_{3'}$) ppm (Lit.[159] 166.2, 165.8, 142.7, 121.4, 103.0, 59.4, 30.8, 28.9, 27.2, 14.4)

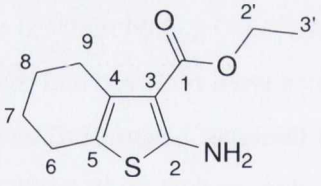
The knoevenagel intermediate, ethyl-(1-cyclopentylidene)-2-cyanoacetate, gave the following spectral data:



^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (q, 2H, *J* = 7.0 Hz, $H_{3'}$), 2.93 (t, 2H, *J* = 7.1 Hz, H_6), 2.74 (t, 2H, *J* = 7.0 Hz, H_3) 2.24 (m, 2H, H), 1.81 (m, 2H, H_4), 1.75 (m, 2H, H_5), 1.26 (t, 3H, *J* = 7.0 Hz, $H_{4'}$) ppm

^{13}C NMR (150 MHz, $CDCl_3$) δ 187.3 (C_1), 161.9 ($C_{2'}$), 115.6 ($C_{2''}$), 100.8 (C_2), 61.5 ($C_{3'}$), 37.8 (C_3), 35.4 (C_6), 26.5 (C_4), 25.1 (C_5), 14.1 ($C_{4'}$) ppm

Ethyl-2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (107)[159]



Synthesised from cyclohexanone (4.2 mL; 3.920 g, 0.04 mol, 1.0 eq.), sulfur powder (1.230 g, 0.04 mol, 1.0 eq.), ethyl cyanoacetate (4 mL; 4.350 g; 0.04 mol; 1 eq.) morpholine (3.4 mL) and EtOH (8 mL) via Method C over 4 h. The reaction mixture was removed from the heat and cooled in a freezer overnight, giving pale yellow crystals, which were collected by vacuum filtration. Yield: 7.150 g (79%)

mp 107 °C (Lit.[159] 113 °C)

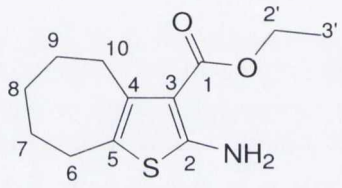
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.99 (brs, 2H, NH_2), 4.27 (q, 2H, *J* = 7.3 Hz, $H_{2'}$), 2.72 (t, 2H, *J* = 5.3 Hz, H_6), 2.52 (t, 2H, *J* = 5.2 Hz, H_9), 1.80-1.75 (m, 4H, $H_{7,8}$), 1.35 (t, 3H, *J* = 7.3 Hz, $H_{3'}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7 (C_1), 161.3 (C_2), 132.0 (C_4), 117.2 (C_5), 105.2 (C_3), 58.9 ($C_{2'}$), 26.5 (C_6), 24.1 (C_9), 22.8 (C_7), 22.4 (C_8), 14.0 ($C_{3'}$) ppm

IR ν_{max} 3399 (NH), 3293 (NH), 2938, 1643 (C=O), 1575 (C=N), 1490, 1427, 1411, 1367, 1294, 1272, 1178, 1152, 1026, 781 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{11}H_{15}N_3O_2S$ [M + H] 226.0902, found 226.0910

Ethyl-2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (108)[159,207]



Synthesised from cycloheptanone (4.7 mL; 4.500 g; 0.04 mol; 1.0 eq.), sulfur powder (1.230 g; 0.04 mol; 1.0 eq.), and ethyl cyanoacetate (4 mL; 4.350 g; 0.04 mol; 1.0 eq.) morpholine (3.4 mL) and EtOH (8 mL) via Method C over 4 h. The reaction mixture was removed from the heat and cooled in a freezer overnight, giving yellow crystals, which were collected by vacuum filtration. Yield: 5.820 g (61%)

mp. 80-81 °C (Lit.[159] 87 °C)

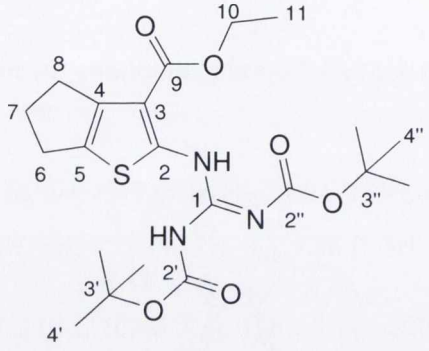
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (brs, 2H, NH_2), 4.31 (q, 2H, *J* = 7.1 Hz, $H_{2'}$), 3.00 (t, 2H, *J* = 5.5 Hz, H_6), 2.61 (t, 2H, *J* = 5.7 Hz, H_{10}), 1.87-1.81 (m, 2H, H_8), 1.70-1.61 (m, 4H, $H_{7,9}$), 1.38 (t, 3H, *J* = 7.1 Hz, $H_{3'}$) ppm (Lit.[207] 5.78, 4.27, 2.98-2.96, 2.58-2.55, 1.85-1.79, 1.66-1.59, 1.34)

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0 (C_1), 159.8 (C_2), 138.0 (C_4), 121.3 (C_5), 107.6 (C_3), 59.6 ($C_{2'}$), 32.1 (C_8), 28.7 (C_6), 28.6 (C_{10}), 27.8 (C_7), 26.9 (C_9), 14.5 ($C_{3'}$) ppm (Lit.[207] 165.9, 159.9, 137.9, 121.2, 107.5, 59.5, 32.1, 28.7, 28.6, 27.8, 26.9, 14.4)

IR ν_{max} 3394 (NH_2), 3299 (NH_2), 2921, 2849, 1650 (C=O), 1591, 1560, 1480, 1407, 1299, 1275, 1159, 1024, 781 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{12}H_{17}NO_2S$ $[M + H]^+$ 240.1058, found 240.1068

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-ethoxycarbonyl-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)guanidine (109).



Synthesised from **106** (400.0 mg; 1.90 mmol; 1.0 eq.), **62** (628.5 mg 2.274 mmol; 1.2 eq.), TEA (1 mL; 767.3 mg; 7.58 mmol; 4.0 eq.) and $HgCl_2$ (771.7 mg; 2.843 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient over 30 CV) giving a white powder. Yield: 308.9 mg (36%)

mp 170-174 °C

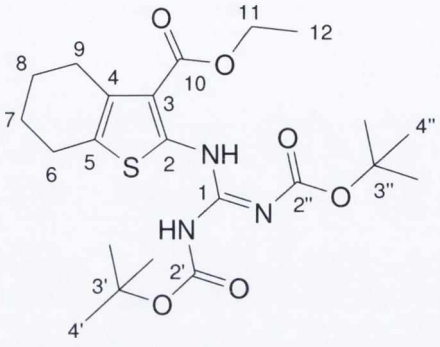
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.64 (s, 1H, N<u>H</u>C_2), 11.36 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 4.40 (q, 2H, *J* = 7.1 Hz, H_{10}), 2.93 (t, 2H, *J* = 6.7, H_6), 2.86 (t, 2H, *J* = 6.7, H_8), 2.34-2.40 (m, 2H, H_7), 1.57 (s, 9H, $H_{4'}$), 1.56 (s, 9H, $H_{4''}$), 1.38 (t, 3H, *J* = 7.1 Hz, H_{11}) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7 (C_9), 162.1 (C_1), 151.4 ($C_{2'}$), 150.1 ($C_{2''}$), 148.9 (C_2), 141.4 (C_4), 134.4 (C_5), 109.6 (C_3), 83.2 ($C_{3'}$), 79.6 ($C_{3''}$), 59.9 (C_{10}), 30.2 (C_6), 28.6 (C_8), 27.7 ($C_{4''}$), 27.7 ($C_{4'}$), 27.3 (C_7), 13.9 (C_{11}) ppm

IR ν_{max} 3150 (NH), 2974 (NH), 2928, 1736 (C=O), 1642 (C=O), 1609 (C=O), 1565, 1392, 1299, 1212, 1150, 1115, 1051, 1028, 807, 761 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{21}H_{31}N_3O_6S$ $[M + Na]^+$ 476.1831, found 476.1829

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-ethoxycarbonyl-4,5,6,7-tetrahydro-4*H*-benzo[*b*]thien-2-yl)guanidine (110)



Synthesised from **107** (500.0 mg; 2.22 mmol; 1.0 eq.), **62** (736.0 mg; 2.66 mmol; 1.2 eq.), TEA (1.24 mL; 898.5 mg; 8.88 mmol; 4.0 eq.) and $HgCl_2$ (903.7 mg; 3.33 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) and was obtained as an off white powder. Yield: 542.0 mg (52%)

mp 165 °C

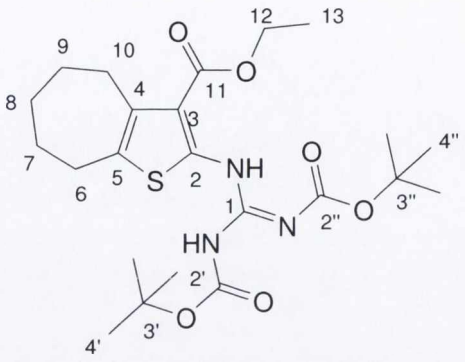
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.71 (brs, 1H, N<u>H</u>C_2), 11.39 (brs, 1H, N<u>H</u>$CO_2{}^t$Bu), 4.44 (q, 2H, *J* = 7.3 Hz, H_{11}), 2.73 (app. brs, 2H, H_6), 2.61 (app. brs, 2H, H_9), 1.73 (app. brs, 4H, $H_{7,8}$), 1.52 (s, 9H, $H_{4'}$), 1.51 (s, 9H, $H_{4''}$), 1.34 (t, 3H, *J* = 7.3 Hz, H_{12}) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7 (C_{10}), 162.1 (C_1), 151.3 ($C_{2'}$), 150.2 ($C_{2''}$), 144.6 (C_2), 130.9 (C_4), 128.7 (C_5), 112.9 (C_3), 83.0 ($C_{3'}$), 79.4 ($C_{3''}$), 59.8 (C_{11}), 27.7 ($C_{4''}$), 27.6 ($C_{4'}$), 25.8 (C_6), 23.8 (C_9), 22.4 (C_8), 22.4 (C_7), 13.9 (C_{12}) ppm

IR ν_{max} 3135 (NH), 2972 (NH), 2928, 1740 (C=O), 1643 (C=O), 1610 (C=O), 1566, 1402, 1306, 1291, 1216, 1152, 1113, 1060, 1027, 804, 780 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_{22}H_{33}N_3O_6S$ $[M+Na]^+$ 490.1988, found 490.1965

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-ethoxycarbonyl-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thien-2-yl)guanidine (111)



Synthesised from **108** (478.0 mg; 2 mmol; 1 eq.), *S*-methylthiopseudourea (639.0 mg; 2.2 mmol; 1.1 eq.), TEA (974 µL; 708.0 mg; 7 mmol; 3.5 eq.) and $HgCl_2$ (815.0 mg; 3 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 472.6 mg (49%)

mp 121-123 °C

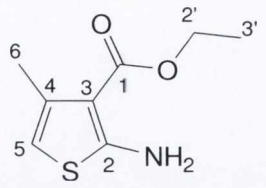
^{1}H NMR (400MHz, $CDCl_3$) δ 12.53 (brs, 1H, N<u>H</u>C_2), 11.39 (brs, 1H, N<u>H</u>$CO_2{}^t$Bu), 4.43 (q, 2H, *J* =7.1 Hz, H_{12}), 3.04 (t, 2H, *J* = 3.2 Hz, H_6), 2.73 (t, 2H, *J* = 3.0 Hz, H_{10}), 1.87-1.81 (m, 2H, H_8), 1.66-1.63 (m, 2H, H_7), 1.61-1.59 (m, 2H, H_9), 1.54 (s, 9H, $H_{4'}$), 1.53 (s, 9H, $H_{4''}$), 1.37 (t, 3H, *J* = 7.1 Hz, H_{13}) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8 (C_{11}), 162.5 (C_1), 151.8 ($C_{2'}$), 150.6 ($C_{2''}$), 142.1 (C_2), 137.1 (C_4), 133.5 (C_5), 115.0 (C_3), 83.4 ($C_{3'}$), 79.8 ($C_{3''}$), 60.4 (C_{12}), 32.3 (C_8), 28.4 (C_{10}), 28.1 ($C_{4''}$), 28.0 ($C_{4'}$), 28.0 (C_6), 27.7 (C_7), 27.0 (C_9), 14.3 (C_{13}) ppm

IR ν_{max} 3180 (NH), 2920 (NH), 1727 (C=O), 1638 (C=O), 1621 (C=O), 1564, 1401, 1367, 1309, 1231, 1151, 1112, 1056, 1027, 804, 779, 760 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{23}H_{35}N_3O_6S$ $[M + H]^+$ 482.2325, found 482.2326

Ethyl-2-amino-4-methylthiophene-3-carboxylate (112)[208]



Synthesised from acetone (3 mL; 2.320 g; 0.04 mol; 1.0 eq.), sulfur powder (1.230 g; 0.04 mol; 1.0 eq.), ethyl cyanoacetate (4 mL; 4.350 g; 0.04 mol; 1.0 eq.) morpholine (3.4 mL) and EtOH (8 mL) via Method C over 20 h. The solvents were removed and the oily residue was purified twice by column chromatography (hexane/EtOAc 1:1), followed by recrystallisation from ether/hexane giving yellow crystals. The filtrate from the recrystallisation was purified by Biotage column (hexane/EtOAc; 0-80% over 30 CV) giving a dark yellow powder. Yield: 1.430 g (19%)

mp 75-76 °C (Lit.[208] 76-78 °C)

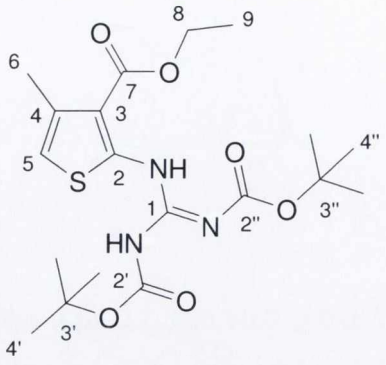
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (brs, 2H, NH_2), 5.86 (s, 1H, H_5), 4.31 (q, 2H, *J* = 7.0 Hz, $H_{2'}$), 2.31 (s, 3H, H_6), 1.38 (t, 3H, *J* = 7.0 Hz, $H_{3'}$) ppm (Lit.[208] 6.02, 5.84, 4.30, 2.29, 1.36)

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 (C_1), 164.1 (C_2), 136.7 (C_4), 106.7 (C_3), 102.8 (C_5), 59.6 ($C_{2'}$), 18.5 (C_6), 14.4 ($C_{3'}$)

IR ν_{max} 3412 (NH_2), 3305 (NH_2), 1641 (C=O), 1589, 1541, 1476, 1441, 1411, 1314, 1271, 1141, 780, 690 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_8H_{11}NO_2S$ $[M + H]^+$ 186.0589, found 186.0587

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-ethoxycarbonyl-4-methylthien-2-yl)guanidine (114)



Synthesised from **112** (370.0 mg; 2 mmol; 1 eq.), *S*-methylthiopseudourea (639.0 mg; 2.2 mmol; 1.1 eq.), TEA (974 µL; 708.0 mg; 7 mmol; 3.5 eq.) and $HgCl_2$ (815.0 mg; 3 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 584.6 mg (68%)

m.p 135-137 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.78 (brs, 1H, N<u>H</u>C_2), 11.35 (brs, 1H, N<u>H</u>$CO_2{}^t$Bu), 6.43 (s, 1H, H_5), 4.42 (q, 2H, *J* = 7.1 Hz, H_8), 2.34 (s, 3H, H_6), 1.54 (s, 9H, $H_{4'}$), 1.52 (s, 9H, $H_{4''}$), 1.37 (t, 3H, *J* = 7.1 Hz, H_9) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2 (C_7), 162.4 (C_1), 151.8 ($C_{2'}$), 150.8 ($C_{2''}$), 148.0 (C_2), 135.3 (C_4), 115.6 (C_5), 113.9 (C_3), 83.6 ($C_{3'}$), 80.0 ($C_{3''}$), 60.5 (C_8), 28.2 ($C_{4''}$), 28.1 ($C_{4'}$), 18.2 (C_6), 14.4 (C_9) ppm

IR ν_{max} 3361 (NH), 3201 (NH), 2976, 2927, 1733 (C=O), 1713 (C=O), 1645 (C=O), 1623, 1569, 1320, 1292, 1229, 1153, 1120, 1060, 801, 777, 718 cm^{-1}

HRMS (ESI) m/z calc. for $C_{19}H_{29}N_3O_6S$ $[M + Na]^+$ 450.1675, found 450.1667

_N_-(3-Carboxy-5,6-dihydro-4_H_-cyclopenta[_b_]thien-2-yl)guanidine hydrochloride (115)

Synthesised using Method G from **105** (77.8 mg; 0.117 mmol; 1.0 eq.) giving a pale brown solid. Yield: 41.0 mg (84%)

mp decomposes 140-150 °C

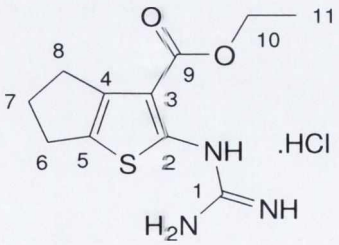
^{1}H NMR (400 MHz, D_2O) δ 2.77 (t, 4H, J = 7.5 Hz, $H_{6,8}$), 2.23 (app. quint., 2H, J = 7.5 Hz, H_7) ppm

^{13}C NMR (100 MHz, D_2O) δ 164.9 (C_9), 156.7 (C_1), 144.5 (C_2), 141.5 (C_4), 139.2 (C_5), 123.5 (C_3), 29.3 (C_6), 28.5 (C_8), 26.5 (C_7) ppm

ν_{max} 3392 (NH), 3188 (NH), 2961 (NH), 2856 (NH), 1704, 1666, 1569, 1549, 1475, 1259, 1196, 1015, 868, 837, 795, 686 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_9H_{11}N_3O_2S$ $[M + H]^+$ 226.0650, found 226.0609

_N_-(3-Ethoxycarbonyl-5,6-dihydro-4_H_-cyclopenta[_b_]thien-2-yl)guanidine hydrochloride (116)



Synthesised using Method G from **109** (200.0 mg: 0.46 mmol; 1 eq.) giving a pale brown solid. Yield: 115.2 mg (90%)

mp 99-102 °C

^{1}H NMR (400 MHz, D_2O) δ 4.20 (q, 2H, *J* = 7.2 Hz, H_{10}), 2.82-2.78 (m, 4H, $H_{6,8}$), 2.24 (app. quint., 2H, *J* = 5.5 Hz, CH_7), 1.23 (t, 3H, *J* = 7.2 Hz, H_{11}) ppm

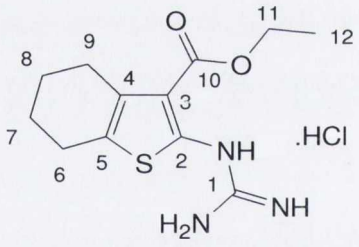
^{13}C NMR (100 MHz, D_2O) δ 163.8 (C_9), 157.4 (C_1), 145.1 (C_2), 141.4 (C_4), 140.2 (C_5), 124.3 (C_3), 61.8 (C_{10}), 29.8 (C_6), 29.0 (C_8), 27.0 (C_7), 13.3 (C_{11}) ppm

IR ν_{max} 3373 (NH), 3154 (NH), 1706, 1672 (C=O), 1630 (C=N), 1583, 1546, 1473, 1267, 1196, 1046, 1013, 782 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_{11}H_{15}N_3O_2S$ $[M + H]^+$ 254.0963, found 254.0958

$C_{11}H_{16}ClN_3O_2S \cdot 1.6D_2O$ requires C, 41.46; H, 5.08; N, 13.19%. Found: C, 41.54; H, 5.38; N, 12.89%

_N_-(3-Ethoxycarbonyl-4,5,6,7-4_H_-tetrahydrobenzo[_b_]thien-2-yl)guanidine hydrochloride (117)



Synthesised using Method G from **110** (500.0 mg; 1.07 mmol; 1.0 eq.) giving a tan solid. Yield: 302.0 mg (93%)

mp 89-92 °C

^{1}H NMR (400 MHz, D_2O) δ 4.13 (q, 2H, *J* = 7.0 Hz, H_{11}), 2.53-2.52 (m, 4H, $H_{6,9}$), 1.61-1.59 (m, 4H, $H_{7,8}$), 1.19 (t, 3H, *J* = 7.0 Hz, H_{12}) ppm

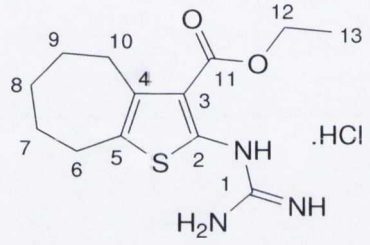
^{13}C NMR (100 MHz, D_2O) δ 164.0 (C_{10}), 157.2 (C_1), 137.3 (C_2), 135.9 (C_4), 135.0 (C_5), 127.2 (C_3), 61.8 (C_{11}), 25.6 (C_6), 24.4 (C_9), 22.2 (C_8), 21.9 (C_7), 13.3 (C_{12}) ppm

IR ν_{max} 3115 (NH), 2934 (NH), 1666 (C=O), 1624 (C=N), 1586, 1416, 1323, 1274, 1191, 1143, 1024 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_{12}H_{17}N_3O_2S$ [M + H]$^+$ 2658.1120, found 268.1114.

$C_{12}H_{18}ClN_3O_2S{\cdot}0.9D_2O$ requires C, 45.04; H, 5.67; N, 13.13%. Found: C, 44.94; H, 5.75; N, 13.33%

_N_-(3-Ethoxycarbonyl-5,6,7,8-tetrahydro-4_H_-cyclohepta[_b_]thien-2-yl)guanidine hydrochloride (118)



Synthesised using Method G from **111** (100.0 mg; 0.208 mmol; 1.0 eq.) giving a yellow gel. Yield: 46.7 mg (71%)

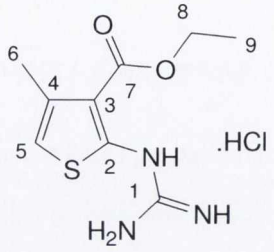
^{1}H NMR (400 MHz, D_2O) δ 4.19 (q, 2H, *J* = 7.0 Hz, H_{12}), 2.72 (t, 2H, *J* = 4.5 Hz, H_6), 2.68 (t, 2H, *J* = 4.5 Hz, H_{10}), 1.74-1.69 (m, 2H, CH_8), 1.53-1.48 (m, 2H, CH_7), 1.46-1.41 (m, 2H, CH_9), 1.18 (t, 3H, *J* = 7.0 Hz, H_{13}) ppm

^{13}C NMR (100 MHz, D_2O) δ 164.5 (C_{11}), 157.0 (C_1), 140.7 (C_2), 139.5 (C_4), 132.4 (C_5), 129.8 (C_3), 61.7 (C_{12}), 31.3 (C_8), 28.5 (C_{10}), 27.2 (C_6), 26.7 (C_7), 26.2 (C_9), 12.7 (C_{13}) ppm

IR ν_{max} 3148 (NH), 2921, 2849, 1666 (CN), 1587 (C=O), 1476, 1444, 1414, 1331, 1283, 1217, 1150, 1019 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{13}H_{19}N_3O_2S$ [M + H]$^+$ 282.1276, found 282.1267

_N_-(3-Ethoxycarbonyl-4-methylthien-2-yl)guanidine hydrochloride (119)



Synthesised using Method G from **114** (100.0 mg; 0.233 mmol; 1.0 eq.) giving a pale yellow solid. Yield: 57.1 mg (93%)

mp decomposes > 240 °C

^{1}H NMR (400 MHz, D_2O) δ 6.97 (s, 1H, H_5), 4.21 (q, 2H, J = 7.0 Hz, H_8), 2.22 (s, 3H, H_6), 1.26 (t, 3H, J = 7.0 Hz, H_9) ppm

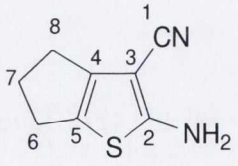
^{13}C NMR (100 MHz, D_2O) δ 164.0 (C_7), 157.4 (C_1), 140.5 (C_2), 138.2 (C_4), 128.1 (C_3), 120.8 (C_5), 61.8 (C_8), 16.1 (C_6), 13.2 (C_9) ppm

IR ν_{max} 3449 (NH), 3294, 3133 (NH), 2859, 2759, 1637, (C=N), 1594 (C=O), 1543, 1487, 1375, 1228, 1163, 1078, 1043, 941, 871, 779, 730 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_9H_{13}N_3O_2S$ $[M + H]^+$ 228.0807, found 228.0807

$C_9H_{14}ClN_3O_2S{\cdot}0.3D_2O$ requires C, 40.08; H, 5.23; N, 15.58%. Found: C, 40.39; H, 5.26; N, 15.75%

2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbonitrile (120)[160,207]



Synthesised from cyclopentanone (3.55 mL; 3.360 g; 0.04 mol; 1.0 eq.), sulfur powder (1.230 g; 0.04 mol; 1.0 eq.), malononitrile (2.52 mL; 2.640 g; 0.04 mol; 1 eq.), morpholine (3.4 mL) and EtOH (8 mL) via Method C over 20 h. The reaction mixture was removed from the heat and cooled in a freezer overnight, giving brown crystals, which were collected by vacuum filtration. Yield: 3.020 g (46%)

mp 150-154 °C (Lit.[160] 153-154 °C)

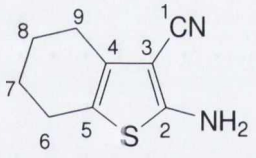
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64 (brs, 2H, NH_2), 2.78 (t, 2H, *J* = 7.3 Hz, H_6), 2.73 (t, 2H, *J* = 7.3 Hz, H_8), 2.39 (quint., 2H, *J* = 7.3 Hz, H_7) ppm (Lit.[207] 4.81, 2.73-2.66, 2.36-2.34)

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2 (C_2), 142.0 (C_4), 125.3 (C_5), 115.6 (C_1), 84.69 (C_3), 29.3 (C_6), 28.5 (C_8), 27.4 (C_7) ppm (Lit.[207] 165.7, 141.9, 125.0, 115.8, 84.1, 29.2, 28.4, 27.3)

ν_{max} 3433 (NH_2), 3332 (NH_2), 3218, 2916, 2858, 2191 (CN), 1613 (C=O), 1506, 1415, 1327, 1139, 1035, 804 cm^{-1}

MS (ESI) *m/z* calc. for $C_8H_8N_2S$ $[M + H]^+$ 165.0486, found 165.0481

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (121)[159,209]



Synthesised from cyclohexanone (4.15 mL; 3.930 g; 0.04 mol; 1.0 eq.), sulfur powder (1.230 g; 0.04 mol; 1.0 eq.), malononitrile (2.52 mL; 2.640 g; 0.04 mol; 1 eq.), morpholine (3.4 mL) and EtOH (8 mL) via Method C over 20 h. The reaction mixture was removed from the heat and cooled in a freezer overnight, giving yellow crystals, which were collected by vacuum filtration. Yield: 3.660 g (51%)

mp 142-145 °C (Lit.[159] 145 °C)

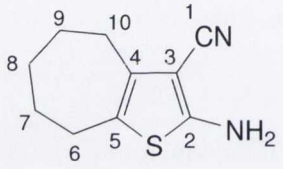
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.57 (brs, 2H, NH_2), 2.54-2.51 (m, 4H, $H_{6,9}$), 1.84-1.79 (m, 4H, $H_{7,8}$) ppm (Lit.[209] 4.41, 2.55-2.43, 1.86-1.72)

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.9 (C_2), 132.4 (C_4), 120.7 (C_5), 115.4 (C_1), 88.8 (C_3), 24.5 (C_6), 24.1 (C_9), 23.4 (C_8), 22.1 (C_7) ppm (Lit.[209] 160.0, 132.4, 120.7, 115.5, 88.8, 24.6, 24.2, 23.4, 22.2)

IR ν_{max} 3443 (NH_2), 3326 (NH_2), 3205, 2930, 2911, 2837, 2195 (CN), 1615 (C=O), 1517, 1432, 1400, 1340, 1128, 853, 831, 819, 765, 657 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_9H_{10}N_2S$ $[M + H]^+$ 179.0643, found 179.0638

2-Amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carbonitrile (122)[159,160]



Synthesised from cycloheptanone (4.71 mL; 4.490 g; 0.04 mol; 1.0 eq.), sulfur powder (1.230 g; 0.04 mol; 1.0 eq.), malononitrile (2.52 mL; 2.640 g; 0.04 mol; 1 eq.), morpholine (3.4 mL) and EtOH (8 mL) via Method C over 20 h. The reaction mixture was removed from the heat and cooled in a freezer overnight, giving salmon crystals, which were collected by vacuum filtration. Yield: 3.280 g (43%)

mp 114-117 °C (Lit.[160] 114 °C)

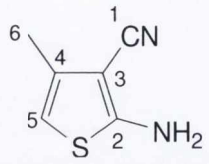
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.47 (brs, 2H, NH_2), 2.65 (t, 2H, *J* = 5.5 Hz, H_6), 2.60 (t, 2H, *J* = 5.5 Hz, H_{10}), 1.87-1.82 (m, 2H, H_8), 1.70-1.64 (m, 4H, $H_{7,9}$) ppm (Lit.[159] 4.26, 2.65-2.57, 1.83-1.79, 1.67-1.63)

^{13}C NMR (100 MHz, $CDCl_3$) 157.8 (C_2), 136.9 (C_4), 123.9 (C_5), 115.8 (C_1), 92.0 (C_3), 31.9 (C_8), 29.4 (C_6), 29.2 (C_{10}), 28.1 (C_9), 27.3 (C_7) ppm (Lit.[159] 157.7, 136.9, 123.9, 115.9, 92.0, 31.9, 29.4, 29.2, 28.1, 27.2)

IR ν_{max} 3442 (NH_2), 3305 (NH_2), 3204, 2927, 2839, 2202 (CN), 1619 (C=O), 1513, 1618, 1340, 1288, 982, 965, 838, 821, 674 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{10}H_{12}N_2S$ $[M + H]^+$ 193.0799, found 193.0795

2-Amino-4-methylthiophene-3-carbonitrile (123)[210]



Synthesised from acetone (2.94 mL; 2.320 g, 0.04 mol, 1.0 eq.), sulfur powder (1.230 g, 0.04 mol, 1.0 eq.), malononitrile (2.52 mL; 2.640 g; 0.04 mol; 1 eq.), morpholine (3.4 mL) and EtOH (8 mL) via Method C over 20 h. The crude material was purified by column chromatography (Hexane:EtOAc 1:1) giving an orange solid. Yield: 108.2 mg (2%)

Or

Compound **124** (2.123 g; 20 mmol; 1 eq.) and sulfur (641.3 mg; 20 mmol; 1 eq.) were dissolved in EtOH (7 mL). Morpholine (3 mL) was added dropwise and the solution was heated to 40 °C for 4 h. The crude material was purified by column chromatography (Hexane:EtOAc 1:1) giving an orange solid. Yield: 424.7 mg (15%)

mp 115-117 °C (Lit.[210] 119-120 °C)

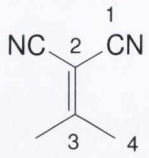
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95 (s, 1H, H_5), 4.95 (brs, 2H, NH_2), 2.18 (s, 3H, H_6) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2 (C_2), 135.6 (C_4), 115.7 (C_1), 105.0 (C_5), 90.9 (C_3), 15.1 (C_6) ppm

IR ν_{max} 3417 (NH_2), 3321 (NH_2), 3211, 3101, 2915, 2202 (CN), 1624 (C=O), 1551, 1513, 1444, 1398, 1376, 1297, 1189, 834, 717, 693 cm^{-1}

HRMS (ESI) m/z calc. for $C_6H_6N_2S$ $[M + H]^+$ 139.0330, found 139.0331

Isopropylidenemalononitrile (124)[164]



A solution of acetone (5.87 mL; 4.650g; 80 mmol; 4 eq.), malononitrile (1.26 mL; 1.320 g; 20 mmol; 1 eq.), sodium acetate trihydrate (612.3 mg; 4.5 mmol; 0.225 eq.), and AcOH (240 µL; 252.0 mg; 4.2 mmol; 0.21 eq.) in benzene was refluxed for 4 h in a Dean Stark apparatus. The reaction was quenched with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and the solvents removed giving an orange oil. This was then purified by vacuum distillation. Yield: 1.520 g (71%)

bp 92 °C at between 0-1 mbar (Lit.[164] 70 °C at 1 mm Hg)

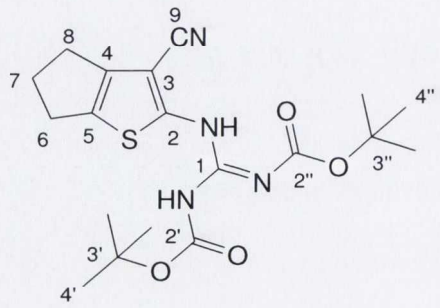
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 6H, H_4) ppm (Lit.[164] 2.24)

^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0 (C_3), 111.3 (C_1), 85.9 (C_2), 24.1 (C_4) ppm

IR ν_{max} 2981, 2232 (CN), 1608, 1435, 1376, 1164, 1124, 1071 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_6H_6N_2$ $[M + H]^+$ 107.0609, found 107.0616

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)guanidine (125)



Synthesised from **120** (328.0 mg; 2 mmol; 1 eq.), *S*-methylthiopseudourea (639.0 mg; 2.2 mmol; 1.1 eq.), TEA (974 µL; 708.0 mg; 7 mmol; 3.5 eq.) and $HgCl_2$ (815.0 mg; 3 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 165.3 mg (20%)

mp decomposes > 180 °C

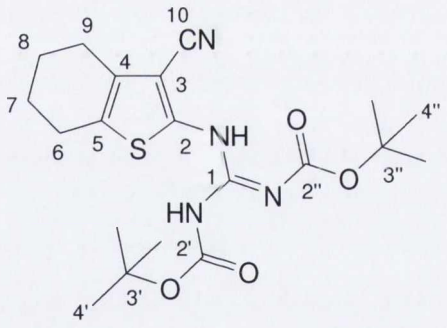
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.54 (brs, 1H, NHC$_2$), 11.30 (s, 1H, NHCO$_2{}^t$Bu), 2.89 (t, 2H, *J* = 7.1 Hz, H_6), 2.82 (t, 2H, *J* = 7.1 Hz, H_8), 2.43 (quint., 2H, *J* = 7.1 Hz, H_7), 1.57 (s, 9H, $H_{4'}$), 1.56 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2 (C_1), 152.8 ($C_{2'}$), 150.9 ($C_{2''}$), 150.5 (C_2), 141.0 (C_4), 136.8 (C_5), 114.4 (C_9), 90.8 (C_3), 84.9 ($C_{3'}$), 80.5 ($C_{3''}$), 29.5 (C_6), 28.1 (C_8 + $C_{4''}$), 28.0 (C_7 + $C_{4'}$) ppm

IR ν_{max} 3436 (NH), 3325 (NH), 2972, 2215 (CN), 1637 (C=O), 1577 (C=O), 1463, 1400, 1256, 1158, 1122, 1088, 1013, 752, 664 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{21}H_{30}N_4O_4S$ $[M + Na]^+$ 429.1572, found 429.1570

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)guanidine (126)



Synthesised from **121** (713.0 mg; 4 mmol; 1 eq.), *S*-methylthiopseudourea (1.278 g; 4.4 mmol; 1.1 eq.), TEA (1.95 mL; 1.416 g; 14 mmol; 3.5 eq.) and $HgCl_2$ (1.682 g; 6 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 611.5 mg (36%)

mp decomposes > 178 °C

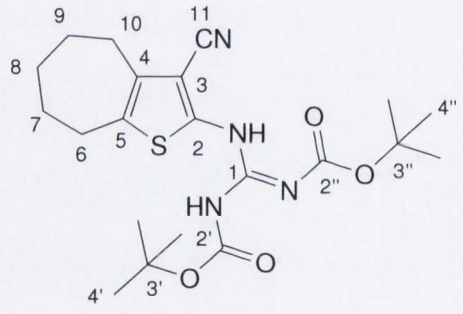
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.52 (brs, 1H, N<u>H</u>C_2), 11.31 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 2.66 (t, 2H, *J* = 5.3 Hz, H_6), 2.61 (t, 2H, *J* = 5.3 Hz, H_9), 1.83-186 (m, 4H, $H_{7,8}$), 1.57 (s, 9H, $H_{4'}$), 1.56 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3 (C_1), 152.8 ($C_{2'}$), 151.0 ($C_{2''}$), 145.6 (C_2), 131.1 (C_4), 130.4 (C_5), 114.2 (C_{10}), 95.1 (C_3), 84.9 ($C_{3'}$), 80.5 ($C_{3''}$), 28.1 ($C_{4''}$), 28.0 ($C_{4'}$), 24.0 (C_6), 23.9 (C_9), 23.1, (C_8), 22.1 (C_7) ppm

IR ν_{max} 3125 (NH), 2982 (NH), 2933, 2213 (CN), 1719, 1648 (C=O), 1634 (C=O), 1579, 1402, 1365, 1314, 1274, 1235, 1148, 1114, 800, 767, 669 cm^{-1}

HRMS (ESI) m/z calc. for $C_{20}H_{28}N_4O_4S$ $[M + Na]^+$ 443.1729, found 443.1730

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-cyano-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thien-2-yl)guanidire (127)



Synthesised from **122** (385.0 mg; 2 mmol; 1 eq.), *S*-methylthiopseudourea (639.0 mg; 2.2 mmol; 1.1 eq.), TEA (974 µL; 708.0 mg; 7 mmol; 3.5 eq.) and $HgCl_2$ (815.0 mg; 3 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 527.8 mg (61%)

mp decomposes > 171 °C

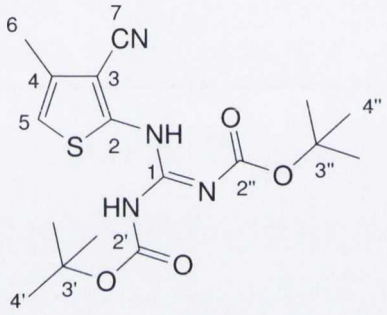
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.48 (brs, 1H, N<u>H</u>C_2), 11.31 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 2.75-2.73 (m, 4H, $H_{5,10}$), 1.90-1.84 (m, 2H, H_8), 1.68-1.66 (m, 4H, $H_{7,9}$), 1.57 (s, 9H, $H_{4'}$), 1.56 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3 (C_1), 152.8 ($C_{2'}$), 151.0 ($C_{2''}$), 143.4 (C_2), 136.0 (C_4), 134.1 (C_5), 114.6 (C_{11}), 97.6 (C_3), 84.8 ($C_{3'}$), 80.4 ($C_{3''}$), 32.1 (C_8), 29.1 (C_6), 29.1 (C_{10}), 28.1 ($C_{4''}$), 28.0 (C_9 + $C_{4'}$), 27.4 (C_7) ppm

IR ν_{max} 3179 (NH), 2979 (NH), 2922, 2852, 2207 (CN), 1719, 1651 (C=O), 1626 (C=O), 1585, 1416, 1368, 1316, 1253, 1234, 1151, 1112 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{21}H_{30}N_4O_4S$ $[M + Na]^+$ 457.1885, found 457.1883

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-cyano-4-methylthien-2-yl)guanidine (128)



Synthesised from **123** (276.0 mg; 2 mmol; 1 eq.), *S*-methylthiopseudourea (639.0 mg; 2.2 mmol; 1.1 eq.), TEA (974 µL; 708.0 mg; 7 mmol; 3.5 eq.) and $HgCl_2$ (815.0 mg; 3 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 218.7 (30%)

mp decomposes > 150 °C

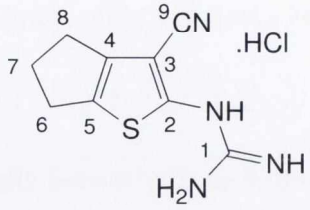
^1H NMR (400 MHz, $CDCl_3$) δ 11.59 (brs, 1H, N<u>H</u>C_2), 11.27 (brs, 1H, N<u>H</u>$CO_2{}^tBu$), 6.51 (s, 1H, H_5), 2.28 (s, 3H, H_6), 1.55 (s, 9H, $H_{4'}$), 1.52 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.1 (C_1), 152.8 ($C_{2'}$), 150.9 ($C_{2''}$), 148.2 (C_2), 134.2 (C_4), 115.6 (C_5), 114.1 (C_7), 96.7 (C_3), 84.9($C_{3'}$), 80.4 ($C_{3''}$), 28.1 ($C_{4''}$), 28.0 ($C_{4'}$), 14.9 (C_6) ppm

IR ν_{max} 3182 (NH), 3099 (NH), 2979, 2926, 2213 (CN), 1725, 1659 (2xC=O), 1585, 1419, 1392, 1368, 1312, 1270, 1251, 1234, 1154, 1114, 1061, 875, 776, 713 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{17}H_{24}N_4O_4S$ $[M + Na]^+$ 403.1416, found 403.1410

_N_-(3-Cyano-5,6-dihydro-4_H_-cyclopenta[_b_]thien-2-yl)guanidine hydrochloride (129)



Synthesised using Method G from **125** (50.0 mg: 0.123 mmol; 1 eq.) giving a yellow gel. Yield: 11.5 mg (39%)

^{1}H NMR (400 MHz, D_2O) δ 2.83 (t, 2H, *J* = 7.3 Hz, H_6), 2.74 (t, 2H, *J* = 7.2 Hz, H_8), 2.31-2.28 (m, 2H, H_7) ppm

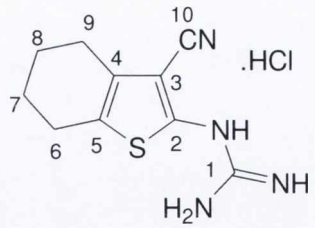
^{13}C NMR (100 MHz, D_2O) δ 156.4 (C_1), 144.6 (C_2), 143.7 (C_4), 142.3 (C_5), 113.1 (C_9), 104.8 (C_3), 29.1 (C_6), 27.2 (C_8), 26.6 (C_7) ppm

IR ν_{max} 3410 (NH), 3302, 3126, 2224 (CN), 1673 (C=N), 1655, 1598, 1564, 1469, 1249, 1156, 1062, 766 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_9H_{10}N_4S$ [M + H] 207.0704, found 207.0701

$C_9H_{11}ClN_4S \cdot 1.5D_2O$ requires C, 39.63; H, 4.07; N, 20.54%. Found: C, 39.91; H, 4.07; N, 20.23%

_N_-(3-Cyano-4,5,6,7-tetrahydrobenzo[_b_]thien-2-yl)guanidine hydrochloride (130)



Synthesised using Method G from **126** (300.0 mg: 0.713 mmol; 1 eq.) followed by recrystallisation from water giving a pale yellow solid. Yield: 41.2 mg (23%)

Or

Synthesised using Method H from **126** (100 mg; 0.238 mmol; 1 eq.) Yield: 26.3 mg (43%)

mp decomposes > 170 °C

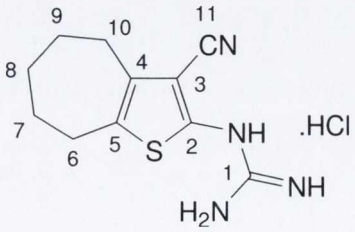
^{1}H NMR (400 MHz, D_2O) δ 2.68-2.64 (m, 2H, H_6), 2.58 (t, 2H, J = 6.0 Hz, H_9), 1.79-1.76 (m, 4H, $H_{7,8}$) ppm

^{13}C NMR (100 MHz, D_2O) δ 156.6 (C_1), 141.1 (C_2), 137.3 (C_4), 134.9 (C_5), 113.4 (C_{10}), 108.7 (C_3), 24.0 (C_6), 23.7 (C_9), 22.3 (C_8), 21.3 (C_7) ppm

IR ν_{max} 3159 (NH), 2938 (NH), 2225 (CN), 1663, 1629, 1885 (C=N), 1438, 1335, 1265, 1140, 765 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{10}H_{12}N_4S$ $[M + H]^+$ 221.0861, found 221.0861

_N_-(3-Cyano-5,6,7,8-tetrahydro-4_H_-cyclohepta[_b_]thien-2-yl)guanidine hydrochloride (131)



Synthesised using Method H from **127** (100.0 mg; 0.23 mmol; 1 eq.) giving a yellow gel. Yield: 36.4 mg (58%)

^{1}H NMR (400 MHz, D_2O) δ 2.78 (t, 2H, *J* = 5.2 Hz, H_6), 2.73 (t, 2H, *J* = 5.2 Hz, H_{10}), 1.83 – 1.81 (m, 2H, H_8), 1.64-1.59 (m, 4H, $H_{7,9}$) ppm

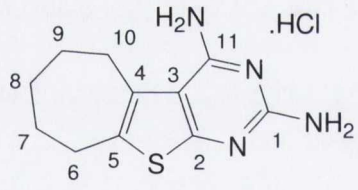
^{13}C NMR (100 MHz, D_2O) 156.7 (C_1), 141.5 (C_2), 140.0 (C_4), 138.3 (C_5), 113.8 (C_{11}), 111.1 (C_3), 31.2 (C_8), 29.1 (C_6), 28.8 (C_{10}), 27.1 (C_9), 26.4 (C_7) ppm

IR ν_{max} 3293 (NH), 3119 (NH), 2924, 2225 (CN), 1675 (C=N), 1627, 1586, 1439, 1256, 1159, 1117 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{11}H_{14}N_4S$ $[M - H]^-$ 233.0861, found 233.0863

$C_{11}H_{15}ClN_4S{\cdot}1.6D_2O{\cdot}0.1C_4H_8O_2$ requires C, 43.94; H, 5.11; N, 17.98%. Found: C, 43.91; H, 5.34; N, 17.89%

2,4-Diamino-6,7,8,9-tetrahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine hydrochloride (133)



Synthesised using Method G from **127** (200.0 mg: 0.46 mmol; 1 eq.). Two reverse phase pencil columns (100% H_2O) were carried out and recrystallisation from water was attempted giving a pale yellow solid. None of these procedures succeeded in purifying the compound sufficiently for biological testing. Yield: 64.0 mg (51%)

mp decomposes > 170 °C

^{1}H NMR (400 MHz, D_2O) δ 2.96 (t, 2H, *J* = 3.7 Hz, H_6), 2.86 (t, 2H, *J* = 3.7 Hz, H_{10}), 1.90–1.87 (m, 2H, H_8), 1.80-1.76 (m, 4H, $H_{7,9}$) ppm

^{13}C NMR (100 MHz, D_2O) 158.6 (C_1), 152.6 (C_{11}), 134.0 (C_2), 132.3 (C_4), 132.3 (C_5), 110.9 (C_3), 29.8 (C_8), 28.3 (C_6), 27.7 (C_{10}), 26.5 (C_9), 25.5 (C_7) ppm

IR ν_{max} 3375 (NH), 3333, 3164 (NH), 2927 , 2849, 1647, 1573, 1533, 1445, 1370, 1140, 769, 739 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{11}H_{14}N_4S$ $[M + H]^+$ 235.1017, found 235.1020

2,4-Diamino-5-methylthieno[2,3-*d*]pyrimidine hydrochloride (134)

Synthesised using Method G from **128** (100.0 mg: 0.23 mmol; 1 eq.) giving a pale yellow solid. Yield: 57.1 mg (93%)

Or

Synthesised using Method H from **128** (50 mg; 0.11 mmol; 1 eq.) Yield: 19.3 mg (68%)

mp decomposes > 200 °C

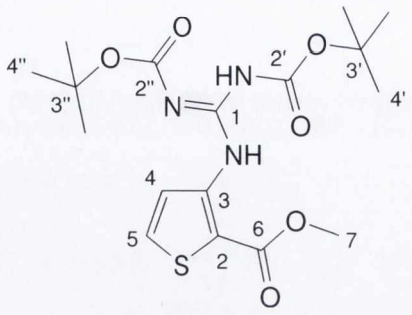
^1H NMR (400 MHz, D_2O) δ 6.71 (s, 1H, H_5), 2.34 (s, 3H, H_6) ppm

^{13}C NMR (100 MHz, D_2O) δ 158.7 (C_1), 157.2 (C_7), 153.2 (C_2), 130.8 (C_4), 114.9 (C_5), 109.8 (C_3), 15.5 (C_6) ppm

IR ν_{max} 3449 (NH), 3294, 3133 (NH), 2859, 2759, 1637, (C=N), 1594 (C=O), 1543, 1487, 1375, 1228, 1163, 1078, 1043, 941, 871, 779, 730 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_7H_8N_4S$ $[M + H]^+$ 181.0548, found 181.0544

[2,3-Di(*tert*-butoxycarbonyl)]-1-(2-methoxycarbonylthien-3-yl)guanidine (136)



Synthesised from methyl-3-amino-2-thiophene carboxylate (300.0 mg; 1.91 mmol; 1.0 eq.), *S*-methylthiopseudourea (665.0 mg; 2.29 mmol; 1.2 eq.), TEA (1.06 mL; 773.0 mg; 7.63 mmol; 4.0 eq.) and $HgCl_2$ (777.0 mg; 2.86 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 431.3 mg (57%)

mp 133-134 °C

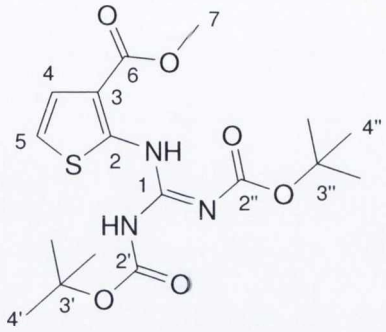
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.92 (s, 1H, N<u>H</u>C_3), 11.60 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 8.37 (d, 1H, *J* = 5.9 Hz, H_4), 7.47 (d, 1H, *J* = 5.9 Hz, H_5), 3.96 (s, 3H, H_7), 1.59 (s, 9H, $H_{4'}$), 1.55 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1 (C_1), 162.6 (C_6), 152.1 ($C_{2'}$), 151.6 ($C_{2''}$), 141.7 (C_3), 130.2 (C_4), 124.6 (C_5), 113.0 (C_2), 83.1 ($C_{3'}$), 79.3 ($C_{3''}$), 51.6 (C_7), 27.7 ($C_{4''}$), 27.6 ($C_{4'}$) ppm

IR ν_{max} 3140 (NH), 2976 (NH), 1730, 1704 (C=O), 1652 (C=O), 1634 (C=O), 1587, 1413, 1391, 1367, 1318, 1277, 1240, 1228, 1153, 1119, 1090, 1060, 805, 767, 750, 729 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{17}H_{25}N_3O_6S$ $[M + Na]^+$ 422.1362, found 422.1362

[2,3-Di(*tert*-butoxycarbonyl)]-1-(3-methoxycarbonylthien-2-yl)guanidine (137)



Synthesised from methyl-2-amino-3-thiophene carboxylate (300.0 mg; 1.91 mmol; 1.0 eq.), *S*-methylthiopseudourea (665.6 mg; 2.29 mmol; 1.2 eq.), TEA (1.06 mL; 773.0 mg; 7.63 mmol; 4.0 eq.) and $HgCl_2$ (777.0 mg; 2.86 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) followed by recrystallisation from EtOAc/hexane giving a white powder. Yield: 279.5 mg (37%)

mp 176-178 °C

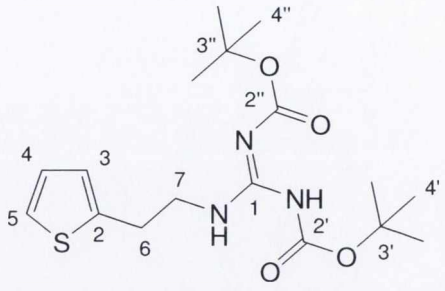
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.72 (s, 1H, N<u>H</u>C$_2$), 11.35 (s, 1H, N<u>H</u>CO$_2$^{t}Bu), 7.25 (d, 1H, *J* = 5.9 Hz, H_4), 6.81 (d, 1H, *J* = 5.9 Hz, H_5), 3.95 (s, 3H, H_7), 1.58 (s, 9H, $H_{4'}$), 1.56 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1 (C_6), 162.4 (C_1), 151.9 ($C_{2'}$), 150.9 ($C_{2''}$), 147.0 (C_2), 124.5 (C_4), 118.5 (C_5), 114.2 (C_3), 83.9 ($C_{3'}$), 80.2 ($C_{3''}$), 51.8 (C_7), 28.2 ($C_{4''}$), 28.1 ($C_{4'}$) ppm

IR ν_{max} 3220 (NH), 2975 (NH), 1733, 1715 (C=O), 1647 (C=O), 1628 (C=O), 1568, 1439, 1404, 1364, 1322, 1280, 1229, 1161, 1116, 1082, 1057, 1029, 804, 777, 756, 714 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{17}H_{25}N_3O_6S$ $[M + H]^+$ 400.1542, found 400.1537

[2,3-Di(*tert*-butoxycarbonyl)]-1-(thien-2-yl)ethylguanidine (138).



Synthesised from 2-thiopheneethylamine (300.0 mg; 2.36 mmol; 1.0 eq.), **62** (782.0 mg; 2.83 mmol; 1.2 eq.), TEA (1.31 mL; 955.0 mg; 9.43 mmol; 4.0 eq.) and $HgCl_2$ (960.0 mg; 3.54 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 693.6 mg (80%)

mp 100-102 °C

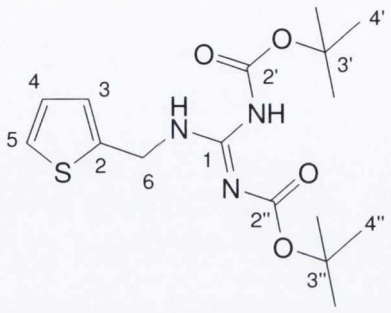
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.51 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 8.46 (t, 1H, *J* = 5.3 Hz, N<u>H</u>C_7), 7.15 (d, 1H, *J* = 4.9 Hz, H_5), 6.93 (dd, 1H, *J* = 3.4, 4.9 Hz, H_4), 6.87 (dt, 1H, *J* = 5.3, 6.6 Hz, H_3), 3.71 (app. q, 2H, *J* = 6.6 Hz, H_7), 3.09 (t, 2H, *J* = 6.6 Hz, H_6), 1.51 (s, 9H, $H_{4''}$), 1.48 (s, 9H, $H_{4'}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1 (C_1), 155.7 ($C_{2''}$), 152.6 ($C_{2'}$), 140.3 (C_2), 126.5 (C_4), 125.0 (C_3), 123.5 (C_5), 82.6 ($C_{3''}$), 78.8 ($C_{3'}$), 41.8 (C_7), 29.1 (C_6), 27.8 ($C_{4''}$), 27.6 ($C_{4'}$) ppm

IR ν_{max} 3321 (NH), 2973 (NH), 1736, 1648 (C=O), 1624 (C=O), 1413, 1369, 1358, 1344, 1300, 1250, 1137, 1096, 1060, 1023, 860, 850, 825, 805, 754, 723 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{17}H_{27}N_3O_4S$ $[M + H]^+$ 370.1801, found 370.1790

[2,3-Di(*tert*-butoxycarbonyl)]-1-(thien-2-yl)methylguanidine (139)



Synthesised from 2-thiophenemethylamine (300.0 mg; 2.65 mmol; 1.0 eq.), *S*-methylthiopseudourea (923.6 mg; 3.18 mmol; 1.2 eq.), TEA (1.48 mL; 1.073 g; 10.6 mmol; 4.0 eq.) and $HgCl_2$ (1.079 g; 3.98 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 886.0 mg (94%)

mp 118-120 °C

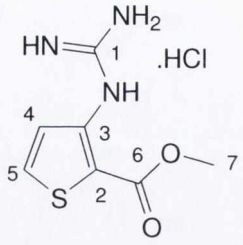
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.51 (s, 1H, N<u>H</u>$CO_2{}^t$Bu), 8.57 (t, 1H, *J* = 4.9 Hz, N<u>H</u>C_6), 7.21 (dd, 1H, *J* = 5.0, ^4J = 0.8 Hz, H_3), 6.99 (d, 1H, *J* = 3.4 Hz, H_5), 6.93 (dd, 1H, *J* = 3.4, 5.0 Hz, H_4), 4.76 (app. d, 2H, *J* = 4.9 Hz, H_6), 1.51 (s, 9H, $H_{4''}$), 1.46 (s, 9H, $H_{4'}$) ppm

^{13}C NMR (400 MHz, $CDCl_3$) δ 163.0 (C_1), 155.2 ($C_{2'}$), 152.6 ($C_{2''}$), 139.0 (C_2), 126.4 (C_3), 125.9 (C_5), 125.0 (C_4), 82.7 ($C_{3'}$), 78.9 ($C_{3''}$), 39.3 (C_6), 27.8 ($C_{4''}$), 27.6 ($C_{4'}$) ppm

IR ν_{max} 3342 (NH), 2979 (NH), 1718, 1640 (C=O), 1610 (C=O), 1559, 1414, 1366, 1317, 1252, 1228, 1148, 1118, 1080, 1054, 1026, 804, 768, 698 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{16}H_{25}N_3O_4S$ $[M + H]^+$ 356.1644, found 356.1639

_N_-(2-Methoxycarbonylthien-3-yl)guanidine hydrochloride (140)



Synthesised using Method G from **136** (300.0 mg; 0.75 mmol; 1 eq.) giving a white solid. Yield: 174.5 mg (99%)

Or

Synthesised using Method H from **136** (100 mg; 0.25 mmol; 1 eq.) Yield: 58.7 mg (99%)

mp 170 °C, decomposes > 172 °C

^{1}H NMR (400 MHz, D_2O) δ 7.66 (d, 1H, J = 5.5 Hz, H_4), 7.08 (d, 1H, J = 5.5 Hz, H_5), 3.74 (s, 3H, H_7) ppm

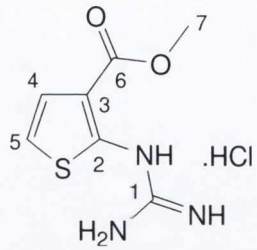
^{13}C NMR (100 MHz, D_2O) δ 163.5 (C_6), 155.6 (C_1), 138.2 (C_3), 133.3 (C_4), 124.4 (C_5), 120.1 (C_2), 52.6 (C_7) ppm

IR ν_{max} 3425 (NH), 3136, 3106 (NH), 1659 (C=O), 1601 (C=N), 1533, 1444, 1397, 1283, 1243, 1083, 1060, 785 cm^{-1}

HRMS (ES TOF) *m/z* calc. for $C_7H_9N_3O_2S$ $[M + H]^+$ 200.0494, found 200.0490

$C_7H_{10}ClN_3O_2S{\cdot}0.4D_2O$ requires C, 34.50; H, 4.14; N, 17.24%. Found: C, 34.62; H, 3.91; N, 17.36%

_N_-(3-Methoxycarbonylthien-2-yl)guanidine hydrochloride (141)



Synthesised using Method G from **137** (300.0 mg; 0.75 mmol; 1 eq.) giving a white solid. Yield: 90.2 mg (51%)

Or

Synthesised using Method H from **137** (100 mg; 0.25 mmol; 1 eq.) Yield: 52.9 mg (90%)

mp 180-181 °C

^{1}H NMR (400 MHz, D_2O) δ 7.27-7.32 (m, 2H, $H_{4,5}$), 3.75 (s, 3H, H_7) ppm

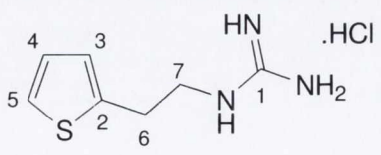
^{13}C NMR (100 MHz, D_2O) δ 163.8 (C_6), 157.0 (C_1), 141.5 (C_2), 127.5 (C_4), 126.9 (C_3), 124.2 (C_5), 52.4 (C_7) ppm

IR ν_{max} 3376, 3184 (NH), 3116 (NH), 2921 (NH), 2850, 2283, 1698, 1666 (C=N), 1642 (C=O), 1592, 1578, 1434, 1389, 1289, 1191, 1150, 1094, 979, 851, 726 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_7H_9N_3O_2S$ $[M + H]^+$ 200.0494, found 200.0498

$C_7H_{10}ClN_3O_2S{\cdot}0.4D_2O$ requires C, 34.50; H, 4.14; N, 17.24%. Found: C, 34.57; H, 3.90; N, 17.42%

2-[Thien-2-yl]ethylguanidine hydrochloride (142)



Synthesised using Method G from **138** (300.0 mg; 0.81 mmol; 1 eq.) and was filtered through a plug of reverse phase silica giving a white solid. Yield: 72.1 mg (43%)

Or

Synthesised using Method H from **138** (100 mg; 0.27 mmol; 1 eq.) Yield: 55.0 mg (99%)

mp 75-78 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, 1H, J = 4.8 Hz, H_5), 6.90 (dd, 1H, J = 2.9, 4.8, H_4), 6.84 (d, 1H, J = 2.9 Hz, H_3), 3.33 (t, 2H, J = 6.5 Hz, H_6), 2.98 (t, 2H, J = 6.5 Hz, H_7) ppm

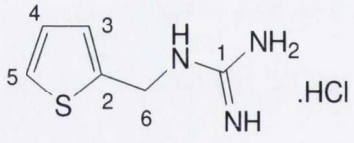
^{13}C NMR (100 MHz, $CDCl_3$) 156.6 (C_1), 140.5 (C_2), 127.3 (C_4), 126.0 (C_3), 124.7 (C_5), 42.4 (C_6), 28.4 (C_7) ppm

IR ν_{max} 3258 (NH), 3148 (NH), 2947, 2422 (C=N), 1662, 1643, 1609, 1567, 1466, 1420, 1355, 1332, 1254, 1207, 1158, 1094, 1029, 845, 821, 705 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_7H_{11}N_3S$ $[M + H]^+$ 170.0752, found 170.0754

Purity by HPLC 97.9% (t_R 19.48 min)

1-[Thien-2-yl]methylguanidine hydrochloride (143)



Synthesised using Method G from **139** (300.0 mg; 0.84 mmol; 1 eq.) and was filtered through a plug of reverse phase silica and the solvent removed giving a white solid. Yield: 88.1 mg (54%)

mp 103-105 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (dd, 1H, J = 5.0, ^4J = 1.0 Hz, H_3), 6.99 (d, 1H, J = 3.3 Hz, H_5), 6.93 (dd, 1H, J = 3.3, 5.0 Hz, H_4), 4.47 (app. brs, 2H, H_6) ppm

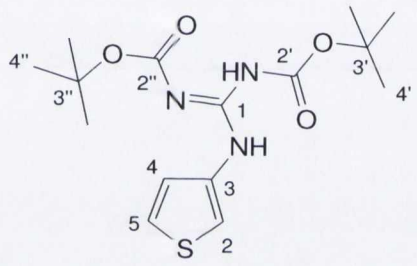
^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (C_1), 138.8 (C_2), 127.3 (C_4), 126.4 (C_5), 126.1 (C_3), 39.8 (C_6) ppm

IR ν_{max} 3398 (NH), 3306 (NH), 3233, 3129 (NH), 3083, 3047, 1665, 1626 (C=N), 1591, 1464, 1363, 1338, 1223, 1173, 1081, 1047, 850, 726, 663 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_6H_9N_3S$ $[M + H]^+$ 156.0595, found 156.0592

$C_6H_{10}ClN_3S{\cdot}0.4D_2O{\cdot}0.2C_4H_8O_2$ requires C, 37.24; H, 5.33; N, 19.16%. Found: C, 37.27; H, 4.98; N, 19.55%

[2,3-Di(*tert*-butoxycarbonyl)]-1-(thien-3-yl)guanidine (145)



Methyl-3-amino-2-thiophene carboxylate (1.600 g; 10.18 mmol; 1 eq.) was refluxed in 2M NaOH (10 mL) using Method F for 0.5 h. Acetone (12.5 mL), 2-propanol (3 mL) and anhydrous oxalic acid dehydrate (1.0 g) were used to generate 629.7 mg (42.9%) of crude lilac salt which was hydrolysed before being treated with **62** (1.207 g; 4.37 mmol; 2.0 eq.), DCM (10 mL), TEA (2.13 mL; 1.547 g; 15.3 mmol; 7.0 eq) and $HgCl_2$ (1.779 g; 6.55 mmol; 3.0 eq.). Work up as in Method F gave a white solid. Yield: 77.7 mg (5%)

mp 139-140 °C

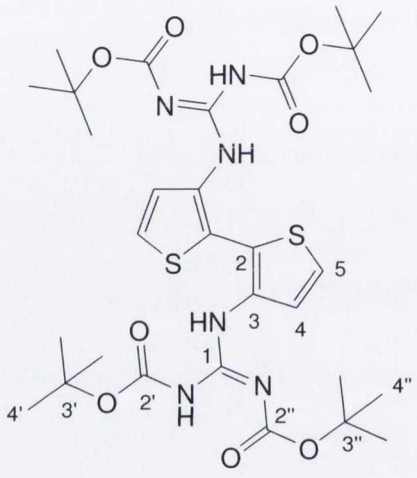
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.56 (brs, 1H, N<u>H</u>$CO_2{}^t$Bu), 10.51 (brs, 1H, N<u>H</u>C_3), 7.75 (dd, 1H, ^4J = 3.3, 1.2 Hz, H_2), 7.23 (dd, 1H, J = 5.2 Hz, ^4J = 3.3, H_5), 7.07 (dd, 1H, J = 5.2, ^4J = 1.2 Hz, H_4), 1.55 (s, 9H, $H_{4'}$), 1.53 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4 (C_1), 153.2 ($C_{2''}$), 152.8 ($C_{2'}$), 134.2 (C_3), 124.3 (C_5), 122.2 (C_4), 112.3 (C_2), 83.7 ($C_{3'}$), 79.5($C_{3''}$), 28.1 ($C_{4''}$), 28.0($C_{4'}$) ppm

IR ν_{max} 3268, 3158 (NH), 2976 (NH), 2930, 1719 (CN), 1625 (C=O), 1596 (C=O), 1557, 1413, 1363, 1276, 1234, 1028, 1152, 1101, 1056, 798, 769, 731, 683 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{15}H_{23}N_3O_4S$ $[M + H]^+$ 342.1488, found 342.1493

2,3-Di(*tert*-butoxycarbonyl)-1-(2-{3-[2',3'-di(*tert*-butoxycarbonyl)guanidino]thien-2-yl}thien-3-yl) guanidine (146)



Methyl-3-aminothiophene-2-carboxylate (1.600 g) was heated to reflux in 2M NaOH (10 mL) for 30 min. The solution was cooled, acidified with concentrated hydrochloric acid and the thick precipitate was filtered off. It was pressed dry as possible before being dissolved in acetone (12.5 mL). The solution was dried ($MgSO_4$), filtered and evaporated at 20 °C. The resulting thick paste was treated with 2-propanol (3 mL) and anhydrous oxalic acid (1.000 g) at 38 °C for 45 min. The mixture was cooled, diluted with ether and the solid filtered off and washed with ether. The salt (500.0 mg) was suspended in DCM (15 mL) and *S*-methylthiopseudourea (1.208 g; 4.16 mmol; 2.4 eq.), TEA (1.93 mL; 1.404 g; 13.9 mmol; 8.0 eq) and $HgCl_2$ (1.412 g; 5.2 mmol; 3.0 eq.) were added sequentially. The solution was stirred for 2 days. The solution was filtered through a pad of celite, washed with methanol and the solvents removed in vacuo. The crude material was columned on a Biotage (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 119.0 mg (10 %)

mp decomposes > 180 °C

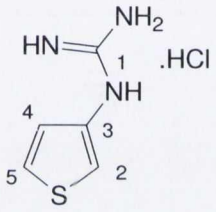
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.69 (brs, 2H, NHCO$_2$^{t}Bu), 10.87 (brs, 2H, NHC$_3$), 7.55 (d, 2H, J = 5.0 Hz, H_5), 7.09 (d, 2H, J = 5.0 Hz, H_4), 1.57 (s, 18H, $H_{4''}$), 1.56 (s, 18H, $H_{4'}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 162.1 (C_1), 153.8 ($C_{2'}$), 153.2 ($C_{2''}$), 138.9 (C_3), 129.2 (C_5), 125.2 (C_2), 123.0 (C_4), 84.5 ($C_{3'}$), 81.4 ($C_{3''}$), 28.1 ($C_{4''}$), 28.0 ($C_{4'}$) ppm

IR ν_{max} 3160 (NH), 2969 (NH), 2922, 1724, 1663 (C=O), 1626 (C=O), 1423, 1396, 1367, 1332, 1277, 1236, 1146, 1113, 1061, 845, 773, 680 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{15}H_{23}N_3O_4S$ $[M + H]^+$ 681.2740, found 681.2507

_N_-(Thien-3-yl)guanidine hydrochloride (147)



Synthesised using Method H from **145** (50.0 mg; 0.146 mmol; 1 eq.) giving a colourless gel. Yield: 22.8 mg (88%)

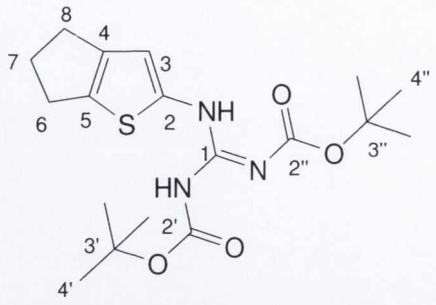
^{1}H NMR (400 MHz, D_2O) δ 7.42 (dd, 1H, J = 5.1, ^4J = 3.1 Hz, H_5), 7.27 (dd, 1H, ^4J = 0.9, 3.1 Hz, H_2), 6.95 (dd, 1H, J = 5.1, ^4J = 0.9 Hz, H_4) ppm

^{13}C NMR (100 MHz, D_2O) δ 156.1 (C_1), 131.5 (C_3), 126.7 (C_2), 124.3 (C_4), 120.2 (C_5) ppm

IR ν_{max} 3301, 3108 (NH), 1664 (C=N), 1600, 1534, 1438, 1408, 1360, 1230, 1185, 1079, 837, 790 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_5H_7N_3S$ $[M + H]^+$ 142.0439, found 142.0436

[2,3-Di(*tert*-butoxycarbonyl)]-1-(5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)guanidine (148)



Compound **106** (3.308 g; 15.66 mmol; 1 eq.) was refluxed in 2M NaOH (5 mL) and ethanol (5 mL) using Method F for 2.25 h. Acetone (20 mL), 2-propanol (4.5 mL) and anhydrous oxalic acid dehydrate (2.5 g) were used to generate 778.6mg (39.8%) of crude yellow salt, which was hydrolysed before being treated with **62** (1.168 g; 4.23 mmol; 2.0 eq.), DCM (10 mL), TEA (2.06 mL; 1.497 g; 14.8 mmol; 7.0 eq) and $HgCl_2$ (1.721 g; 6.33 mmol; 3.0 eq.). Work up as in Method F gave a white solid which darkened to pale orange over time. Yield: 60.1 mg (4%)

mp decomposes > 125 °C

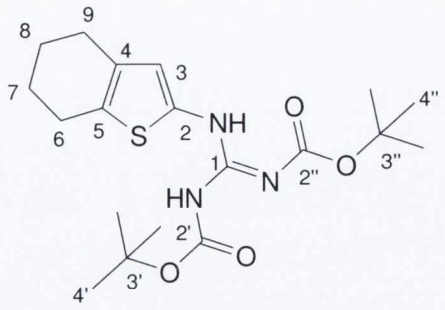
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.47 (brs, 1H, N<u>H</u>$CO_2{}^t$Bu), 10.61 (brs, 1H, N<u>H</u>C_2), 6.53 (s, 1H, H_3), 2.87 (t, 2H, *J* = 4.8 Hz, H_6), 2.68 (t, 2H, *J* = 4.8 Hz, H_8), 2.36 (app. quint., 2H, *J* = 4.8 Hz, H_7), 1.54 (s, 9H, $H_{4'}$), 1.53 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0 (C_1), 153.1 ($C_{2'}$), 151.6 ($C_{2''}$), 142.0 (C_2), 139.3 (C_4), 136.7 (C_5), 110.7 (C_3), 83.8 ($C_{3'}$), 79.6 ($C_{3''}$), 29.3 (C_6), 28.7 (C_8), 28.3 (C_7), 28.1 ($C_{4''}$), 28.0 ($C_{4'}$) ppm

IR ν_{max} 3230 (NH), 2975 (NH), 2930 (NH), 2855 (NH), 1716 (C=N), 1620 (C=O), 1618 (C=O), 1589, 1406, 1367, 1326, 1272, 1249, 1232, 1141, 1101, 1057, 1028, 795 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{18}H_{27}N_3O_4S$ $[M + H]^+$ 382.1801, found 382.1782

[2,3-Di(*tert*-butoxycarbonyl)]-1-(4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)guanidine (149)



Compound **107** (1.126 g; 5 mmol; 1 eq.) was refluxed in 2M NaOH (5 mL) and ethanol (5 mL) using Method F for 2.5 h. Acetone (6.5 mL), 2-propanol (1.5 mL) and anhydrous oxalic acid dehydrate (600.0 mg) were used to generate 726.2 mg (73.3 %) of crude pink salt, of which 585.4 mg was hydrolysed before being treated with **62** (816.0 mg; 2.95 mmol; 2.0 eq.), DCM (10 mL), TEA (1.439 mL; 1.046 g; 10.33 mmol; 7.0 eq) and $HgCl_2$ (1.202 g; 4.43 mmol; 3.0 eq.). Work up as in Method F gave a white solid which darkens to pink. Yield: 72.9 mg (23%)

mp 114-116 °C

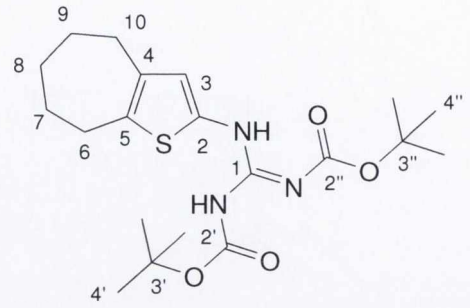
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.47 (brs, 1H, N<u>H</u>$CO_2{}^t$Bu), 10.62 (brs, 1H, N<u>H</u>C_2), 6.42 (s, 1H, H_3), 2.69 (t, 2H, J = 5.9 Hz, H_6), 2.51 (t, 2H, J = 5.9 Hz, H_9), 1.84-1.75 (m, 4H, $H_{7,8}$), 1.54 (s, 9H, $H_{4'}$), 1.54 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0 (C_1), 153.1 ($C_{2'}$), 151.5 ($C_{2''}$), 134.2 (C_2), 131.8 (C_4), 130.2 (C_5), 115.2 (C_3), 83.7 ($C_{3'}$), 79.5 ($C_{3''}$), 28.1 ($C_{4''}$), 28.0 ($C_{4'}$), 25.2 (C_9), 24.3 (C_6), 23.5 (C_7), 22.9 (C_8) ppm

IR ν_{max} 3236 (NH), 2936 (NH), 1715 (C=N), 1643 (C=O), 1624 (C=O), 1395, 1369, 1331, 1305, 1277, 1250, 1143, 1103, 1058, 795, 775, 751 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{19}H_{29}N_3O_4S$ $[M + H]^+$ 396.1957, found 396.1963

[2,3-Di(*tert*-butoxycarbonyl)]-1-(5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thien-2-yl)guanidine (150)



Compound **108** (1.197 g; 5 mmol; 1 eq.) was refluxed in 2M NaOH (5 mL) and ethanol (5 mL) using Method F for 2.5 h. Acetone (6.5 mL), 2-propanol (1.5 mL) and anhydrous oxalic acid dehydrate (600.0 mg) were used to generate 715.9 mg (67.5 %) of crude lilac salt which was hydrolysed before being treated with **62** (932.0 mg; 3.37 mmol; 2.0 eq.), DCM (10 mL), TEA (1.643 mL; 1.195 g; 11.8 mmol; 7.0 eq) and $HgCl_2$ (1.373 g; 5.06 mmol; 3.0 eq.). Work up as in Method F gave a white solid which darkens to pale yellow. Yield: 483.3 mg (35%)

mp 122-124 °C

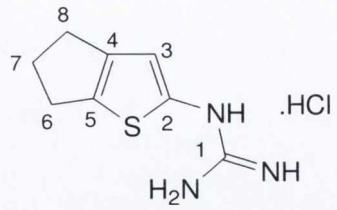
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.47 (brs, 1H, N<u>H</u>$CO_2{}^t$Bu), 10.58 (brs, 1H, N<u>H</u>C_2), 6.40 (s, 1H, H_3), 2.69 (t, 2H, J = 5.4 Hz, H_6), 2.56 (t, 2H, J = 5.4 Hz, H_{10}), 1.80-1.77 (m, 2H, H_8), 1.66-1.64 (m, 2H, H_7), 1.59-1.57 (m, 2H, H_9), 1.51 (s, 9H, $H_{4'}$), 1.51 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0 (C_1), 153.1 ($C_{2'}$), 151.3 ($C_{2''}$), 137.0 (C_2), 133.8 (C_4), 131.8 (C_5), 117.8 (C_3), 83.6 ($C_{3'}$), 79.4 ($C_{3''}$), 32.4 (C_8), 30.8 (C_{10}), 29.2 (C_6), 28.4 (C_7), 28.1 ($C_{4''}$), 27.9 ($C_{4'}$), 27.9 (C_9) ppm

IR ν_{max} 3255 (NH), 2976, 2931, 2915 (NH), 2846, 1717 (C=N), 1631 (C=O), 1613 (C=O), 1589, 1405, 1368, 1330, 1316, 1254, 1230, 1146, 1134, 1102, 1057, 1028, 780 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{20}H_{31}N_3O_4S$ $[M + H]^+$ 410.2114, found 410.2108

_N_-(5,6-Dihydro-4_H_-cyclopenta[_b_]thien-2-yl)guanidine hydrochloride (152)



Synthesised using Method H using **148** (50.0 mg; 0.131 mmol; 1 eq.) giving a yellow gel. Yield: 11.7mg (60%)

^{1}H NMR (400 MHz, D_2O) δ 6.73 (s, 1H, H_3), 2.76 (t, 2H, J = 6.9 Hz, H_6), 2.59 (t, 2H, J = 6.9 Hz, H_8), 2.24-2.20 (m, 2H, H_7) ppm

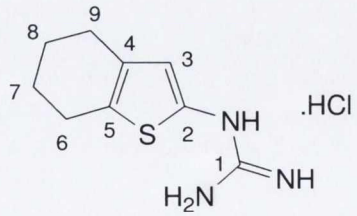
^{13}C NMR (100 MHz, D_2O) δ 157.6 (C_1), 144.1 (C_2), 141.8 (C_4), 135.1 (C_5), 123.1 (C_3), 29.2 (C_6), 28.3 (C_8), 27.5 (C_7) ppm

IR ν_{max} 3318 (NH), 2953 (NH), 2855, 2456, 1654, 1586 (C=N), 1439, 1194, 1154 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_8H_{12}N_3S$ $[M + H]^+$ 182.0752, found 182.0751

$C_8H_{13}ClN_3S{\cdot}2.7D_2O{\cdot}0.4C_4H_8O_2$ requires C, 37.56; H, 5.00; N, 13.69%. Found: C, 37.76; H, 4.69; N, 13.37%

_N_-(4,5,6,7-4_H_-Tetrahydrobenzo[_b_]thien-2-yl)guanidine hydrochloride (151)



Synthesised via Method H from **149** (100.0 mg; 0.252 mmol; 1 eq.) giving a yellow gel. Yield: 46.0 mg (79%)

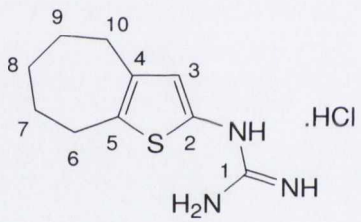
^{1}H NMR (400 MHz, D_2O) δ 6.59 (s, 1H, H_3), 2.56 (t, 2H, *J* = 5.5 Hz, H_6), 2.41 (t, 2H, *J* = 5.6 Hz, H_9), 1.69-1.63 (m, 4H, $H_{7,8}$) ppm

^{13}C NMR (100 MHz, D_2O) δ 156.9 (C_1), 135.6 (C_2), 134.1 (C_4), 130.1 (C_5), 126.9 (C_3), 24.4 (C_9), 23.8 (C_6), 22.4 (C_7), 21.7 (C_8) ppm

IR ν_{max} 3129, 2930 (NH), 2843, 1665 (CN), 1591, 1439, 1207, 1135, 844 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_9H_{14}N_3S$ $[M + H]^+$ 196.0908, found 196.0902

_N_-(5,6,7,8-Tetrahydro-4_H_-cyclohepta[_b_]thien-2-yl)guanidine hydrochloride (153)



Synthesised using Method H from **150** (100.0 mg; 0.244 mmol; 1 eq.) giving a yellow gel. Yield: 59.6 mg (99%)

^{1}H NMR (400 MHz, D_2O) δ 6.69 (s, 1H, H_3), 2.70 (t, 2H, *J* = 5.6 Hz, H_6), 2.59 (t, 2H, *J* = 5.6 Hz, H_{10}), 1.80-1.74 (m, 2H, H_8), 1.61-1.55 (m, 2H, H_7), 1.54-1.49 (m, 2H, H_9) ppm

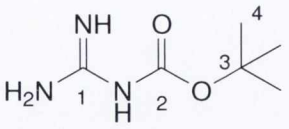
^{13}C NMR (100 MHz, D_2O) δ 157.5 (C_1), 140.2 (C_2), 140.2 (C_4), 130.1 (C_3), 128.0 (C_5), 31.7 (C_8), 20.1 (C_{10}), 29.2 (C_6), 27.8 (C_7), 27.3 (C_9) ppm

IR ν_{max} 3289 (NH), 2918 (NH), 2842, 2418, 1642, 1576 (C=N), 1433, 1211, 717 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{10}H_{15}N_3S$ $[M + H]^+$ 210.1065, found 210.1069

$C_{10}H_{16}ClN_3S{\cdot}0.5D_2O$ requires C, 46.96; H, 6.30; N, 16.43%. Found: C, 47.35; H, 6.04; N, 16.37%

N-tert-Butoxycarbonyl guanidine (154)[183]



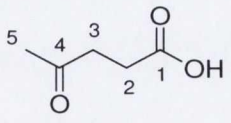
Guanidine hydrochloride (7.500 g; 78.5 mmol; 1.25 eq.) was dissolved in a 4M sodium hydroxide solution (40 mL) and cooled to 0 °C. Di-*tert*-butyldicarbonate (13.706 g; 62.8 mmol; 1 eq.) was dissolved in dioxane (80 mL) and added dropwise with stirring. The reaction was left stirring at r.t. overnight. Excess solvent was removed under reduced pressure. The white solid was suspended in water (50 mL) and sonicated for 10 min. The solid was filtered and resuspended in ether (50 mL) and sonicated for a further 10 min. Finally the white solid was filtered and dried in a dessicator overnight. Yield: 6.100 g (61%)

mp > 300 °C (Lit.[183] 165 °C decomposes)

^{1}H NMR (400 MHz; DMSO-d_6) δ 6.78 (brs, 4H, NH_2, NH, NH), 1.34 (s, 9H, H_4) ppm (Lit.[183] 6.82, 1.34)

HRMS (ESI) calc. for $C_6H_{13}N_3O_2$ $[M + H]^+$ 160.1086, found 160.1082

Levulinic acid or 4-oxo-pentanoic acid (156)[188,211]



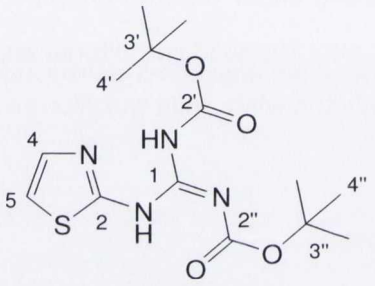
Methyl levulinate (10 mL; 9.515 g; 73.2 mmol; 1 eq.) was heated in refluxing aqueous KOH (20%; 100 mL) for 1 h. The mixture was cooled down and acidified with conc HCl. The mixture was then extracted with EtOAc (4x250 mL) and the solvents removed. Yield: 8.010 g (94%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (t, 2H, *J* = 6.5, H_2), 2.61 (t, 2H, *J* = 6.5, H_3), 2.20 (s, 3H, H_5) ppm (Lit.[188] 2.73, 2.59, 2.17)

IR ν_{max} 2922 (OH), 1702 (C=Ox2), 1400, 1366, 1206, 1160, 1063, 1022, 988 cm^{-1} (Lit.[211] 2941-3333, 1695-1754, 1404, 1370, 1211, 1166, 1064, 1026, 990)

HRMS (ESI) *m/z* calc. for $C_5H_8O_3$ $[M + Na]^+$ 139.0371, found 139.0375

[2,3-Di(*tert*-butoxycarbonyl)]-1-(thiazol-2-yl)guanidine (160)



Synthesised from 2-aminothiazole (300.0 mg; 3 mmol; 1.0 eq.), **62** (994.0 mg; 3.59 mmol; 1.2 eq.), TEA (1.67 mL; 1.213 g; 11.98 mmol; 4.0 eq.) and $HgCl_2$ (1.220 g; 4.49 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 360.5 mg (35%)

Or

Synthesised from 2-aminothiazole (300.4 mg; 3 mmol; 1 eq.) via Method E. Yield: 529.4 mg (52%)

mp decomposes 126 °C

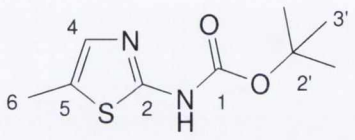
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.33 (brs, 1H, N<u>H</u>$CO_2{}^t$Bu), 9.62 (brs, 1H, N<u>H</u>C_2), 8.14 (d, 1H, *J* = 5.0 Hz, H_4), 6.48 (d, 1H, *J* = 5.0 Hz, H_5), 1.56 (s, 9H, $H_{4'}$), 1.54 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8 (C_1), 170.5 (C_2), 160.3 ($C_{2''}$), 157.4 ($C_{2'}$), 123.6 (C_4), 106.4 (C_5), 81.0 ($C_{3'}$), 80.0 ($C_{3''}$), 28.2 ($H_{4''}$), 28.1 ($H_{4'}$) ppm

IR ν_{max} 2980 (NH), 2730 (NH), 1711, 1562 (2xC=O), 1366, 1320, 1289, 1249, 1150, 1076, 1053, 860, 794, 766, 724, 711, 679 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{14}H_{22}N_4O_4S$ $[M + H]^+$ 343.1440, found 343.1433

tert-Butyl-_N_-(5-methylthiazol-2-yl)carbamate (161)



Synthesised from 5-methyl-2-aminothiazole (228.3 mg; 2 mmol; 1.0 eq.), *S*-methylthiopseudourea (696.9 mg; 2.4 mmol; 1.2 eq.), TEA (1.11 mL; 809.8 mg; 8 mmol; 4.0 eq.) and $HgCl_2$ (814.5 mg; 3 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 240.9 mg (56%)

mp 163-165 °C

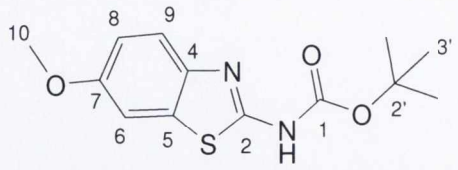
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.20 (brs, 1H, NH), 7.00 (app. d, 1 H, ^4J = 1.5 Hz, H_4), 2.37 (d, 3H, ^4J = 1.5 Hz, H_6), 1.58 (s, 9H, $H_{3'}$) ppm

^{13}C NMR (400 MHz, $CDCl_3$) δ 160.3 (C_2), 153.0 (C_1), 133.4 (C_4), 125.7 (C_5), 81.5 ($C_{2'}$), 28.3 ($C_{3'}$), 11.6 (C_6) ppm

IR ν_{max} 3168 (NH), 3060, 2980, 2927, 2782, 2720, 1709 (C=O), 1571, 1366, 1306, 1284, 1248, 1159, 1055, 823, 835, 804, 781, 747 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_9H_{14}N_2O_2S$ $[M + Na]^+$ 215.0854, found 215.0854

tert-Butyl-(6-methoxybenzothiazol-2-yl)carbamate (162)



Synthesised from 2-amino-6-methoxybenzothiazole (360.5 mg; 2 mmol; 1.0 eq.), *S*-methylthiopseudourea (696.9 mg; 2.4 mmol; 1.2 eq.), TEA (1.11 mL; 809.8 mg; 8 mmol; 4.0 eq.) and $HgCl_2$ (814.5 mg; 3 mmol; 1.5 eq.) using Method D. The product was purified by Biotage column chromatography (hexane/EtOAc; 3 CV/% gradient) giving a white powder. Yield: 281.0 mg (50%)

mp 174-176 °C

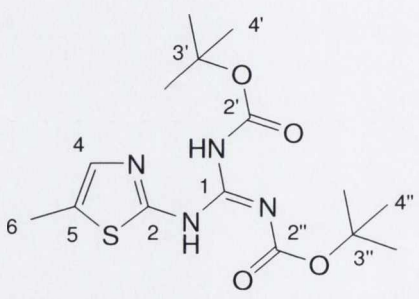
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.62 (brs, 1H, NH), 7.86 (d, 1H, J = 8.5 Hz, H_9), 7.28 (d, 1H, ^4J = 2.5 Hz, H_6), 7.00 (dd, 1H, J = 8.5, ^4J = 2.5 Hz, H_8), 3.89 (s, 3H, H_{10}), 1.61 (s, 9H, $H_{3'}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6 (C_2), 156.4 (C_7), 152.9 (C_1), 142.9 (C_4), 132.7 (C_5), 121.5 (C_6), 114.5 (C_8), 104.1 (C_9), 83.1 ($C_{2'}$), 55.9 (C_{10}), 28.4 ($C_{3'}$) ppm

IR ν_{max} 2980 (NH), 1708 (C=O), 1611, 1561, 1475, 1366, 1275, 1247, 1219, 1160, 1053, 1027, 824, 813, 790, 750 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{13}H_{16}N_2O_3S$ $[M + H]^+$ 281.0960, found 281.0952

[2,3-Di(tert-butoxycarbonyl)]-1-(5-methylthiazol-2-yl)guanidine (168)



Synthesised from 5-methyl-2-aminothiazole (342.5 mg; 3 mmol; 1 eq.) via Method E giving a white powder. Yield: 715.0 mg (67%)

mp 117-119 °C

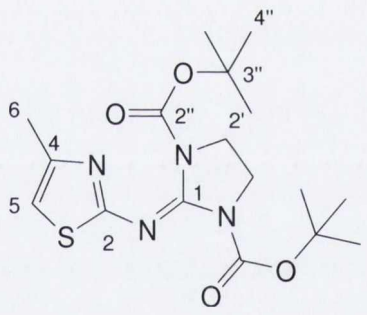
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.23 (brs, 1H, N<u>H</u>$CO_2{}^{t}$Bu), 9.60 (brs, 1H, N<u>H</u>C_2), 7.82 (app. d, 1H, ^{4}J = 1.4 Hz, H_4), 2.20 (d, 3H, ^{4}J = 1.4 Hz, H_6), 1.55 (s, 9H, $H_{4'}$), 1.54 (s, 9H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0 (C_2), 162.4 (C_1), 159.7 ($C_{2'}$), 156.9 ($C_{2''}$), 118.6 (C_4), 117.9 (C_5), 80.4 ($C_{3'}$), 79.4 ($C_{3''}$), 27.7 ($C_{4''}$), 27.6 ($C_{4'}$), 12.0 (C_6) ppm

IR ν_{max} 3333 (NH), 2972 (NH), 2925, 1663 (C=O), 1648 (C=O), 1519, 1334, 1279, 1236, 1167, 1117, 1052, 816, 659 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{15}H_{24}N_4O_4S$ $[M + Na]^+$ 379.1416, found 379.1423

[1,3-Di-*tert*-butoxycarbonyl]-2-(4-methylthiazol-2-ylimino)imidazolidine (176)



Synthesised from 4-methyl-2-aminothiazole (228.3 mg; 2 mmol; 1.0 eq.), **63** (544.4 mg; 1.8 mmol; 0.9 eq.), TEA (1.11 mL; 809.7 mg; 8 mmol; 4.0 eq.) and $HgCl_2$ (814.5 mg; 3 mmol; 1.5 eq.) using Method D. A neutral alumina column (hexane/EtOAc 1:1) was run for purification giving a pale yellow powder. Yield: 174.0 mg (25%)

mp 84-86 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.43 (app. d, 1H, J = 1.1 Hz, H_5), 3.86 (s, 4H, $H_{2'}$), 2.24 (d, 3H, J = 1.1 Hz, H_6), 1.40 (s, 18H, $H_{4''}$) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.59 (C_2), 149.66 (C_1), 148.51 ($C_{2''}$), 142.69 (C_4), 108.41 (C_5), 83.03 ($C_{3''}$), 43.07 ($C_{2'}$), 27.81 ($C_{4''}$), 17.51 (C_6) ppm

IR ν_{max} 3087 (NH), 2975 (NH), 2927, 1715, 1704 (C=O), 1654 (C=O), 1455, 1383, 1368, 1306, 1255, 1221, 1207, 1150, 1103, 1091, 988, 967, 850, 770, 761, 695 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{17}H_{16}N_4O_4S$ $[M + H]^+$ 383.1753, found 383.1749

9.2 Pharmacology

Materials and Methods.

[^{3}H]RX821002 (specific activity 55 Ci/mmol) was obtained from GE Healthcare, UK. [^{35}S]GTPγS (1250 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences, Inc., RX821002 HCl was synthesized by Dr. F. Geijo at SA Lasa Laboratories (Barcelona, Spain) and UK14304 was purchased from Sigma (St.Louis, USA).

Preparation of Membranes for [^{3}H]RX821002 Binding Assays

Neural membranes (P2 fractions) were prepared from the PFC of human brains obtained at autopsy in the Instituto Vasco de Medicina Legal, Bilbao, Spain. Postmortem human brain samples of each of 12 subjects (~ 300 mg) were homogenized using an Ultra-Turrax T8 (IKA Labortechnik, Staufen, Germany) at maximum speed for 10 seconds (4 °C) in 30 volumes of homogenization buffer (1 mM MgCl2, and 5 mM Tris-HCl, pH 7.4) supplemented with 0.25 M sucrose. The crude homogenate was centrifuged for 5 min at 1000 X g (4 °C) and the supernatant was centrifuged again for 10 min at 40,000 X g (4 °C). The resultant pellet was washed twice in 5 volumes of incubation buffer (50 mM Tris-HCl; pH 7.5) and recentrifuged in similar conditions. Aliquots of 1.2 mg protein were stored at -70 °C until assay. Protein content was measured according to the method Bradford using BSA as standard.

The day of the experiment the membrane pellets were defrosted (4 °C), thawed and re-suspended in incubation buffer reaching a final protein concentration of 0.4 mg/ml approximately. The real final protein content was measured after the experiment according to Bradford's method.

[^{3}H]RX821002 Binding Assays.

Specific [^{3}H]RX821002 binding was measured in 0.250 mL aliquots (50 mM Tris-HCl, pH 7.5) of the neural membranes, which were incubated in 96-well plates with [^{3}H]RX821002 (2 nM) for 30 min at 25 °C in the absence or presence of the competing compounds (10^{-12} to 10^{-3} M, 10 concentrations). Free ligand was separated from bound ligand by rapid filtration under vacuum (1450 FilterMate Harvester, PerkinElmer) through GF/C glass fiber filters (Printed Filtermat A) presoaked with 0.5% polyethylenimine. The filters were then rinsed three times with 300 µL of ice-cold Tris incubation buffer (4 °C), air dried and counted for radioactivity by liquid scintillation spectrometry using a MicroBeta TriLux counter (PerkinElmer). Specific binding was determined and plotted as a function of the compound concentration. Nonspecific binding was determined in the presence of adrenaline (10^{-5} M).

Analysis of Binding Data

Analysis of competition experiments to obtain the inhibition constant (K_i) were performed by nonlinear regression using GraphPad Prism® software. All experiments were analysed assuming a one-site model of radioligand binding.

Preparation of Membranes for [^{35}S]GTPγS Assays

Preparation of plasma membranes (P2 fraction) and [^{35}S]GTPγS assays were performed as previously described (Gonzalez-Maeso et al.[106]) with minor modifications. Tissue samples of each subject (~200 mg) were homogenized using a Teflon-glass grinder (10 up-and-down strokes at 1,500 rpm) in 30 volumes of homogenization buffer (1 mM EGTA, 3 mM $MgCl_2$, 1 mM DTT, and 50 mM Tris-HCl, pH 7.4) supplemented with 0.25 M sucrose. The crude homogenate was centrifuged for 5 min at 1,000 g at 4 °C and the supernatant layer was re-centrifuged for 10 min at 40,000 g (4 °C). The resultant pellet was washed twice in 20 volumes of homogenization buffer and recentrifuged in similar conditions. Protein content was measured according to the method of Bradford (1976) using BSA as standard. Samples were aliquoted in order to have a protein content of 1.2 mg and then centrifuged in a benchtop centrifuge (EBA 12 R, Hettich Instruments, Tuttlingen, Germany) at highest speed (14.000 rpm) during 15 min at 4 °C. The supernatant layer was carefully discarded and the pellets stored at -70 °C until the assays were carried out.

The day of the experiment the membrane pellets were defrosted (4 °C), thawed and re-suspended in 12 ml of buffer containing 1 mM EGTA, 3 mM $MgCl_2$, 100 mM NaCl, and 50 mM Tris-HCl, pH 7.4, reaching a final protein concentration of 0.8 mg/mL approximately. The real final protein content was measured after the experiment according to Bradford's method.

[^{35}S]GTPγS Activity Assays.

[^{35}S]GTPγS binding assays were performed for all compounds which reached at least a K_i value of 1 μM in the competition binding assays with [^{3}H]RX821002.

The incubation buffer for measuring [^{35}S]GTPγS binding to brain membranes contained, in a total volume of 250 μL, 1 mM EGTA, 3 mM $MgCl_2$, 100 mM NaCl, 50 mM GDP, 50 mM Tris-HCl at pH 7.4, and 0.5 nM [^{35}S]GTPγS. Protein aliquots were thawed and resuspended in the same buffer. The incubation was started by addition of the membrane suspension (20 μg of membrane proteins per well) to the previous mixture and was performed in 96-well plates at 30 °C for 120 min with shaking. In order to evaluate the influence of the compounds on [^{35}S]GTPγS binding, ten concentrations (10^{-12} to 10^{-3} M) of the different compounds were added to the assay.

All compounds that showed antagonistic activity in the [^{35}S]GTPγS binding assays (no stimulation nor inhibition of the basal binding) were also assayed at a constant concentration (10^{-5} M) for [^{35}S]GTPγS binding in the presence of increasing concentrations of the α_2-AR receptor agonist UK14304 (10^{-13}-10^{-4} M). These experiments were performed in order to investigate the potential of the compounds to specifically modify the EC_{50} or the E_{max} values in the UK14304 stimulation curve.

Incubations were terminated by rapid filtration under vacuum through Whatman GF/C filters (FilterMate A) presoaked in the same buffer. The filters were then rinsed three times with 300 μL of ice-cold incubation buffer (4 °C), air dried and counted for radioactivity by liquid scintillation spectrometry using a MicroBeta TriLux counter (PerkinElmer). The [^{35}S]GTPγS bound was about 7-14% of the total [^{35}S]GTPγS added. Nonspecific binding (NS) of the radioligand was defined as the remaining [^{35}S]GTPγS binding in the presence of 100 μM unlabeled GTPγS.

Mathematical and Statistical Analysis of the Results

The NS was subtracted from the net experimental stimulations (NStim). In order to allow a good interpretation of the data, they are displayed as relative stimulations over basal values (B) (% over basal). The basal binding of [^{35}S]GTPγS, defined in the absence of agonist, was considered the 100% and the percentage of stimulation (or inhibition) over the basal level was calculated as follows: The pharmacological parameters of the stimulation or inhibition of the [^{35}S]GTPγS binding, the maximal effect (E_{max}) and the concentration of the drug that determines the half maximal effect (EC_{50}), were obtained by non-linear analysis using GraphPad Prism® software. The points fit to a concentration-response curve, shown in Equation 9.1 . The pharmacological parameters E_{max} and EC_{50} are expressed as means ± S.E.M.

Equation 9.1: % over basal= ((NStim - NS)/B - NS) x 100

10 References

1. Elhwuegi, A. S., *Prog. Neuro-psychoph.* **2004,** 28, 435-451.
2. http://zelitenutrition.com/images/Neurotransmitters.png (2/7/2012),
3. http://2.bp.blogspot.com/-TRQWk4SewVU/TZtRhvyKxHI/AAAAAAAAABg/DucxX0X8FX8/s1600/ader.JPG (8/7/12),
4. Patrick, G. L., *An Introduction to Medicinal Chemistry*. Second Edition ed.; Oxford University Press: Oxford, 2001.
5. Hieble, J. P.; Bondinell, W. E.; Ruffolo, R. R. J., *J. Med. Chem.* **1995,** 38, 3415-3444.
6. Bahia, D. S.; Wise, A.; Fanelli, F.; Lee, M.; Rees, S.; Milligan, G., *Biochemistry-US* **1998,** 37, 11555-11562.
7. Wong, W. C.; Sun, W.; Cui, W.; Chen, Y.; Forray, C.; Vaysse, P. J. J.; Branchek, T. A.; Gluchowski, C., *J. Med. Chem.* **2000,** 43, 1699-1704.
8. Vonvoigtlander, P. F.; Triezenberg, H. J.; Losey, E. G.; Gay, D. D., *Drug Develop. Res.* **1983,** 3, 545-554.
9. Hertel, P.; Fagerquist, M. V., *Science* **1999,** 286, 105-107.
10. Tellez, S.; Colpaert, F.; Marien, M., *J. Neurochem.* **1997,** 68, 778-785.
11. Raiteri, M.; Maura, G.; Folghera, S.; Cavazzani, P.; Andrioli, G. C.; Schlicker, E.; Schalnus, R.; Gothert, M., *N-S Arch. Pharmacol.* **1990,** 342, 508-512.
12. Premont, R. T.; Inglese, J.; Lefkowitz, R. J., *FASEB J.* **1995,** 9, 175-182.
13. Liang, M.; Eason, M. G.; Theiss, C. T.; Liggett, S. B., *Eur. J. Pharmacol.* **2002,** 437, 41-46.
14. http://abcd.math.vanderbilt.edu/~signaltr/virtual_rod/pic/GPCR.jpg (3/9/12),
15. Ribas, C.; Miralles, A.; Busquets, X.; García-Sevilla, J. A., *Brit. J. Pharmacol.* **2001,** 132, 1467-1476.
16. Callado, L. F.; Meana, J. J.; Grijalba, B.; Pazos, A.; Sastre, M.; Garcia-Sevilla, J. A., *J. Neurochem.* **1998,** 70, 1114-1123.
17. Lohse, M. J.; Krasel, C.; Winstel, R.; Mayor Jr., F., *Kidney Int.* **1996,** 49, 1047-1052.
18. Scheinin, M.; Sallinen, J.; Haapalinna, A., *Life Sci.* **2001,** 68, 2277-2285.
19. Trendelenburg, A.; Limberger, N.; Starke, K., *N-S Arch. Pharmacol.* **1993,** 348, 35-45.
20. Uhlen, S.; Xia, Y.; Chhajlani, V.; Felder, C. C.; Wikberg, J. E. S., *Brit. J. Pharmacol.* **1992,** 106, 986-995.
21. Esteban, S.; Lladó, J.; Sastre-Coll, A.; García-Sevilla, J. A., *N-S Arch. Pharmacol.* **1999,** 360, 135-143.
22. Esteban, S.; Lladó, J.; Garcia-Sevilla, J. A., *N-S Arch. Pharmacol.* **1996,** 353, 391-399.

23. Gonzales-Maeso, J.; Rodriguez-Puertas, R.; Meana, J. J.; Garcia-Sevilla, J. A.; Gulmon, J., *Mol. Psychiatr.* **2002,** 7, 755-767.
24. García-Vallejo, P.; Gómez, F. M.; Infante, C.; Ginestal, E.; Giralt, M. T., *Brain Res.* **1998,** 801, 72-77.
25. Mateo, Y.; Fernandez-Pastor, B.; Meana, J. J., *Brit. J. Pharmacol.* **2001,** 133, 1362-1370.
26. Rang, H. P.; Dale, M. M.; Ritter, J. M.; Moore, P. K., *Pharmacology*. 5th ed.; Churchill Livingstone: 2003.
27. Fernandez-Pastor, B.; Meana, J. J., *Eur. J. Pharmacol.* **2002,** 442, 225-229.
28. Devoto, P.; Flore, G.; Pani, L.; Gessa, G. L., *Mol. Psychiatr.* **2001,** 6, 657-664.
29. Mateo, Y.; Pineda, J.; Meana, J. J., *J. Neurochem.* **1998,** 71, 790-798.
30. Thompson, C., *Hum. Psychopharm. Clin.* **2002,** 17, S27-S32.
31. Carpenter, L. L.; Yasmin, S.; Price, L. H., *Biol. Psychiat.* **2002,** 51, 183-188.
32. Sanacora, G.; Berman, R. M.; Cappiello, A.; Oren, D. A.; Kugaya, A.; Liu, N.; Gueorguieva, R.; Fasula, D.; Charney, D. S., *Neuropsychopharmacol.* **2004,** 29, 1166-1171.
33. http://www.nlm.nih.gov/medlineplus/depression.html (3/9/12),
34. Nestler, E. J.; Barrot, M.; DiLeone, R. J.; Eisch, A. J.; Gold, S. J.; Monteggia, L. M., *Neuron* **2002,** 34, 13-25.
35. http://www.nhs.uk/Conditions/Depression/Pages/Introduction.aspx (3/9/12),
36. Gater MD, R.; Tansella, M.; Korten, A.; Tiemens, B. G.; Mavreas, V. G.; Olatawura, M. O., *Arch. Gen. Psychiatry* **1998,** 55, 405-413.
37. http://www.suicideprevention.ie/pages/?id=52&tid=13 (3/9/12),
38. http://www.cso.ie/en/releasesandpublications/health/nationaldisabilitysurvey2006-firstresults/ (3/9/12),
39. http://www.who.int/mental_health/management/depression/definition/en/ (3/9/12),
40. *Mental Health Global Action Programme. Close the gap, dare to care*, Geneva: World Health Organization, 2002.
41. Berto, P.; D'Ilario, D.; Ruffo, P.; Di Virgilio, R.; Rizzo, F., *J. Ment. Health Policy* **2000,** 3, 3–10.
42. Sullivan, P. F.; Neale, M. C.; Kendler, K. S., *Am. J. Psychiat.* **2000,** 157, 1552-1562.
43. Gutman, D. A.; Nemeroff, C. B., *Physiol. Behav.* **2003,** 79, 471-478.
44. Berton, O.; Nestler, E. J., *Nat. Rev. Neurosci.* **2006,** 7, 137-151.
45. Schildkraut, J. J., *Am. J. Psychiat.* **1965,** 122, 509-522.
46. Airan, R. D.; Meltzer, L. A.; Roy, M.; Gong, Y.; Chen, H.; Deisseroth, K., *Science* **2007,** 317, 819-823.

47. Nestler, E. J.; Carlezon, J. W. A., *Biol. Psychiat.* **2006,** 59, 1151-1159.

48. Krishnan, V.; Nestler, E. J., *Nature* **2008,** 455, 894-902.

49. Shirayama, Y.; Chen, A. C. H.; Nakagawa, S.; Russell, D. S.; Duman, R. S., *J. Neurosci.* **2002,** 22, 3251-3261.

50. Santarelli, L.; Saxe, M.; Gross, C.; Surget, A.; Battaglia, F.; Dulawa, S.; Weisstaub, N.; Lee, J.; Duman, R.; Arancio, O.; Belzung, C.; Hen, R., *Science* **2003,** 301, 805-809.

51. Malberg, J. E.; Eisch, A. J.; Nestler, E. J.; Duman, R. S., *J. Neurosci.* **2000,** 20, 9104-9110.

52. Nibuya, M.; Morinobu, S.; Duman, R. S., *J. Neurosci.* **1995,** 15, 7539-7547.

53. Warner-Schmidt, J. L.; Duman, R. S., *P. Natl. Acad. Sci. USA* **2007,** 104, 4647-4652.

54. Yanpallewar, S. U.; Fernandes, K.; Marathe, S. V.; Vadodaria, K. C.; Jhaveri, D.; Rommelfanger, K.; Ladiwala, U.; Jha, S.; Muthig, V.; Hein, L.; Bartlett, P.; Weinshenker, D.; Vaidya, V. A., *J. Neurosci.* **2010,** 30, 1096-1109.

55. Jhaveri, D. J.; Mackay, E. W.; Hamlin, A. S.; Marathe, S. V.; Nandam, L. S., *J. Neurosci.* **2010,** 30, 2795-2806.

56. Sahay, A.; Hen, R., *Nat. Neurosci.* **2007,** 10, 1110-1115.

57. Gillespie, C. F.; B, N. C., *Psychosom. Med.* **2005,** 67, S26-S28.

58. Carroll, B. J., *Lancet* **1968,** 1, 1373-1374.

59. Carroll, B. J., *J. Clin. Psychiat.* **1982,** 43, 44-50.

60. Arana, G. W.; Baldessarini, J. R.; Ornsteen, M., *Arch. Gen. Psychiat.* **1985,** 42, 1193-1204.

61. Tsatsanis, C.; Dermitzaki, E.; Venihaki, M.; Chatzaki, E.; Minas, V.; Gravanis, A.; Margioris, A., *Cell. Mol. Life Sci.* **2007,** 64, 1638-1655.

62. Lemonde, S.; Turecki, G.; Bakish, D.; Du, L.; Hrdina, P. D.; Bown, C. D.; Sequeira, A.; Kushwaha, N.; Morris, S. J.; Basak, A.; Ou, X.-M.; Albert, P. R., *J. Neurosci.* **2003,** 23, 8788-8799.

63. Zill, P.; Baghai, T. C.; Zwanzger, P.; Schüle, C.; Eser, D.; Rupprecht, R.; Möller, H.-J.; Bondy, B.; Ackenheil, M., *Mol. Psychiatr.* **2004,** 9, 1030-1036.

64. Perroud, N.; Neidhart, E.; Petit, B.; Vessaz, M.; Laforge, T.; Relecom, C.; La Harpe, R.; Malafosse, A.; Guipponi, M., *Am. J. Med. Genet. B* **2010,** 153B, 909-918.

65. Eley, T. C.; Sugden, K.; Corsico, A.; Gregory, A. M.; Sham, P.; McGuffin, P.; Plomin, R.; Craig, I. W., *Mol. Psychiatr.* **2004,** 9, 908-915.

66. Mann, J. J.; Huang, Y.-y.; Underwood, M. D.; Kassir, S. A.; Oppenheim, S.; Kelly, T. M.; Dwork, A. J.; Arango, V., *Arch. Gen. Psychiat.* **2000,** 57, 729-738.

67. Homberg, J.; Nijman, I. J.; Kuijpers, S.; Cuppen, E., *BMC Genet.* **2010,** 11, 37-47.

68. Sallinen, J.; Haapalinna, A.; MacDonald, E.; Viitamaa, T.; Lahdesmaki, J.; Rybnikova, E.; Pelto-Huikko, M.; Kobilka, B. K.; Scheinin, M., *Mol. Psychiatr.* **1999,** 4, 443-452.

69. Martin-Guerrero, I.; Callado, L. F.; Saitua, K.; Rivero, G.; Garcia-Orad, A.; Meana, J. J., *Psychopharmacology* **2006,** 184, 82-86.

70. Haefner, S.; Baghai, T. C.; Schule, C.; Eser, D.; Spraul, M.; Zill, P.; Rupprecht, R.; Bondy, B., *Neuropsychobiology* **2008,** 58, 154-162.

71. Zill, P.; Baghai, T. C.; Zwanzger, P.; Schüle, C.; Minov, C.; Riedel, M.; Neumeier, K.; Rupprecht, R.; Bondy, B., *NeuroReport* **2000,** 11, 1893-1897.

72. Binder, E. B.; Nemeroff, C. B., *Mol. Psychiatr.* **2010,** 15, 574-588.

73. Domschke, K.; Dannlowski, U.; Hohoff, C.; Ohrmann, P.; Bauer, J.; Kugel, H.; Zwanzger, P.; Heindel, W.; Deckert, J.; Arolt, V.; Suslow, T.; Baune, B. T., *Eur. Neuropsychopharm.* **2010,** 20, 301-309.

74. Selikoff, I. J.; Robitzek, E. H., *Dis. Chest* **1952,** 21, 385-438.

75. Rozas, I., *Expert Opin. Ther. Pat.* **2009,** 19, 827-845.

76. http://www.nlm.nih.gov/medlineplus/druginfo/meds/a695033.html (3/9/12),

77. http://www.tianeptine.com/ (3/9/12),

78. Frazer, A., *J. Clin. Psychiat.* **1997,** 58, 9-25.

79. Mustafa, S. M.; Bavadekar, S. A.; Ma, G.; Moore, B. M.; Feller, D. R.; Miller, D. D., *Bioorg. Med. Chem. Lett.* **2005,** 15, 2758-2760.

80. Pinder, R. M.; van Delft, A. M. L., *Br. J. Clin. Pharmaco.* **1983,** 15, 269S–276S.

81. Leonard, B. E., *Hum. Psychopharm. Clin.* **1999,** 14, 75-81.

82. de Boer, T. H.; Nefkens, F.; van Helvoirt, A.; van Delft, A. M., *J. Pharmacol. Exp. Ther.* **1996,** 277, 852–860.

83. Irena, N.; El; Zdot; Bieta, C.-F.; Jerzy, V., *Hum. Psychopharm. Clin.* **1996,** 11, 273-282.

84. Maes, M. M. D. P.; Libbrecht, I. M. D.; van Hunsel, F. M. D.; Campens, D. M. D.; Meltzer, H. Y. M. D., *J. Clin. Psychopharm.* **1999,** 19, 177-182.

85. Andrés, J. I.; Alcázar, J.; Alonso, J. M.; Alvarez, R. M.; Cid, J. M.; De Lucas, A. I.; Fernández, J.; Martínez, S.; Nieto, C.; Pastor, J.; Bakker, M. H.; Biesmans, I.; Heylen, L. I.; Megens, A. A., *Bioorg. Med. Chem. Lett.* **2003,** 13, 2719-2725.

86. Andres, J. I.; Alcazar, J.; Alonso, J. M.; Alvarez, R. M.; Bakker, M. H.; Biesmans, I.; Cid, J. M.; De Lucas, A. I.; Fernandez, J.; Font, L. M.; Hens, K. A.; Iturrino, L.; Lenaerts, I.; Martinez, S.; Megens, A. A.; Pastor, J.; Vermote, P. C. M.; Steckler, T., *J. Med. Chem.* **2005,** 48, 2054-2071.

87. Andrés, J. I.; Alcázar, J.; Alonso, J. M.; De Lucas, A. I.; Iturrino, L.; Biesmans, I.; Megens, A. A., *Bioorgan. Med. Chem.* **2006,** 14, 4361-4372.

88. Andrés, J. I.; Alcázar, J.; Alonso, J. M.; Alvarez, R. M.; Bakker, M. H.; Biesmans, I.; Cid, J. M.; De Lucas, A. I.; Drinkenburg, W.; Fernández, J.; Font, L. M.; Iturrino, L.; Langlois, X.; Lenaerts, I.; Martínez, S.; Megens, A. A.; Pastor, J.; Pullan, S.; Steckler, T., *Bioorg. Med. Chem.* **2007,** 15, 3649-3660.

89. Pastor, J.; Alcázar, J.; Alvarez, R. M.; Andrés, J. I.; Cid, J. M.; De Lucas, A. I.; Diaz, A.; Fernández, J.; Font, L. M.; Iturrino, L.; Lafuente, C.; MartInez, S.; Bakker, M. H.; Biesmans, I.; Heylen, L. I.; Megens, A. A., *Bioorg. Med. Chem. Lett.* **2004,** 14, 2917-2922.

90. Hoglund, I. P. J.; Silver, S.; Engstrom, M. T.; Salo, H.; Tauber, A.; Kyyronen, H.-K.; Saarenketo, P.; Hoffren, A.-M.; Kokko, K.; Pohjanoksa, K.; Sallinen, J.; Savola, J.-M.; Wurster, S.; Kallatsa, O. A., *J. Med. Chem.* **2006,** 49, 6351-6363.

91. Van der Berg, W. J. WO2006006858, 2006.

92. Wikstrom, H. V.; Mensonides-Harsema, M. M.; Cremers, T. I. F. H.; Moltzen, E. K.; Arnt, J., *J. Med. Chem.* **2002,** 45, 3280-3285.

93. Szántay, C.; Szabó, L.; Zsilla, G.; Vizi, S. E., *Arch. Pharm.* **2002,** 335, 22-26.

94. Cordi, A. A.; Berque-Bestel, I.; Persigand, T.; Lacoste, J.-M.; Newman-Tancredi, A.; Audinot, V.; Millan, M. J., *J. Med. Chem.* **2001,** 44, 787-805.

95. Mayer, P.; Brunel, P.; Chaplain, C.; Piedecoq, C.; Calmel, F.; Schambel, P.; Chopin, P.; Wurch, T.; Pauwels, P. J.; Marien, M.; Vidaluc, J.-L.; Imbert, T., *J. Med. Chem.* **2000,** 43, 3653-3664.

96. Kennis, L. E. J.; Bischoff, F. P.; Mertens, C. J.; Love, C. J.; Van den Keybus, F. A. F.; Pieters, S.; Braeken, M.; Megens, A. A. H. P.; Leysen, J. E., *Bioorg. Med. Chem. Lett.* **2000,** 10, 71-74.

97. Shiue, C.-y.; Pleus, R. C.; Shiue, G. G.; Rysavy, J. A.; Sunderland, J. J.; Cornish, K. G.; Young, S. D.; Bylund, D. B., *Nucl. Med. Biol.* **1998,** 25, 127-133.

98. Marthi, K.; Bender, D.; Watanabe, H.; Smith, D. F., *Nucl. Med. Biol.* **2002,** 29, 317-319.

99. Hume, S. P.; Hirani, E.; Opacka-Juffry, J.; Osman, S.; Myers, R.; Gunn, R. N.; McCarron, J. A.; Clark, R. D.; Melichar, J.; Nutt, D. J.; Pike, V. W., *Eur. J. Nucl. Med.* **2000,** 27, 475-484.

100. Robinson, E. S. J.; Tyacke, R. J.; Finch, L.; Willmott, G.; Husbands, S.; Nutt, D. J.; Hudson, A. L., *Neuropharmacology* **2004,** 46, 847-855.

101. Marthi, K.; Bender, D.; Gjedde, A.; Smith, D. F., *Eur. Neuropsychopharm.* **2002,** 12, 427-432.

102. Van der Mey, M.; Windhorst, A. D.; Klok, R. P.; Herscheid, J. D. M.; Kennis, L. E.; Bischoff, F.; Bakker, M.; Langlois, X.; Heylen, L.; Jurzak, M.; Leysen, J. E., *Bioorg. Med. Chem.* **2006,** 14, 4526-4534.

103. Rodriguez, F.; Rozas, I.; Ortega, J. E.; Meana, J. J.; Callado, L. F., *J. Med. Chem.* **2007,** 50, 4516-4527.

104. Rodriguez, F.; Rozas, I.; Ortega, J. E.; Erdozain, A. M.; Meana, J. J.; Callado, L. F., *J. Med. Chem.* **2008,** 51, 3304-3312.

105. Rodriguez, F.; Rozas, I.; Ortega, J. E.; Erdozain, A. M.; Meana, J. J.; Callado, L. F., *J. Med. Chem.* **2009,** 52, 601–609.

106. González-Maeso, J.; Rodríguez-Puertas, R.; Gabilondo, A. M.; Meana, J. J., *Eur. J. Pharmacol.* **2000,** 390, 25-36.

107. Fillenz, M., *Neurosci. Biobehav. Rev.* **2005,** 29, 949-962.

108. Boyd, R. E.; Rasmussen, C. R.; Press, J. B.; Raffa, R. B.; Codd, E. E.; Connelly, C. D.; Li, Q. S.; Martinez, R. P.; Lewis, M. A.; Almond, H. R.; Reitz, A. B., *J. Med. Chem.* **2001,** 44, 863-872.

109. Barbaro, R.; Betti, L.; Botta, M.; Corelli, F.; Giannaccini, G.; Maccari, L.; Manetti, F.; Strappaghetti, G.; Corsano, S., *J. Med. Chem.* **2001,** 44, 2118-2132.

110. Patani, G. A.; LaVoie, E. J., *Chem. Rev.* **1996,** 96, 3147-3176.

111. Hoechst, A. DE 1941761, 1971.

112. Kim, K. S.; Qian, L., *Tetrahedron Lett.* **1993,** 48, 7677-7680.

113. Kaspady, M.; Narayanaswamy, V. K.; Raju, M.; Rao, G. K., *Lett. Drug Des. Discov.* **2009,** 6, 21-28.

114. Gewald, K.; Jansch, H.-J., *J. Prakt. Chem.* **1973,** 315, 779-785.

115. Dardonville, C.; Goya, P.; Rozas, I.; Alsasua, A.; Martín, M. I.; Borrego, M. J., *Bioorg. Med. Chem.* **2000,** 8, 1567-1577.

116. Beyer, H.; Hantschel, H., *Chem. Ber.* **1962,** 95, 893-901.

117. Dolzhenko, A. V.; Chui, W.-K.; Dolzhenko, A. V., *Synthesis* **2006,** 2006, 597–602.

118. www.sigmaaldrich.com (3/9/12),

119. www.acros.com (3/9/12),

120. Matsuno, K.; Nakajima, T.; Ichimura, M.; Giese, N. A.; Yu, J.-C.; Lokker, N. A.; Ushiki, J.; Ide, S.-i.; Oda, S.; Nomoto, Y., *J. Med. Chem.* **2002,** 45, 4513-4523.

121. Schnute, M. E.; Cudahy, M. M.; Brideau, R. J.; Homa, F. L.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Pitts, T. W.; Poorman, R. A.; Wathen, M. W.; Wieber, J. L., *J. Med. Chem.* **2005,** 48, 5794-5804.

122. Rahaim, R. J.; Maleczka, R. E., *Org. Lett.* **2005,** 7, 5087-5090.

123. Poss, M. A.; Iwanowicz, E.; Reid, J. A.; Lin, J.; Gu, Z., *Tetrahedron Lett.* **1992,** 33, 5933-5936.

124. Hall, H. K., *J. Am. Chem. Soc.* **1957,** 79, 5441-5444.

125. Albert, A.; Goldacre, R.; Phillips, J., *J. Chem. Soc.* **1948**, 2240-2249.

126. Le Merrer, Y.; Gauzy, L.; Gravier-Pelletier, C.; Depezay, J.-C., *Bioorg. Med. Chem.* **2000,** 8, 307-320.

127. O' Connor, C. J.; Roydhouse, M. D.; Przybyl,, A. M.; Wall, M. D.; Southern, J. M., *J. Org. Chem.* **2010,** 75, 2534-2538.

128. Baldwin, J. E., *J. Chem. Soc. Chem. Comm.* **1976**, 734-736.

129. Gronowitz, S.; Gjos, N., *Acta Chem. Scand.* **1967,** 21, 2823-2833.

130. Vanderzee, C. E.; Edgell, W. F., *Anal. Chem.* **1950,** 22, 572-574.

131. Vanderzee, C. E.; Edgell, W. F., *J. Am. Chem. Soc.* **1950,** 72, 2916-2923.

132. Kenning, D. D.; Mitchell, K. A.; Calhoun, T. R.; Funfar, M. R.; Sattler, D. J.; Rasmussen, S. C., *J. Org. Chem.* **2002,** 67, 9073-9076.

133. O'Connor, C.; Roydhouse, M.; Przybyl, A.; Wall, M. D.; Southern, M., *Unpublished work*.

134. Yoshikawa, Y.; Tomiya, K.; Katsuta, H.; Kawashima, H.; Takahashi, T.; Inami, S.; Yanase, Y.; Takashi, A.; Shimotori, H.; Tomura, N. EP19960105345 19960403 1996-10-16 2006.

135. Cope, O. J.; Brown, R. K., *Can. J. Chemistry* **1961,** 39, 1695-1710.

136. Tang, H.; Ning, F.-X.; Wei, Y.-B.; Huang, S.-L.; Huang, Z.-S.; Chan, A. S.-C.; Gu, L.-Q., *Bioorg. Med. Chem. Lett.* **2007,** 17, 3765-3768.

137. Yong, Y. F.; Kowalski, J. A.; Lipton, M. A., *J. Org. Chem.* **1997,** 62, 1540-1542.

138. Clayden, J.; Greeves, N.; Warren, S.; Wothers, P., *Organic Chemistry*. 3rd ed.; Oxford: 2001.

139. Ashworth, I. W.; Cox, B. G.; Meyrick, B., *J. Org. Chem.* **2010,** 75, 8117-8125.

140. Brugier, D.; Outurquin, F.; Paulmier, C., *Tetrahedron* **1997,** 53, 10331-10344.

141. Boyle, P. H.; Daly, K. M.; Leurquin, F.; Robinson, J. K.; Scully, D. T., *Tetrahedron Lett.* **2001,** 42, 1793-1795.

142. Schadendorf, T.; Hoppmann, C.; Rück-Braun, K., *Tetrahedron Lett.* **2007,** 48, 9044-9047.

143. Gever, G., *J. Am. Chem. Soc.* **1955,** 77, 577-578.

144. Taylor, R., In *Thiophene and Its Derivatives*, 5th ed.; Gronowitz, S., Ed. Wiley-Interscience: New York, 1992.

145. Gowda, S.; Gowda, D. C., *Indian J. Chem.* **2003,** 42B, 180-183.

146. Silverstein, R. M.; Webster, F. X., *Spectrometric Identification of Organic Compounds*. 6th ed.; John Wiley & Sons: 1997.

147. Bellamy, F. D.; Ou, K., *Tetrahedron Lett.* **1984,** 25, 839-842.

148. Vogel, A. I.; Tatchell, A. R.; Furnis, B. S.; Hannaford, A. J.; PWG, S., *Textbook of Practical Organic Chemistry*. 5th ed.; Prentice Hall: 1989.

149. Armarego, W. L. F.; Chai, C. L. L., *Purification of Laboratory Chemicals*. 5th ed.; Butterworth-Heinemann: 2003.

150. Malicorne, G.; Bompart, J.; Giral, L.; Despaux, E., *Eur. J. Med. Chem.* **1991,** 26, 3-11.

151. King, W. J.; Nord, F. F., *J. Org. Chem.* **1949,** 14, 638-642.

152. Kumar, P. R.; Raju, S.; Goud, P. S.; Sailaja, M.; Sarma, M. R.; Reddy, G. O.; Kumar, M. P.; Reddy, V. V. R. M. K.; Suresh, T.; Hegde, P., *Bioorg. Med. Chem.* **2004,** 12, 1221-1230.

153. Yuquan, S.; Yuxia, Z.; Zao, L.; Jianghong, W.; Ling, Q.; Shixiong, L.; Jianfeng, Z.; Jiayun, Z., *J. Chem. Soc. Perk. T. 1* **1999,** 24, 3691-3695.

154. Campaigne, E.; Grose, H. G., *J. Am. Chem. Soc.* **1951,** 73, 3812-3814.

155. Babasinian, V. S., *J. Am. Chem. Soc.* **1928,** 50, 2748-2753.

156. Glaxo Group LTD WO2006/65788, 2006.

157. Goya, P.; Lissavetzky, J.; Rozas, I.; Stud, M., *Arch. Pharm.* **1984,** 317, 777-781.

158. Gewald, K.; Schinke, E.; Böttcher, H., *Chem. Ber.* **1966,** 99, 94-100.

159. Mojtahedi, M. M.; Abaee, M. S.; Mahmoodi, P.; Adib, M., *Synthetic Commun.* **2010,** 40, 2067-2074.

160. Rozas, I. PhD thesis: 1,1-Dioxidos de 1,2,5-Tiadiazina: Sintesis de Nuevos Derivados y Estudio de Propiedades Fisico-Quimicas. Universidad Complutense de Madrid, Madrid, 1987.

161. Scheib, S.; Bäuerle, P., *J. Mater. Chem.* **1999,** 9, 2139-2150.

162. Dinescu, L.; Maly, K. E.; Lemieux, R. P., *J. Mater. Chem.* **1999,** 9, 1679-1686.

163. Miel, H.; Rault, S., *Tetrahedron Lett.* **1997,** 38, 7865-7866.

164. Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A.; Schulz, D. W., *J. Med. Chem.* **1997,** 40, 1749-1754.

165. Link, H., *Helv. Chim. Acta* **1990,** 73, 797-803.

166. Barker, J. M.; Huddleston, P. R.; Wood, M. L., *Synthetic Commun.* **1995,** 25, 3729-3734.

167. Lobana, T. S.; Sharma, R.; Sharma, R.; Sultana, R.; Butcher, R. J., *Z. Anorg. Allg. Chem.* **2008,** 634, 718-723.

168. Guram, A. S.; Rennels, R. A.; Buchwald, S. L., *Angew. Chem. Int. Ed.* **1995,** 34, 1348-1350.

169. Louie, J.; Hartwig, J. F., *Tetrahedron Lett.* **1995,** 36, 3609-3612.

170. Wolfe, J. P.; Wagaw, S.; Buchwald, S. L., *J. Am. Chem. Soc.* **1996,** 118, 7215-7216.

171. Driver, M. S.; Hartwig, J. F., *J. Am. Chem. Soc.* **1996,** 118, 7217-7218.

172. Christmann, U.; Vilar, R., *Angew. Chem. Int. Ed.* **2005,** 44, 366-374.

173. Surry, D. S.; Buchwald, S. L., *Angew. Chem. Int. Ed.* **2008,** 47, 6338-6361.

174. Jonckers, T. H. M.; Maes, B. U. W.; Lemière, G. L. F.; Dommisse, R., *Tetrahedron* **2001,** 57, 7027-7034.

175. Bailey, P. J.; Pace, S., *Coordin. Chem. Rev.* **2001,** 214, 91-141.

176. Halepoto, D. M.; Larkworthy, L. F.; Povey, D. C.; Smith, G. W.; Ramdas, V., *Polyhedron* **1995,** 14, 1453-1460.

177. Yang, H.; Han, X.; Ma, Z.; Wang, R.; Liu, J.; Ji, X., *Green Chem.* 12, 441-451.

178. Jesberger, M.; Davis, T. P.; Barner, L., *Synthesis* **2003,** 2003, 1929-1958.

179. Grubb, A. M.; Hasan, S.; Kiryanov, A. A.; Sampson, P.; Seed, A. J., *Liq. Cryst.* **2009,** 36, 443 - 453.

180. Sosnicki, J. G., *Tetrahedron Lett.* **2009,** 50, 178-181.

181. Oh, C.-H.; Cho, J.-H., *Eur. J. Med. Chem.* **2006,** 41, 50-55.

182. Makino, E.; Iwasaki, N.; Yagi, N.; Ohashi, T.; Kato, H.; Ito, Y.; Azuma, H., *Chem. Pharm. Bull.* **1990,** 38, 201-207.

183. Carsten, S.; Volker, B.; Michael, M.; Lars, G.; Daniel, R.; Jürgen, D.; Peter, W.; Thomas, R.; Uwe, M., *Eur. J. Org. Chem.* **2008,** 2008, 324-329.

184. Basha, A.; Lipton, M.; Weinreb, S. M., *Tetrahedron Lett.* **1977,** 18, 4171-4172.

185. Gustafsson, T.; Ponten, F.; Seeberger, P. H., *Chem. Commun.* **2008**, 1100-1102.

186. Tietze, L. F.; Braun, H.; Steck, P. L.; El Bialy, S. A. A.; Tölle, N.; Düfert, A., *Tetrahedron* **2007,** 63, 6437-6445.

187. Cytokinetics, I. WO2008/16676 A2, 2008.

188. The Regents of the Unversity of California US2009/234142, 2009.

189. Michel Brunel, J.; Salmi, C.; Letourneux, Y., *Tetrahedron Lett.* **2005,** 46, 217-220.

190. Yin, G.; Wang, Z.; Chen, A.; Gao, M.; Wu, A.; Pan, Y., *J. Org. Chem.* **2008,** 73, 3377-3383.

191. Yang, X.-D.; Zeng, X.-H.; Zhao, Y.-H.; Wang, X.-Q.; Pan, Z.-Q.; Li, L.; Zhang, H.-B., *J. Comb. Chem.* **2010,** 12, 307-310.

192. Beemelmanns, C.; Reissig, H.-U., *Org. Biomol. Chem.* **2009,** 7, 4475-4480.

193. Beemelmanns, C.; Blot, V.; Gross, S.; Lentz, D.; Reissig, H.-U., *Eur. J. Org. Chem.* **2010,** 2010, 2716-2732.

194. Nagano, M.; Matsui, T.; Tobitsuka, J.; Oyamada, K., *Chem. Pharm. Bull.* **1972,** 20, 2626-2633.

195. Mei, Y.; Bentley, P. A.; Du, J., *Tetrahedron Lett.* **2008,** 49, 3802-3804.

196. Sreedhar, B.; Surendra Reddy, P.; Madhavi, M., *Synthetic Commun.* **2007,** 37, 4149-4156.

197. Stork, G.; Brizzolarha, A.; Landesman, H.; Szmuszkovanicz, J.; Terrell, R., *J. Am. Chem. Soc.* **1963,** 85, 207-222.

198. Meshram, H. M.; Reddy, P. N.; Sadashiv, K.; Yadav, J. S., *Tetrahedron Lett.* **2005,** 46, 623-626.

199. Actelion Pharmaceuticals LTD WO2004/83218, 2004.

200. http://boa06elg.webnode.com/mechanism-of-action/ (3/9/12),

201. Kelly, B.; Muguruza Millan, C.; Rozas, I.; Callado, L. F.; Meana, J. J., Unpublished work, 2011.

202. Satonaka, H., *B. Chem. Soc. Jpn.* **1983,** 56, 2463-2468.

203. Jen, A. K. Y.; Joel, D. K.; Gerolamo, F. EP 0 647 874 A1, 1994.

204. Katritzky, A. R.; Vakulenko, A. V.; Sivapackiam, J.; Draghici, B.; Damavarapu, R., *Synthesis* **2008,** 5, 699-706.

205. Pietsch, M.; Gutschow, M., *J. Med. Chem.* **2005,** 48, 8270-8288.

206. Baty, J. D.; Jones, G.; Moore, C., *J. Org. Chem.* **1969,** 34, 3295-3302.

207. Wang, T.; Huang, X.-G.; Liu, J.; Li, B.; Wu, J.-J.; Chen, K.-X.; Zhu, W.-L.; Xu, X.-Y.; Zeng, B.-B., *Synlett* **2010**, 1351-1354.

208. Gutschow, M.; Kuerschner, L.; Neumann, U.; Pietsch, M.; Loser, R.; Koglin, N.; Eger, K., *J. Med. Chem.* **1999,** 42, 5437-5447.

209. Sopbué Fondjo, E.; Döpp, D.; Henkel, G., *Tetrahedron* **2006,** 62, 7121-7131.

210. Gerald, E. R.; Ervin, H. R.; Viktors, K. E. EP0452002, 1991.

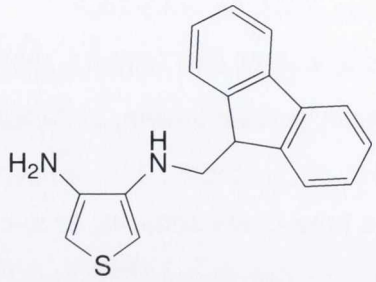
211. Stevens, R., *J. Chem. Soc.* **1960**, 1118-1125.

212. Díaz-Gavilán, M.; Gómez-Vidal, J. A.; Rodríguez-Serrano, F.; Marchal, J. A.; Caba, O.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M., *Bioorg. Med. Chem. Lett.* **2008,** 18, 1457-1460.

213. Abbott Laboratories US2009/18114, 2009.

Appendix

Partial synthesis of 9-Fluorenylmethyl-(4-aminothien-3-yl)carbamate (83)[212]



(a) A mixture of NaOH (224.4 mg; 5.61 mmol; 3.5 eq.) in dry THF (10 ml) under argon was cooled to 0°C, before **72**.HCl (300.0 mg; 1.6 mmol; 1 eq.) was added slowly. A solution of Fmoc-Cl (416.4mg; 1.6 mmol; 1 eq.) in dry THF was added dropwise. The solution was allowed to stir for 2 days, and was examined by TLC. After 2 days, the TLC showed no or little product formation, so Fmoc-Cl (100 mg) was added followed by the dropwise addition of TEA (781 µl; 567.8 mg; 5.6 mmol; 3.5 eq.) in THF (30 ml). TLC after a further day showed some product present. A further 200 µl of TEA was added and the mixture stirred overnight. The TLC (Hex/EtOAc 3:1) on the fourth day showed 5 spots.

The reaction mixture was extracted in 1N HCl, the aqueous layer was washed with DCM, and the aqueous layer was basified to pH 10, extracted in DCM, dried and the solvents removed giving a beige powder. Yield: 40.6 mg (8%)

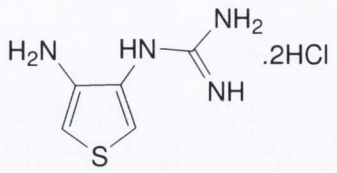
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, 2H, *J* = 7.3 Hz, Ar-CH), 7.64 (app. brs, 2H, Ar-CH), 7.44 (t, 2H, *J* = 7.3 Hz, Ar-CH), 7.36 (t, 2H, *J* = 6.9 Hz, Ar-CH), 7.29 (s, 1H, Ar-CH), 7.21 (brs, 1H, NH), 6.83 (brs, 1H, NH_2), 6.36 (s, 1H, Ar-CH), 4.59 (d, 2H, *J* = 6.5 Hz, CH_2), 4.30 (t, 1H, *J* = 6.2 Hz, CH) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 143.7 (qAr), 141.4 (qAr), 127.8 (Ar-CH), 127.8 (Ar-CH), 127.2 (Ar-CH), 127.1 (Ar-CH), 125.0 (Ar-CH), 125.0 (Ar-CH), 120.1 (qAr), 120.0 (qAr), 66.9 (CH_2), 47.1 (CH) ppm

The material, once obtained was used straight away in the next reaction

(b) The reaction was repeated at twice the original scale over one night. TLC following day showed multiple spots, so the reaction was stopped. 3 columns were carried out to purify the product however no more material was isolated.

Attempted synthesis of *N*-(4-aminothien-3-yl)guanidine hydrochloride (86)



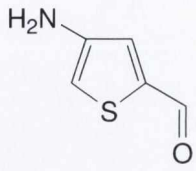
(a) Compound **79** (100.0 mg; 0.22 mmol; 1 eq.) was dissolved in 1.25 M HCl in methanol (7.5 mL) and was stirred overnight. The solvents were removed and the crude material purified by reverse phase silica column chromatography (Biotage). The sample turned brown on heating to remove the solvents and the spots on the TLC plate also turned brown over time. No product was observed via ^{1}H NMR spectroscopy.

(b) Synthesis attempted using Method G from **79** (100 mg; 0.22 mmol; 1 eq.) with no product observed by ^{1}H NMR.

(c) Compound **85** (100 mg; 0.22 mmol; 1 eq.) was dissolved in DCM/TFA (10 mL/0.1 mL), and stirred for 2 hours. The solvents were then removed and the crude material dissolved in deionised water and stirred with activated Amberlyte resin in the chloride form (10:1 ratio). The resin was then filtered and the filtrate washed through a plug of reverse phase silica and the solvent removed giving a brown amorphous material (6.7 mg). Two reverse phase columns were run (Biotage) on the material, however it remained impure as determined by ^{1} NMR spectroscopy

HRMS (ES TOF) *m/z* calc. for $C_5H_9N_4S$ $[M + H]^+$ 157.0548, found 157.0542

Attempted synthesis of 4-amino-2-thiophene carboxaldehyde (92) *via* reduction of 4-nitro-2-thiophene carboxaldehyde (91) using Zn/hydrazine (Including synthesis of 4-amino-2-thiophenecarboxylate hydrazone (96)



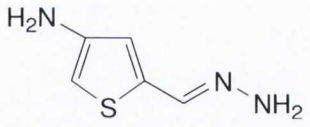
A suspension of **91** (50.0 mg; 0.3 mmol; 1 eq.), zinc powder (41.6 mg: 0.6 mmol; 2 eq.) and 98% hydrazine hydrate (0.2 ml) in MeOH (0.5 ml) was stirred at r.t. for 10 min, after which all the starting material had been used up (TLC). The reaction mixture was filtered through celite, washed with MeOH.

(a) The solvents removed from the filtrate giving a residue. The residue was dissolved in DCM, washed with brine and the organic layer dried over Na_2SO_4 and the solvents removed. No product was obtained (NMR) and the product no longer moved via TLC.

(b) The filtrate was acidified with 1 M HCl and the solvents removed giving 205.0 mg salts, which were examined by TLC (alumina acetonitrile, 3:1 and 1:1 acetonitrile/MeOH, EtOAc 1:1 acetonitrile/MeOH and 1:1 and 1:3 EtOAc/acetonitrile). Column chromatography was attempted using 1:1 acetonitrile/MeOH and 1:3 EtOAc/acetonitrile but no product was obtained from either.

(c) The reaction mixture was washed through celite using NaOH saturated MeOH. The solvent was removed and the solid washed with EtOAc and the filtrate kept and the solvent removed. The remaining solid dissolved in MeOH. Addition of EtOAc to the MeOH solution resulted in the formation of a ppt. All fractions were examined by TLC showing highly complex mixtures.

(d) The crude product was examined by NMR showing a complex mixture. The mixture was washed with EtOAc and filtered, and the solvents removed from the filtrate. This was repeated using MeOH leaving 20 mg of material which is only soluble in basic water. All fractions were analysed by NMR. The aqueous base soluble material showed one product, which was deemed to be **96** based on spectral information.

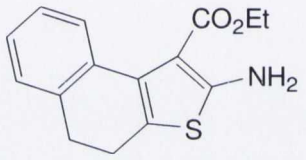


^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (1H, brs, CH=N), 9.76 (1H, brs, NH), 7.42 (2H, s, Ar-H), 7.21 (2H, brs, NH_2), 5.98 (1H, brs, NH) ppm

^{13}C NMR (400 MHz, DMSO-d_6) δ 144.8 (q), 141.1 (q), 139.2 (CH), 129.5 (CH), 96.1 (CH) ppm

IR ν_{max} 3408, 3309, 2788, 1619, 1597, 1492, 1437, 1376, 1098, 810 cm^{-1}

Ethyl 2-amino-4,5-dihydro-naphtho[2,1-*b*]thiophene-1-carboxylate (113)[213]



To a mixture of alpha tetralone (5.3 mL; 5.84 g, 0.04 mol, 1.0 eq.), sulfur powder (1.23 g, 0.04 mol, 1.0 eq.) and ethyl cyanoacetate (4 mL; 4.35 g; 0.11 mol; 1 eq.) and EtOH (8 mL) was added dropwise morpholine (3.4 mL). The mixture was stirred at 30-40 °C for 20h. The solvents were removed and the oily residue was purified by column chromatography hexane/EtOAc 3:1.This gave approx 2.5 g impure product. A Biotage column (hexane/EtOAc 1.5 CV/%) was then carried out giving a colourless oil, which turned tan overnight. Yield: 309.7 mg (3%)

^{1}H NMR (400 Mhz, $CDCl_3$) δ 7.40 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.20 (t, 2H, *J* = 7.5 Hz, Ar-H), 7.14 (t, 1H, *J* = 7.5 Hz, Ar-H), 5.93 (brs, 2H, NH_2), 4.33 (q, 2H, *J* = 7.3 Hz, CH_2), 2.93 (t, 2H, *J* = 7.3 Hz, CH_2), 2.61 (t, 2H, *J* = 7.3 Hz, CH_2), 1.28 (t, 3H, *J* = 7.3 Hz, CH_3) ppm

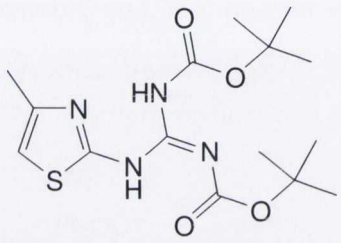
IR ν_{max} 3437, 3326, 1638 (C=O), 1585, 1481, 1464, 1399, 1257, 1159, 1127, 1087, 1014, 752 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{15}H_{15}NO_2SNa$ $[M + Na]^+$ 296.0721, found: 296.0720

Attempted Synthesis of thiazole and benzothiazole guanidines

To a solution of thiourea (182.64 mg; 2.4 mmol; 1.2 eq.), the relevant 2-aminothiazole or 2-aminobenzothiazole (2 mmol; 1.0 eq.) and TEA (1.114 µL; 809.76 mg; 8 mmol; 4.0 eq.) in MeOH (15 ml) was added $HgCl_2$ (814.47 mg; 3 mmol; 1.5 eq.). The solution was refluxed overnight. The solution was filtered through a pad of celite, washed with methanol and the solvents removed. Column chromatography (Biotage DCM/Methanol 3%/CV) was attempted for purification.

[2,3-di(*tert*-butoxycarbonyl)]-1-(4-methylthiazol-2-yl)guanidine (163)



Synthesised from 4-methyl-2-aminothiazole (342.5 mg; 3 mmol; 1 eq.) via Method E giving a white powder. Yield: 95.6 mg (6%)

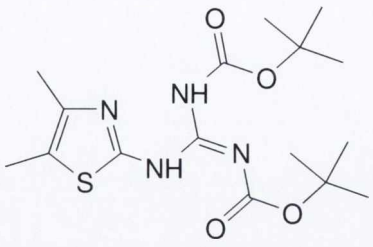
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.77 (brs, 0.2H, NH), 11.43 (brs, 0.5H, NH), 11.28 (brs, 0.7H, NH), 10.19 (brs, 0.2H, NH), 6.57 – 6.44 (m, 1H, Ar-H), 2.34 (s, 3H, CH_3), 1.55 (s, 9H, CH_3), 1.54 (s, 9H, CH_3) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9 (CN), 151.9 (CN), 150.8 (C=O), 147.2 (C=O), 109.7 (Ar-CH), 108.2 (qAr), 84.0 (qC), 83.2 (qC), 27.6 (CH_3), 27.5 (CH_3), 16.7 (CH_3) ppm

IR ν_{max} 3236, 2980, 2930, 1774 (C=O), 1728 (C=O), 1645 (CN), 1532, 1366, 1318, 1306, 1246, 1160, 1143, 1120, 1059, 765 cm^{-1}

HRMS (ESI) *m/z* calc. for $C_{15}H_{25}N_4O_4S$ $[M + H]^+$ 357.1597, found 357.1599

[2,3-di(*tert*-butoxycarbonyl)]-1-(4,5-dimethylthiazol-2-yl)guanidine (169)



To a solution of 4,5-dimethyl-2-aminothiazole hydrochloride (494.0 mg; 3 mmol; 1 eq.) in DCM (25 mL) were added **62** (994.9 mg; 3.6 mmol; 1.2 eq.), TEA (1340 µL; 971.0 mg; 6.6 mmol; 2.2 eq.) and Mukaiyama's reagent (919.76 mg; 3.6 mmol; 1.2 eq.) and the reaction was allowed to stir at r.t. over 3 nights.

The reaction was monitored by TLC, and upon completion, the solvents were removed and the product dissolved in ether (25 mL) washed with water (25 mL) and the aqueous layer extracted with ether (2 x 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents removed. The crude material was purified by Biotage column (hexane/EtOAc 3CV/%) giving a Yield: 223.9 mg (20%)

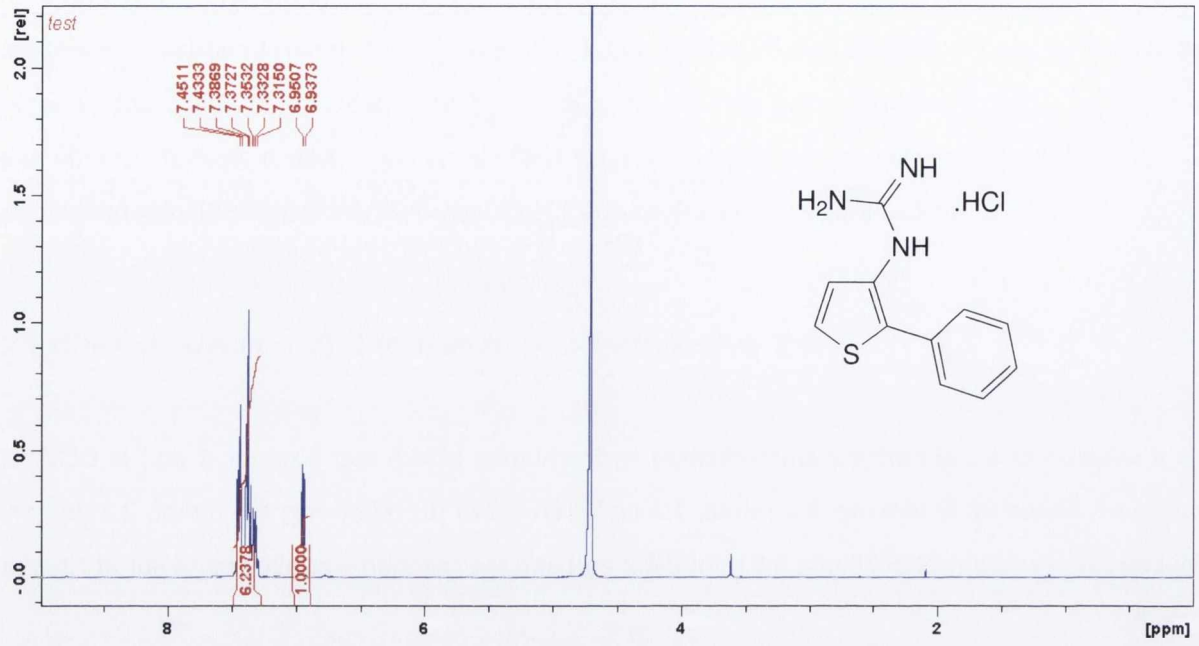
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.81 (brs, 0.3H, NH), 12.26 (brs, 0.1H, NH), 11.29 (brs, 1H, NH), 10.16 (brs, 0.3H, NH), 2.29 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 1.55 (s, 9H, CH_3), 1.55 (s, 9H, CH_3) ppm

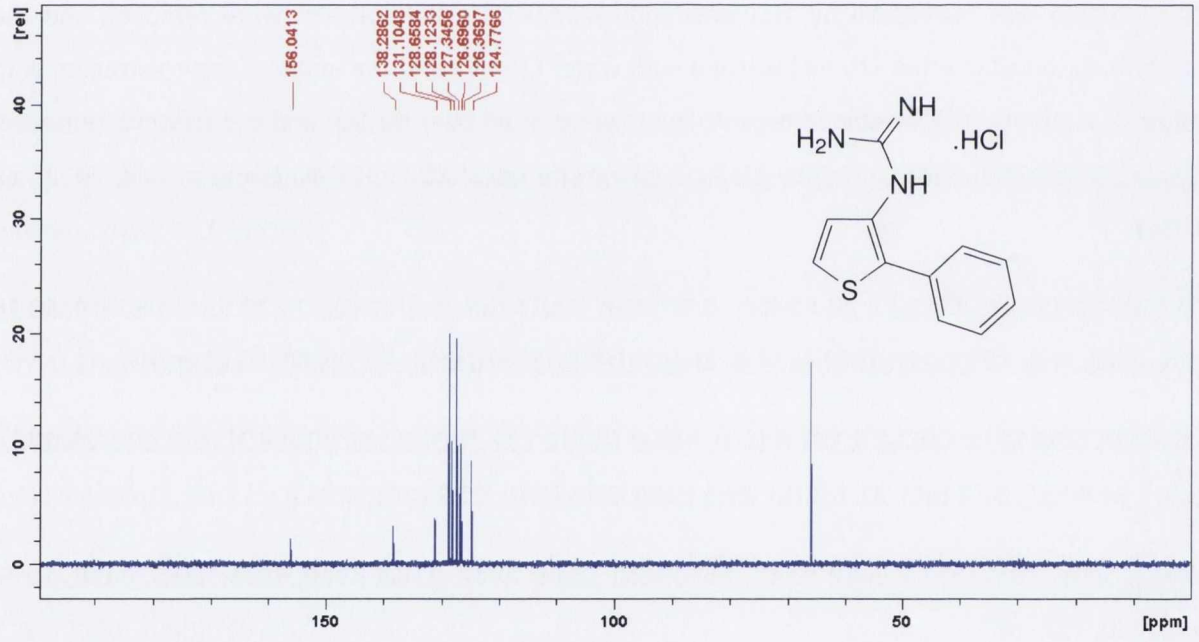
^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6 (CN), 154.0 (C=N), 152.2 (C=O), 151.0 (C=O), 142.4 (qAr), 120.6 (qAr), 84.4 (qC), 82.0 (qC), 31.1 (CH_3), 27.5 (CH_3), 27.5 (CH_3), 22.2 (CH_3) ppm

IR ν_{max} 3245, 2981, 2923, 1717 (C=O), 1630 (CN), 1566, 1410, 1368, 1310, 1235, 1150, 1118, 1059, 775, 750, 717 cm^{-1}

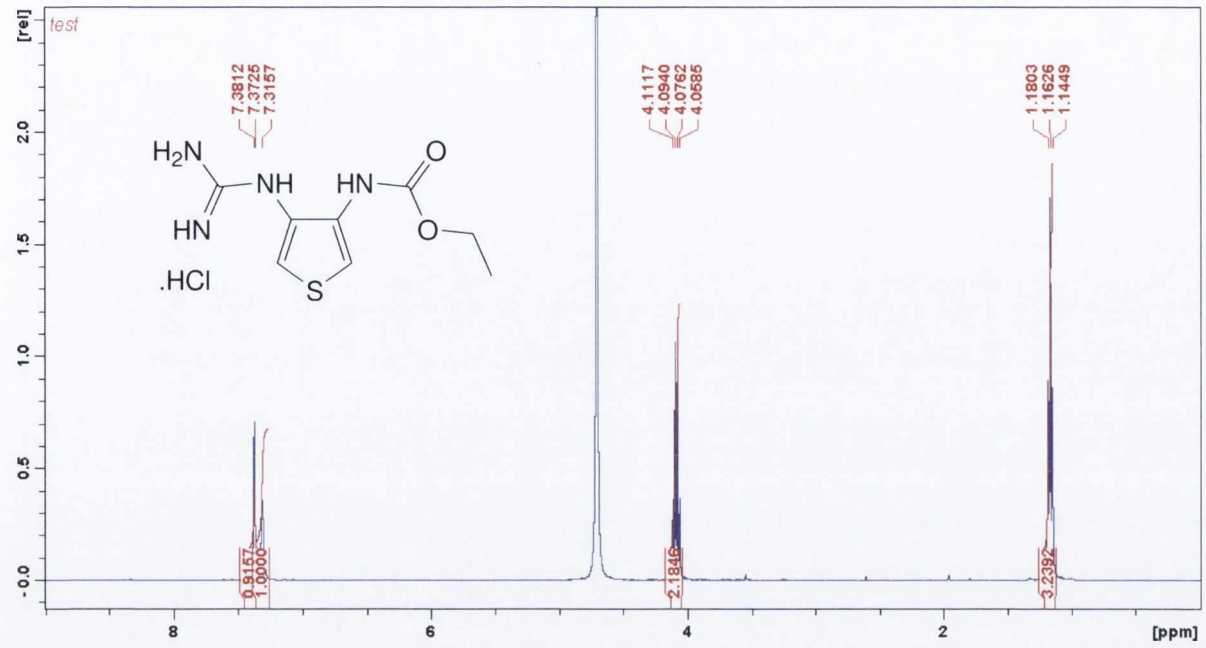
HRMS (ESI) *m/z* calc. for $C_{15}H_{27}N_4O_4S$ $[M + H]^+$ 371.1753, found: 371.1754

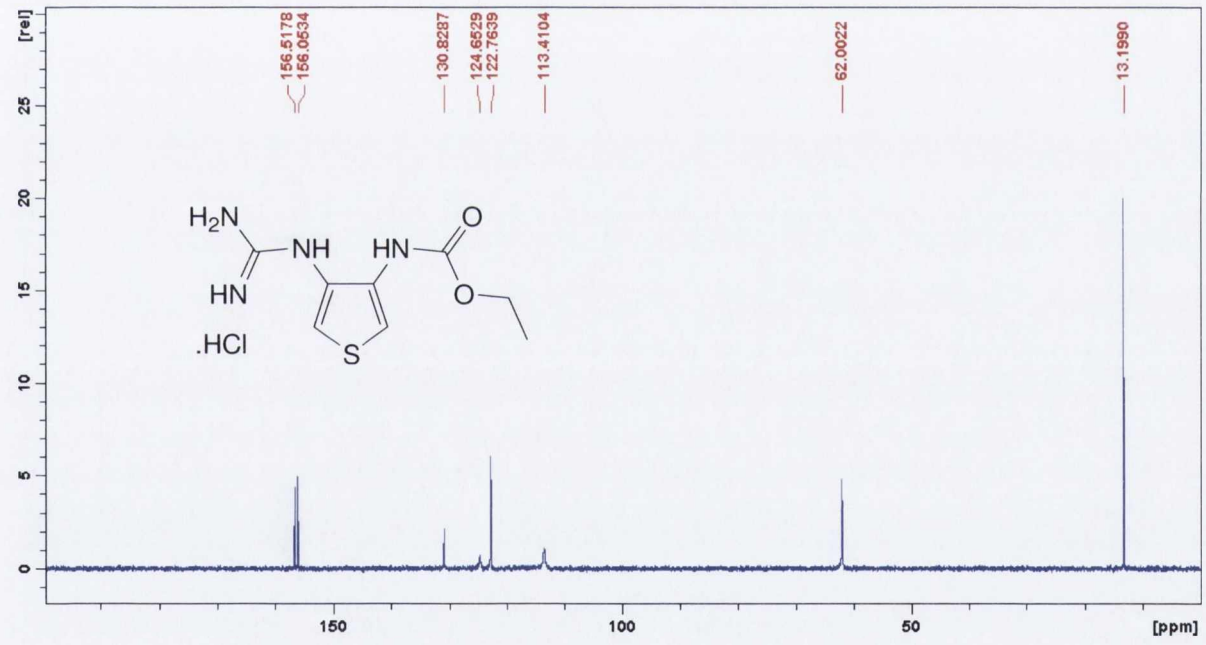
_N_-(2-Phenylthien-3-yl)guanidine hydrochloride (70)



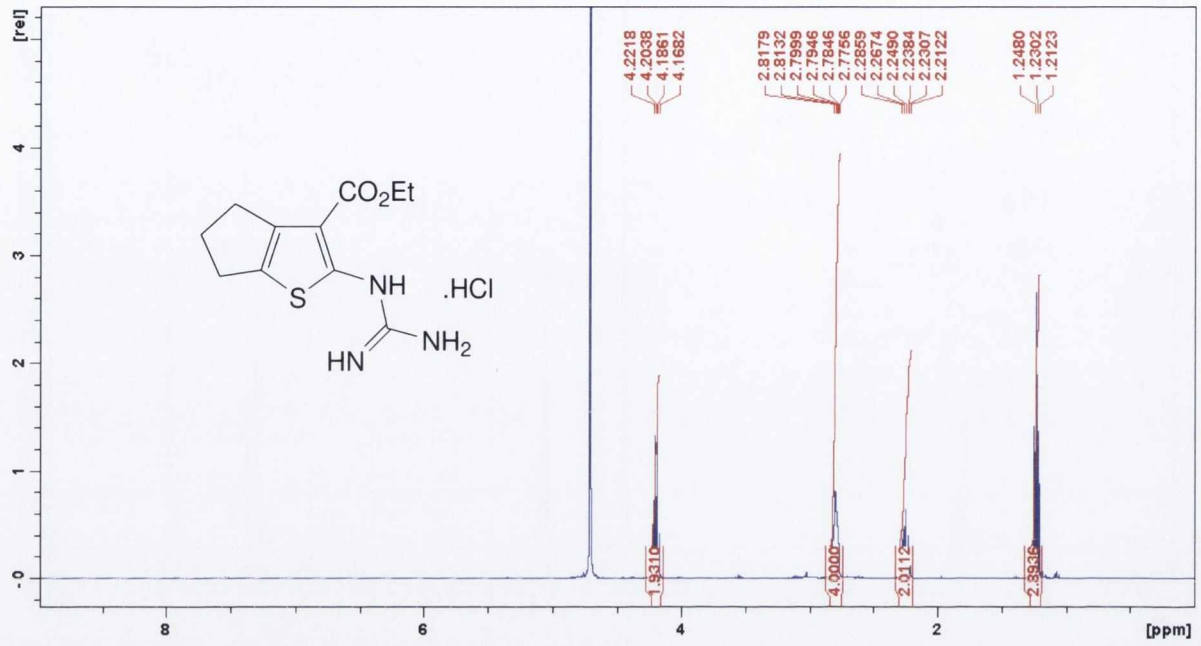


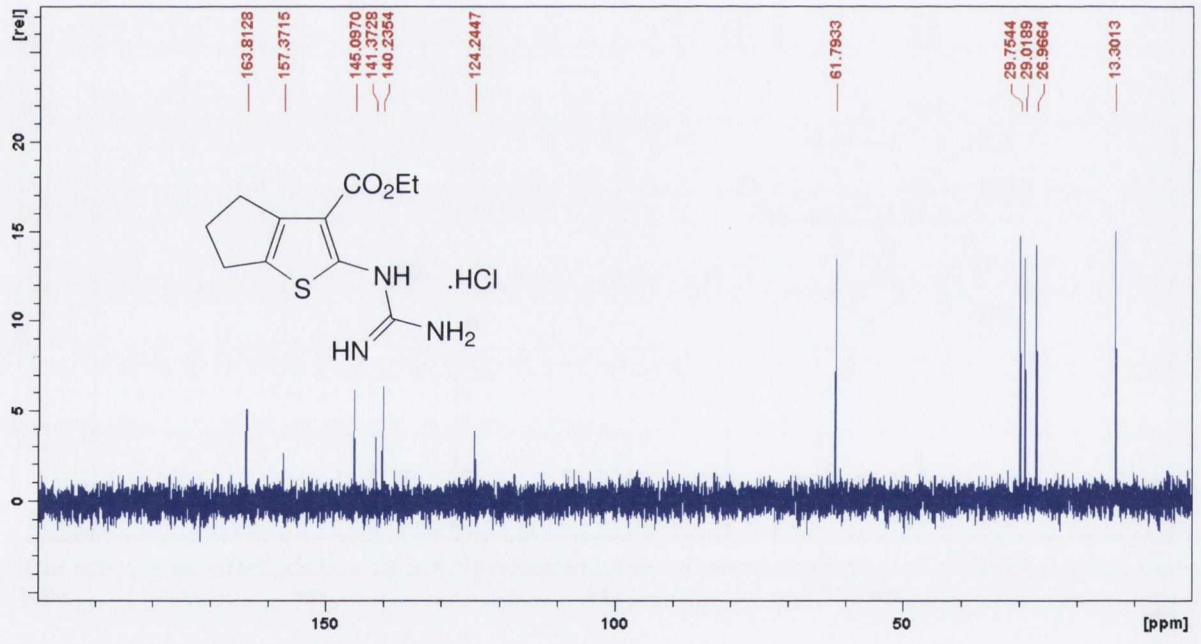
_N_-(4-Ethoxycarbonylaminothien-3-yl)guanidine hydrochloride (87) CHN



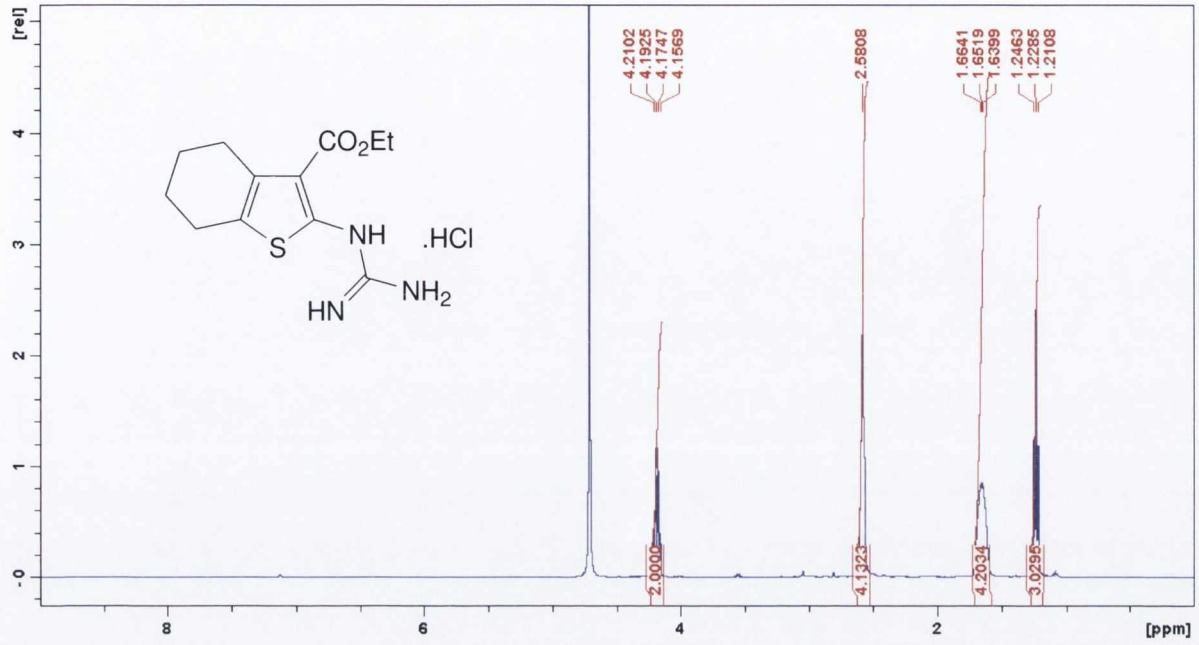


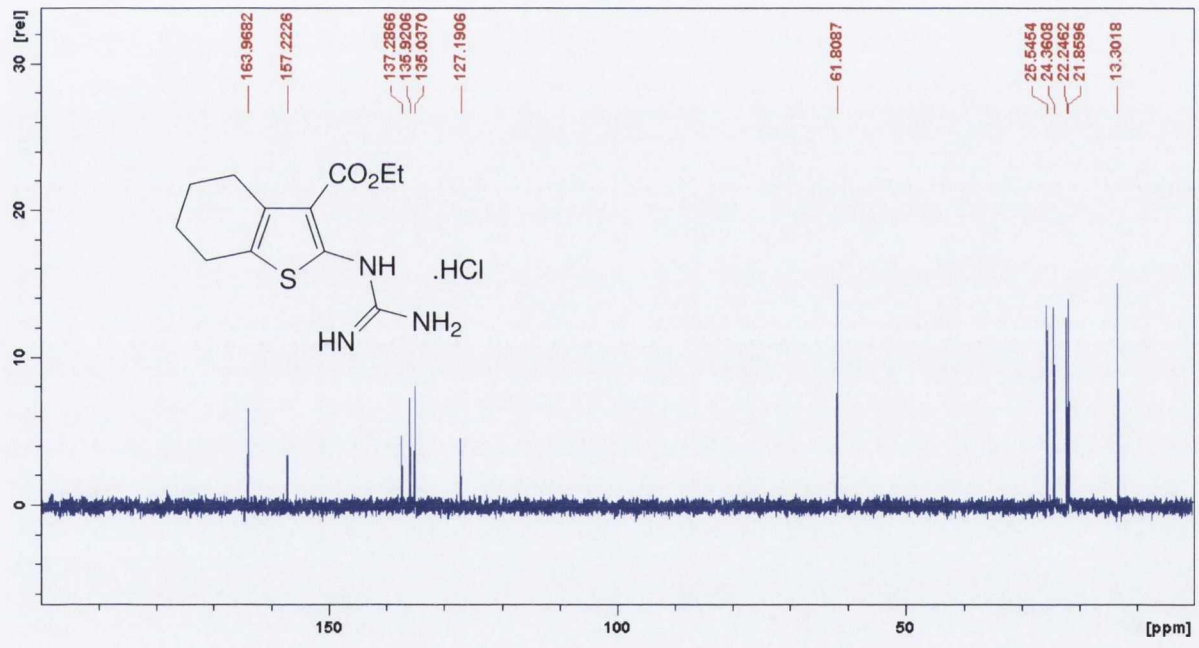
_N_-(3-Ethoxycarbonyl-5,6-dihydro-4_H_-cyclopenta[_b_]thien-2-yl)guanidine hydrochloride (116) CHN



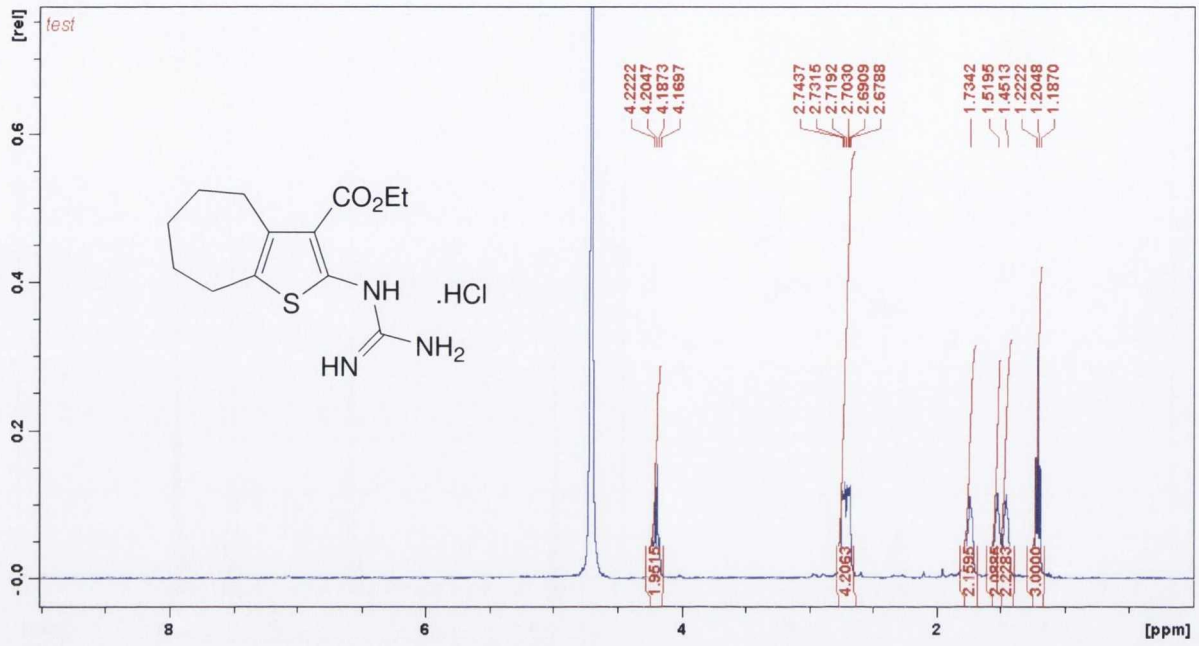


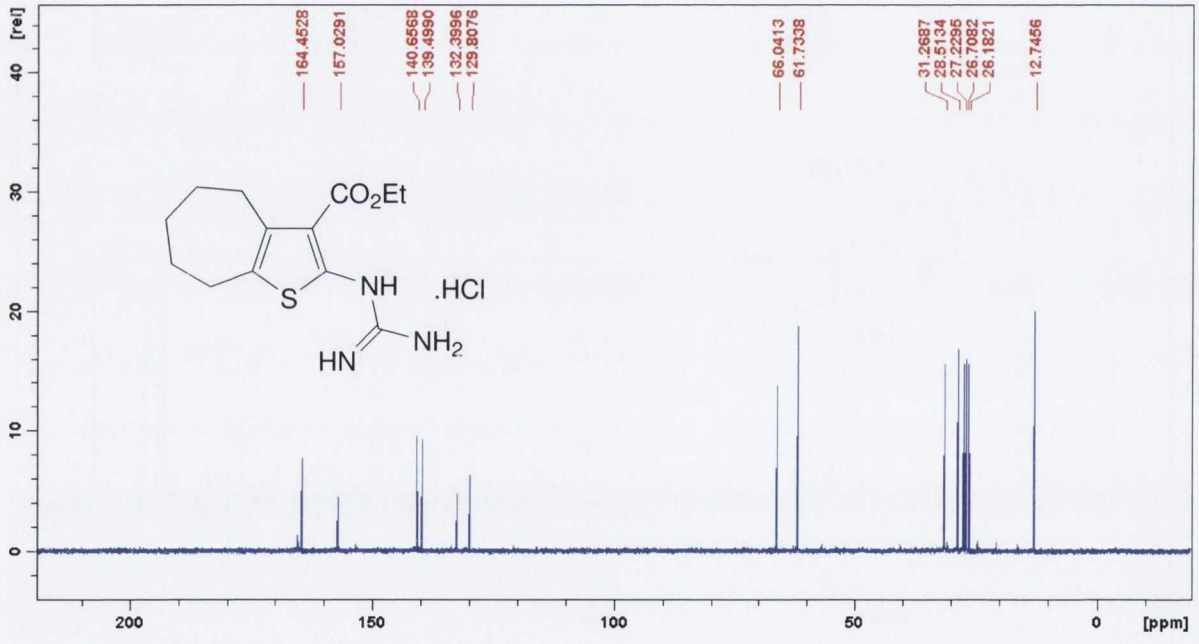
_N_-(3-Ethoxycarbonyl-4,5,6,7-4_H_-tetrahydrobenzo[_b_]thien-2-yl)guanidine hydrochloride (117) CHN



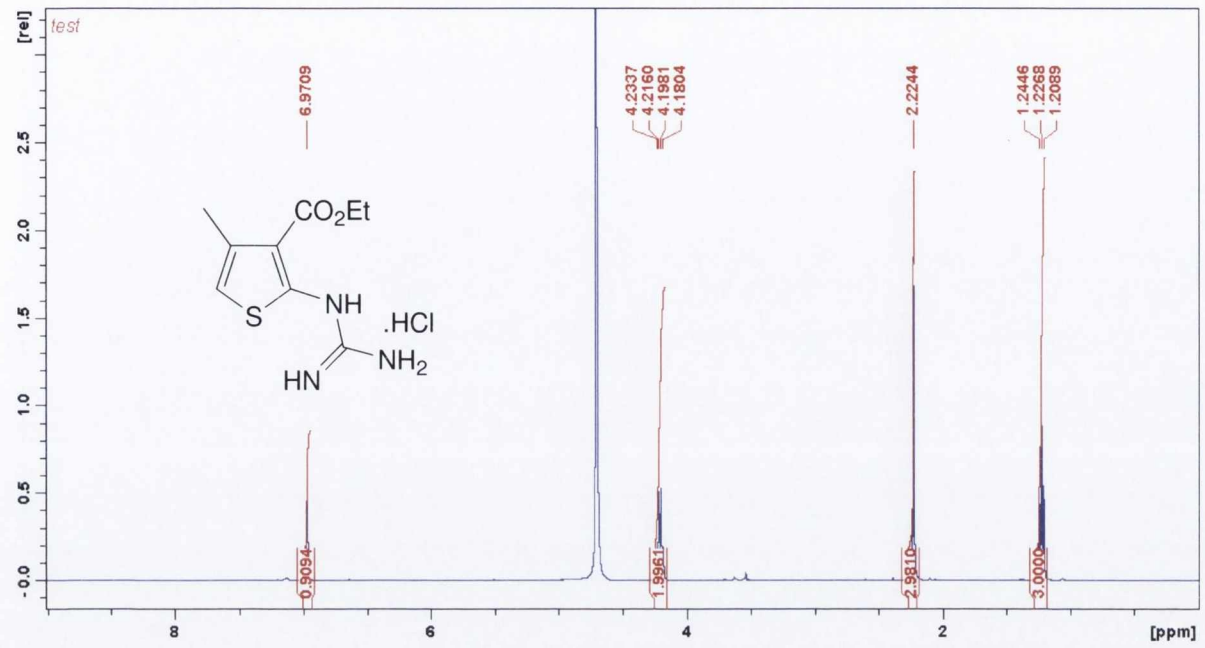


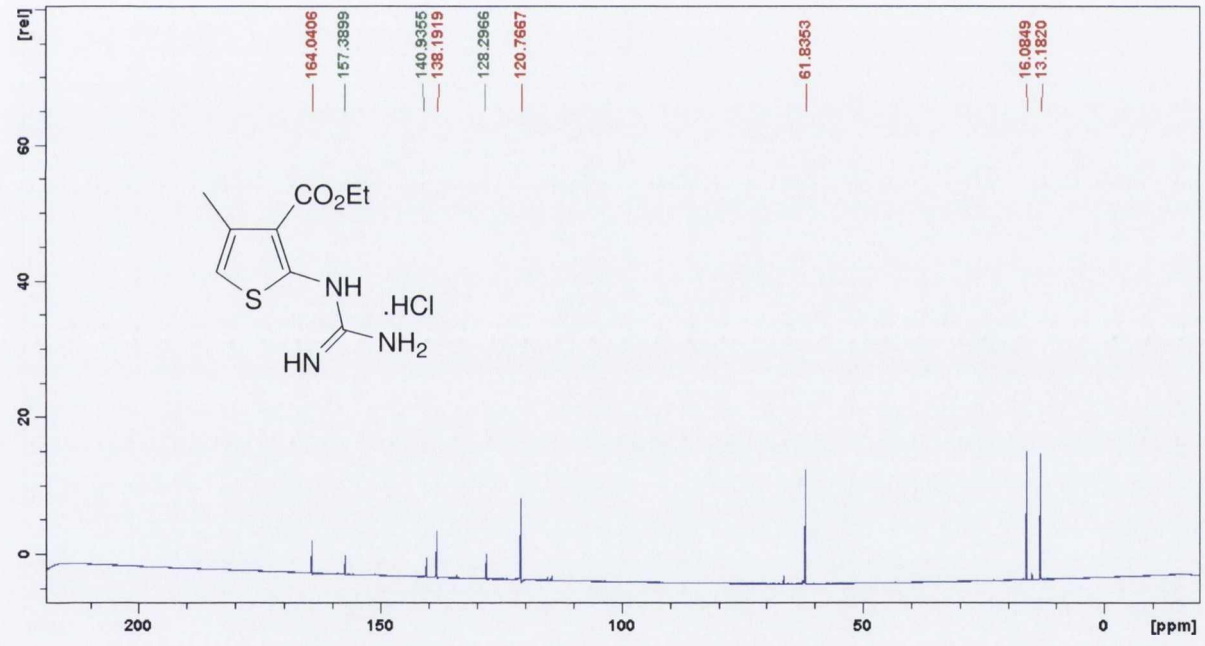
_N_-(3-Ethoxycarbonyl-5,6,7,8-tetrahydro-4_H_-cyclohepta[_b_]thien-2-yl)guanidine hydrochloride (118)



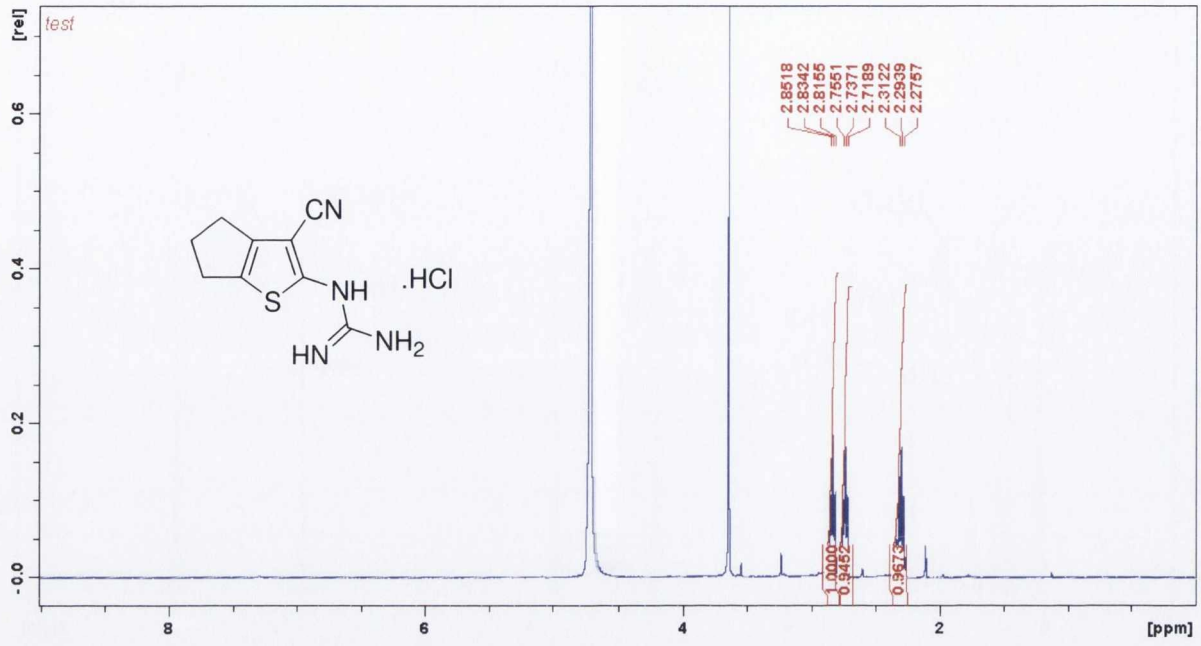


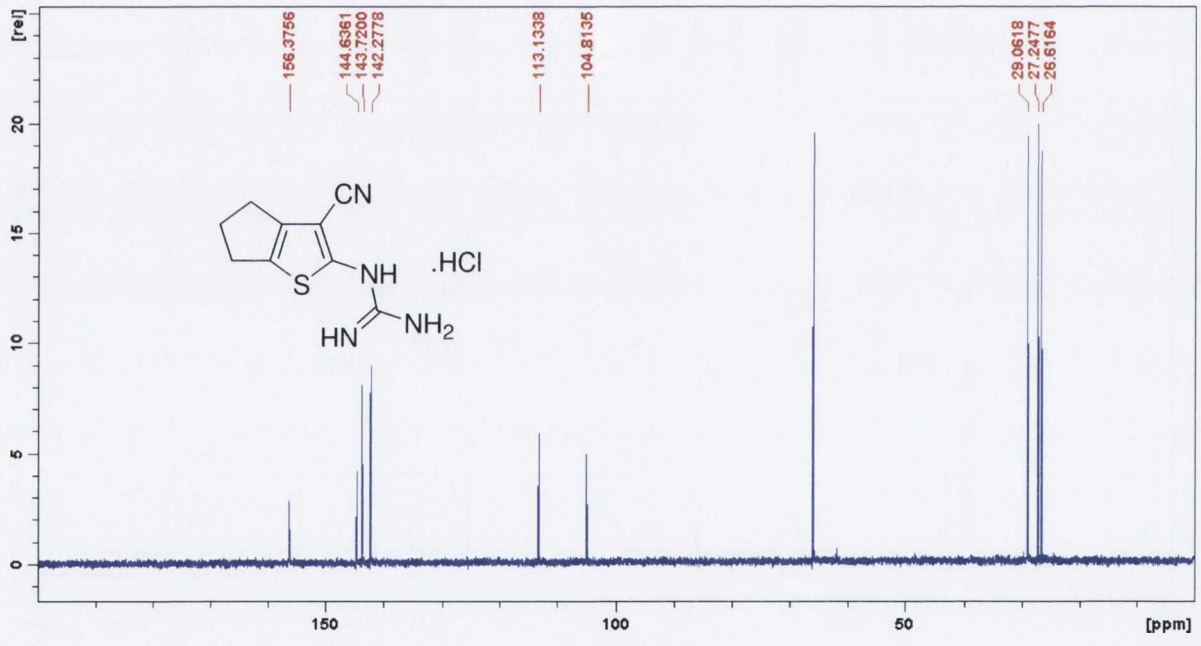
_N_-(3-Ethoxycarbonyl-4-methylthien-2-yl)guanidine hydrochloride (119) CHN



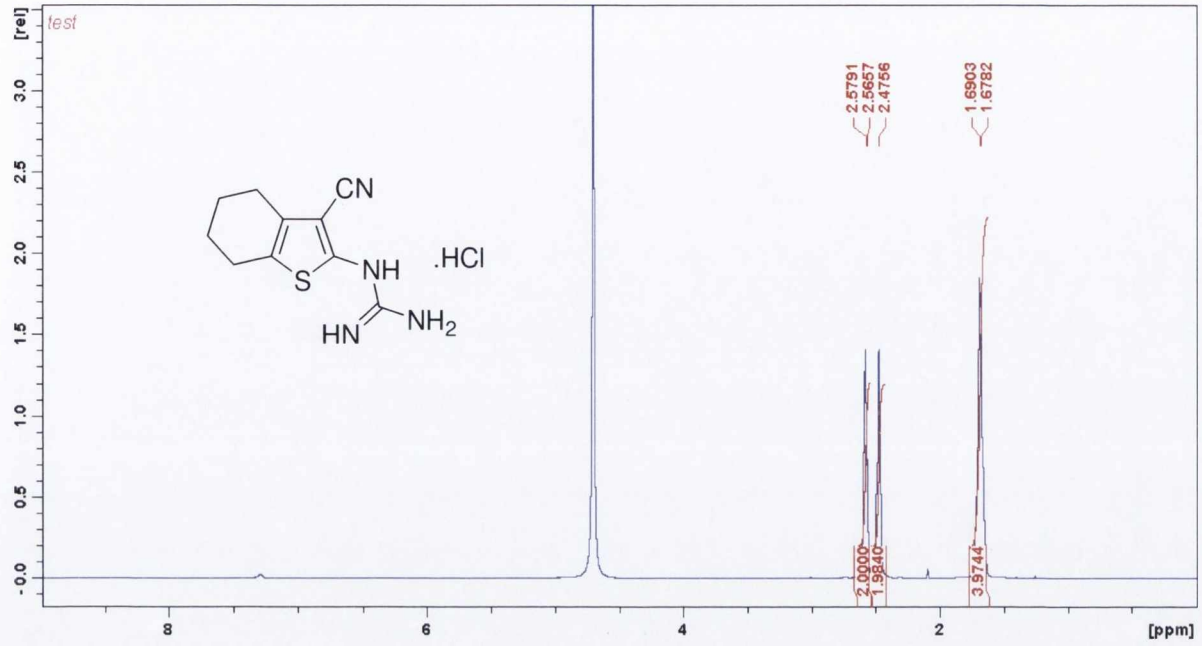


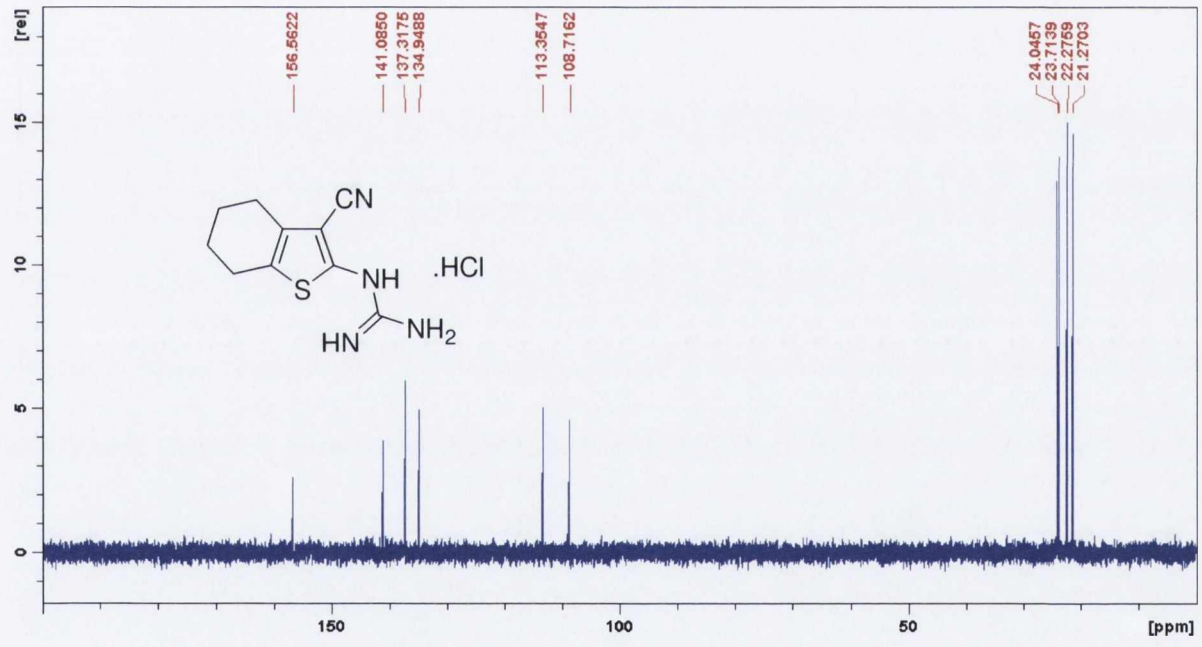
_N_-(3-Cyano-5,6-dihydro-4_H_-cyclopenta[_b_]thien-2-yl)guanidine hydrochloride (129) CHN



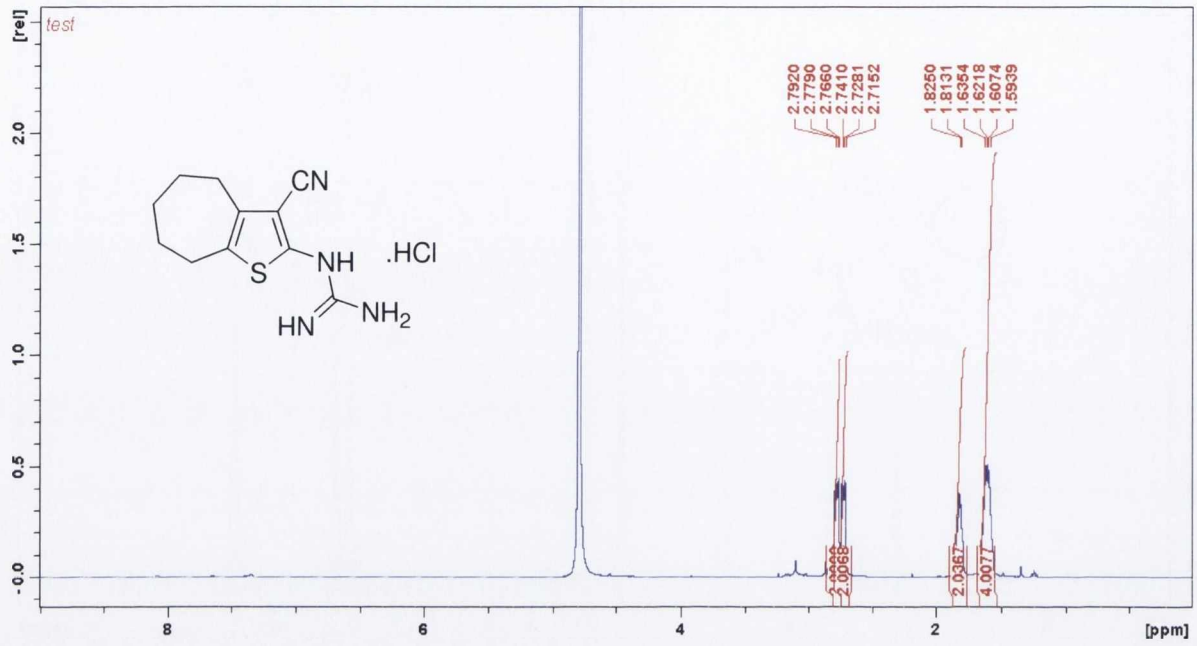


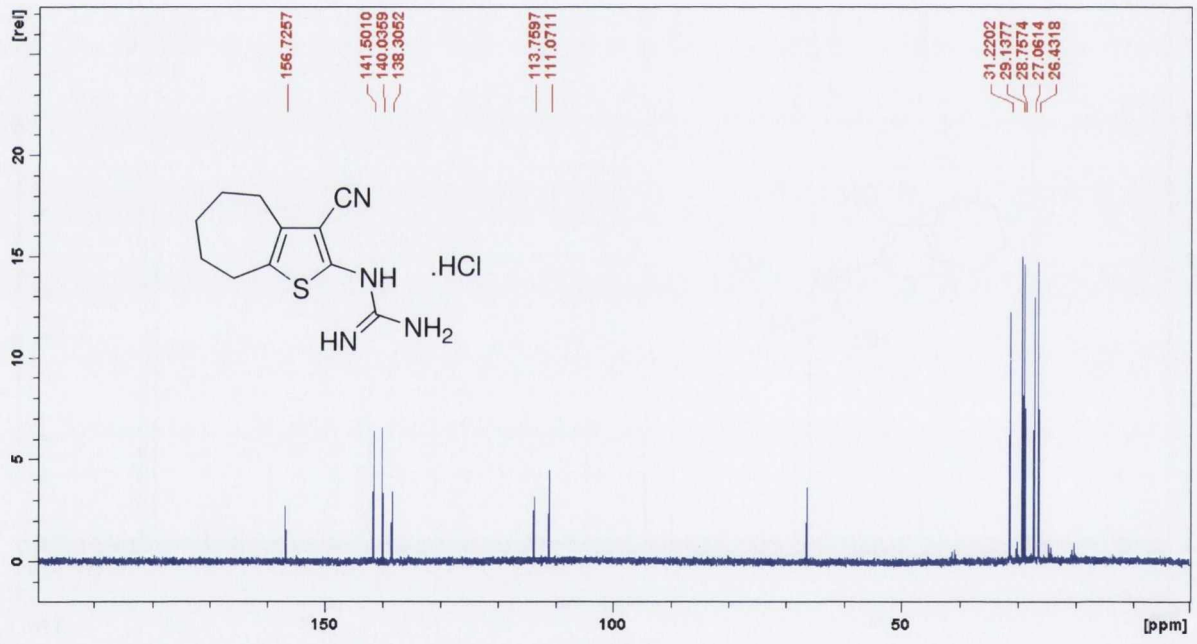
_N_-3-Cyano-4,5,6,7-tetrahydrobenzo[_b_]thien-2-yl)guanidine hydrochloride (130)



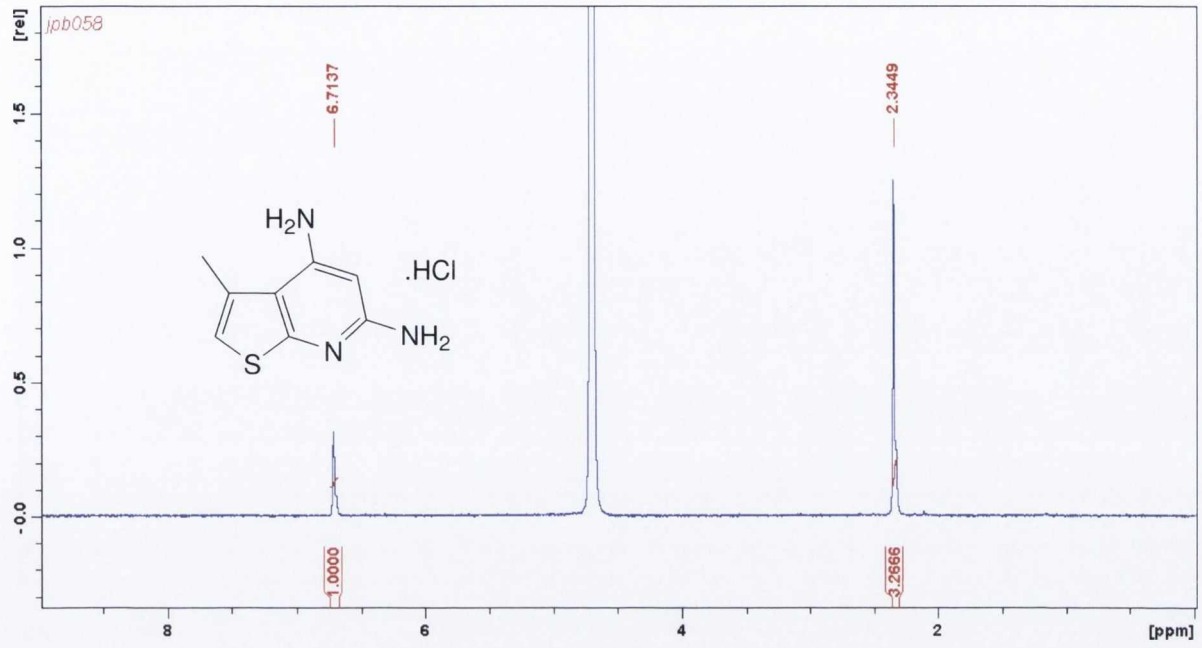


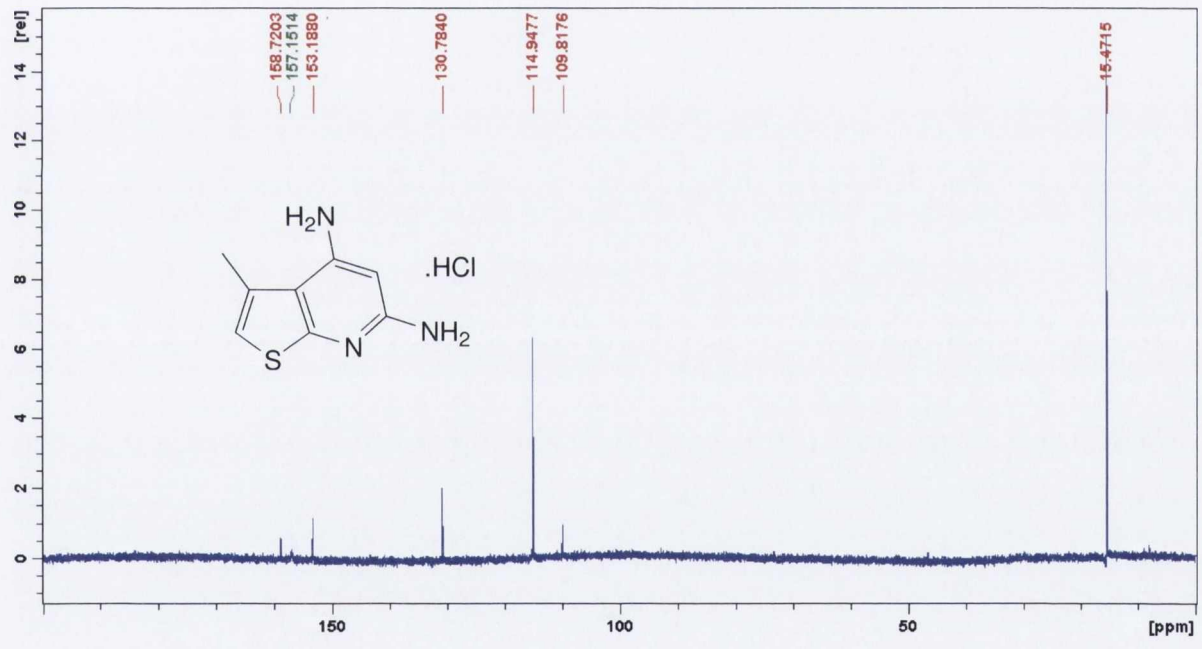
_N_-(3-Cyano-5,6,7,8-tetrahydro-4_H_-cyclohepta[_b_]thien-2-yl)guanidine hydrochloride (131)



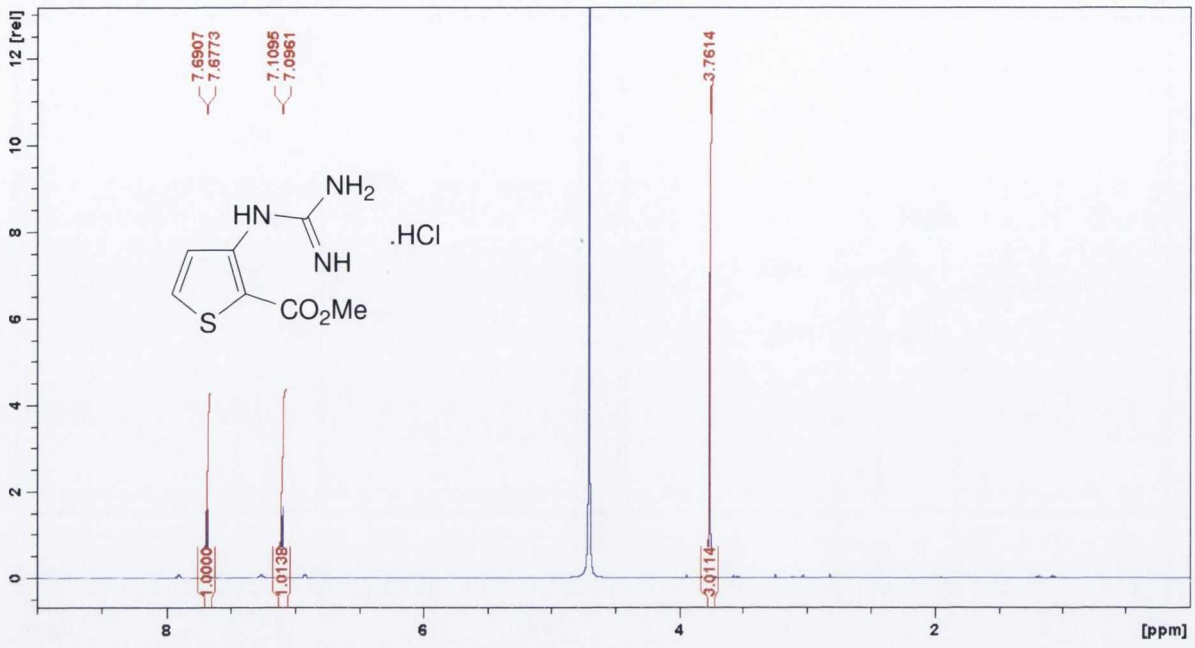


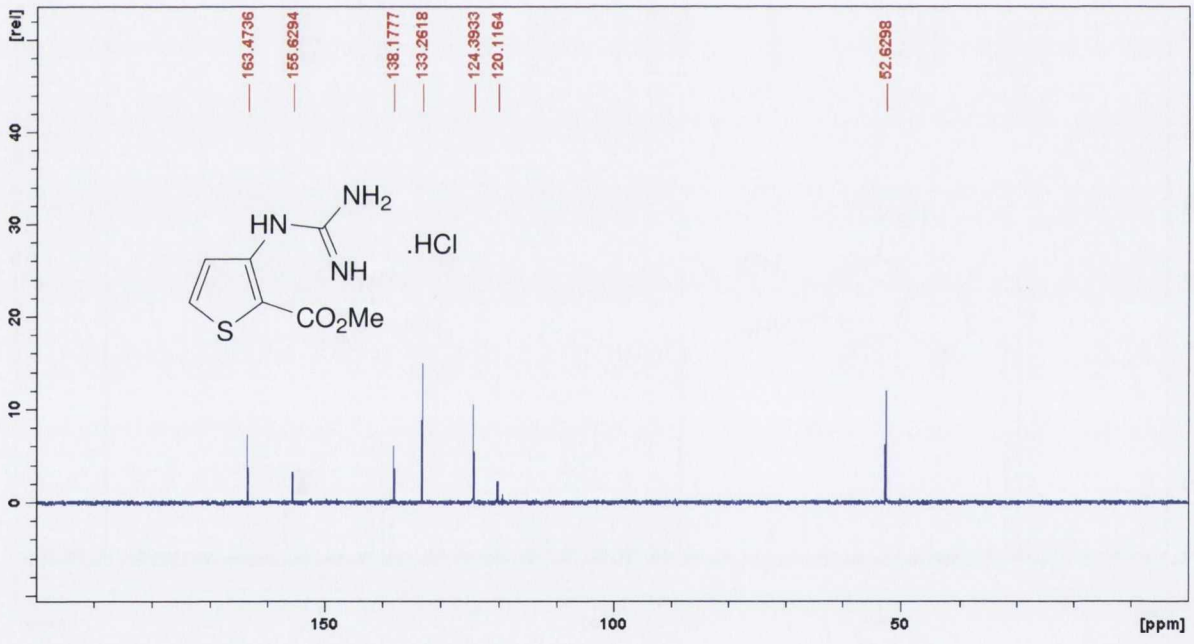
2,4-Diamino-5-methylthieno[2,3-*d*]pyrimidine hydrochloride (134)



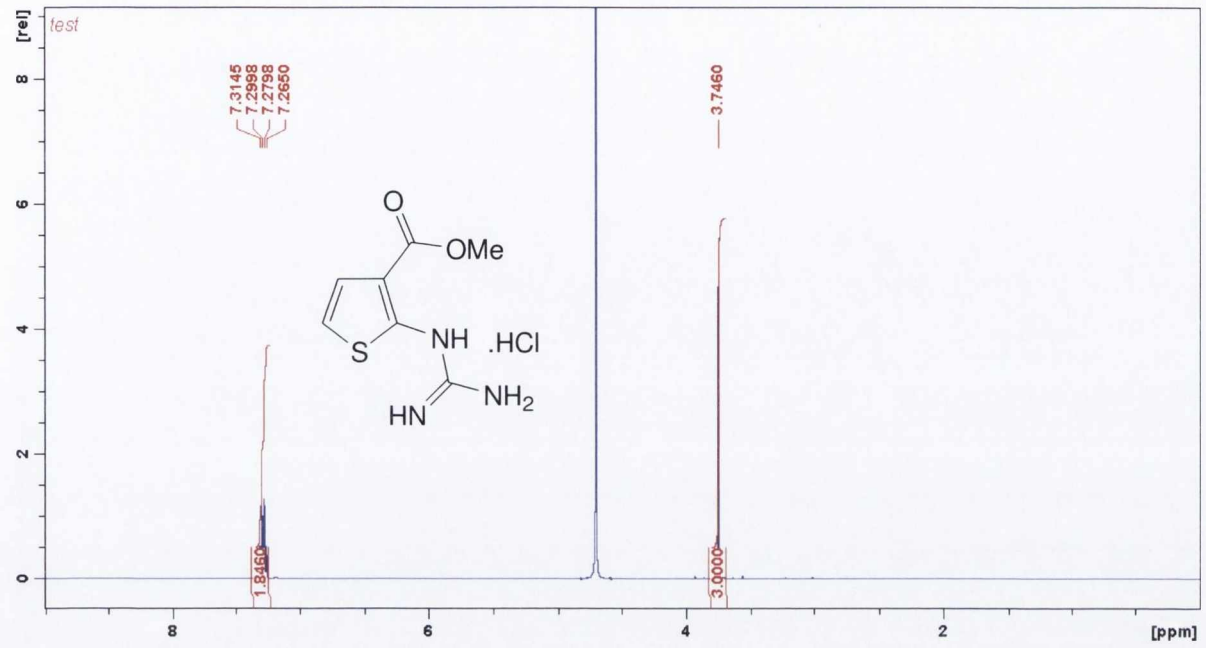


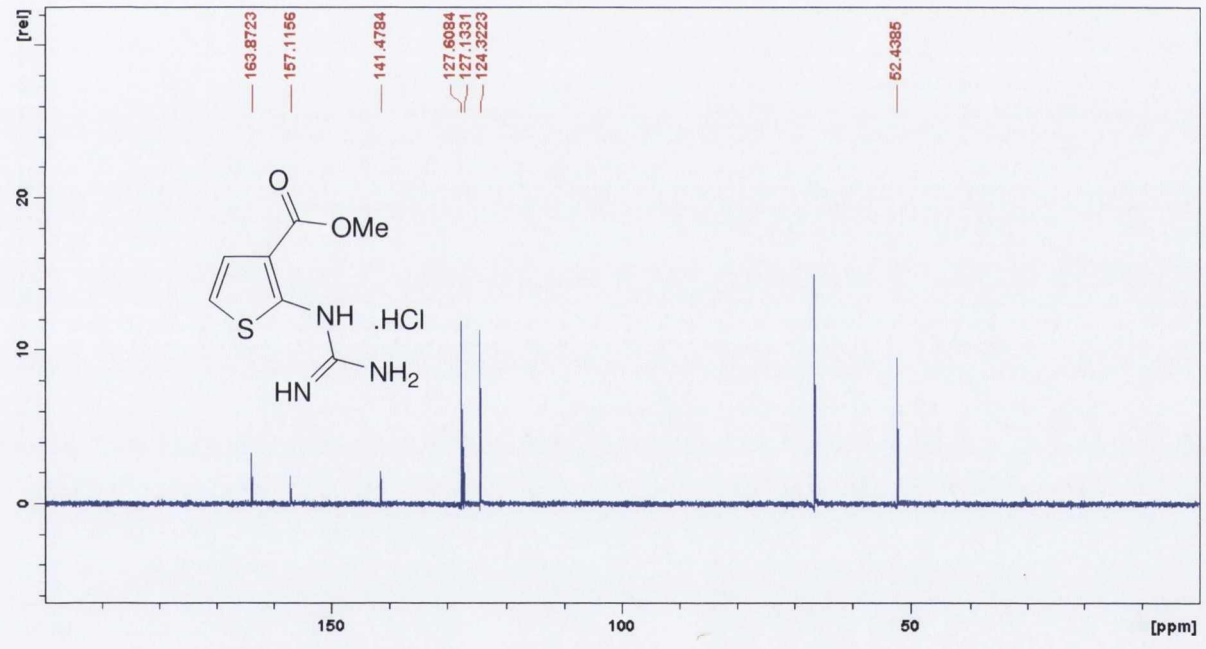
_N_-(2-Methoxycarbonylthien-3-yl)guanidine hydrochloride (140) CHN



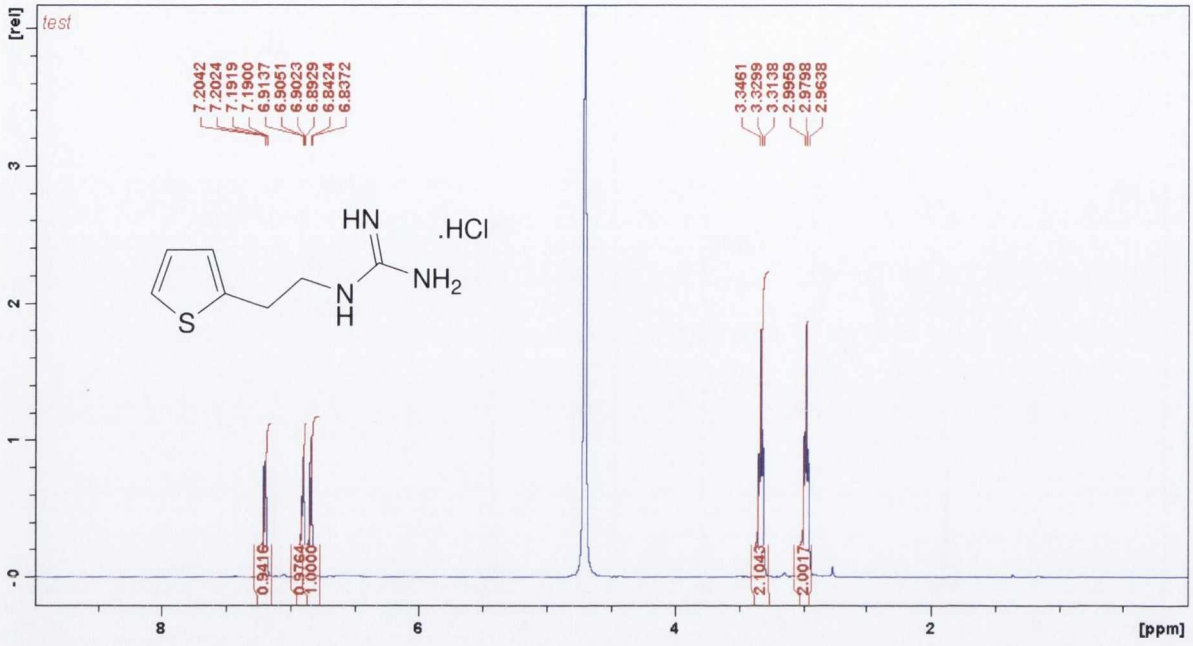


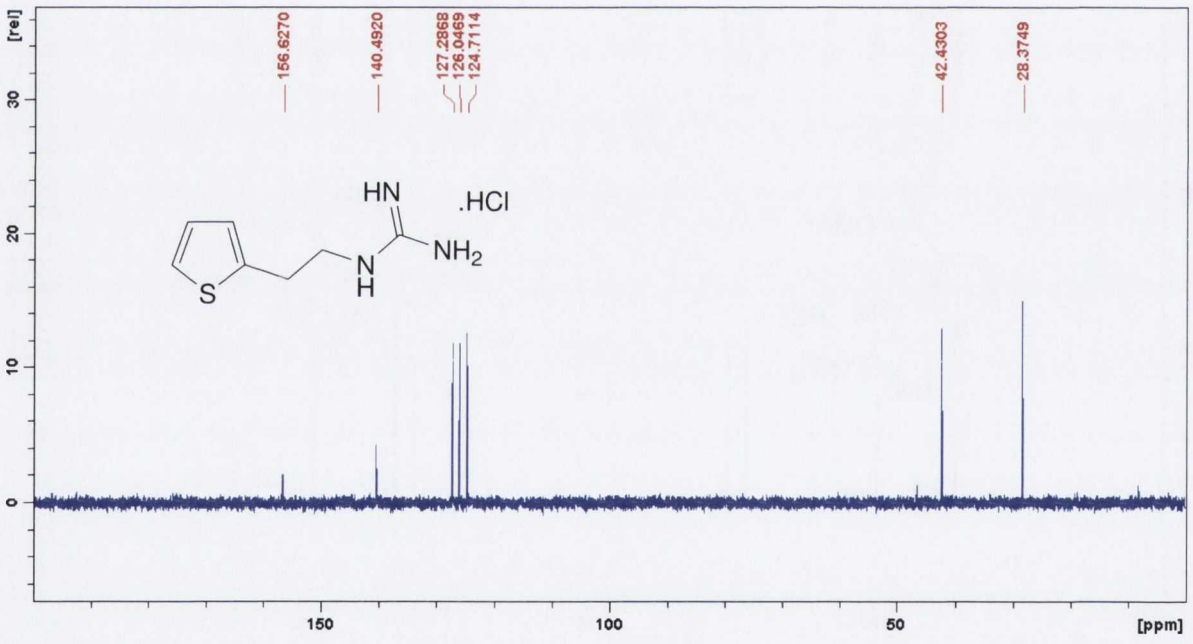
_N_-(3-Methoxycarbonylthien-2-yl)guanidine hydrochloride (141) CHN



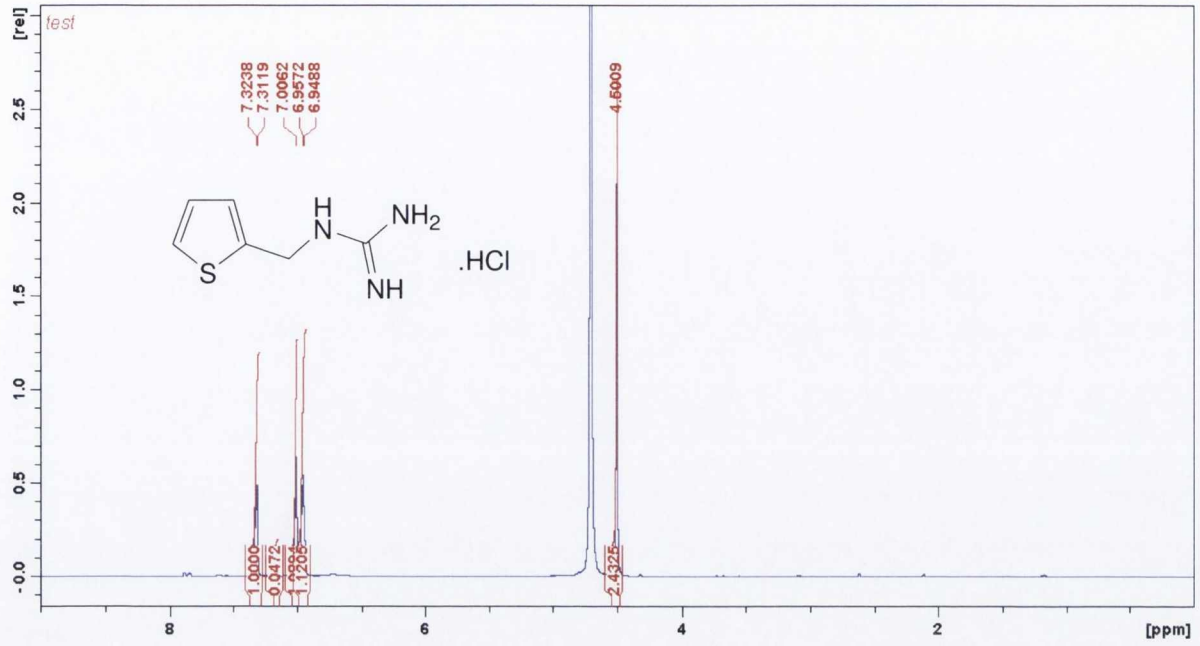


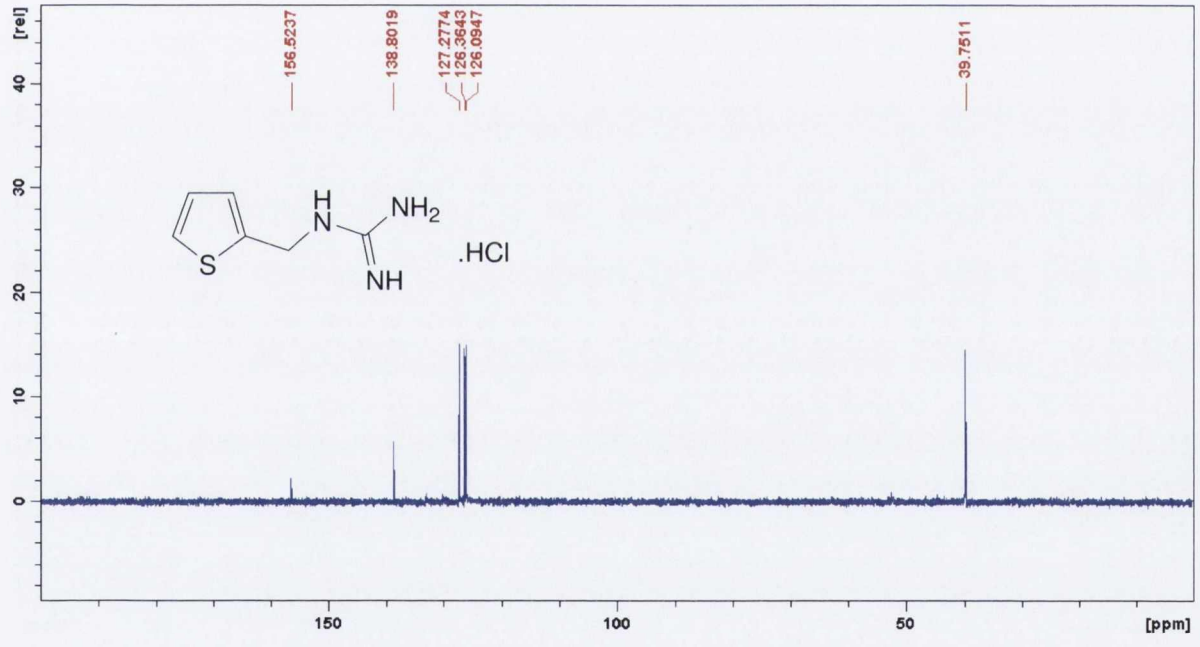
2-[Thien-2-yl]ethylguanidine hydrochloride (142) HPLC



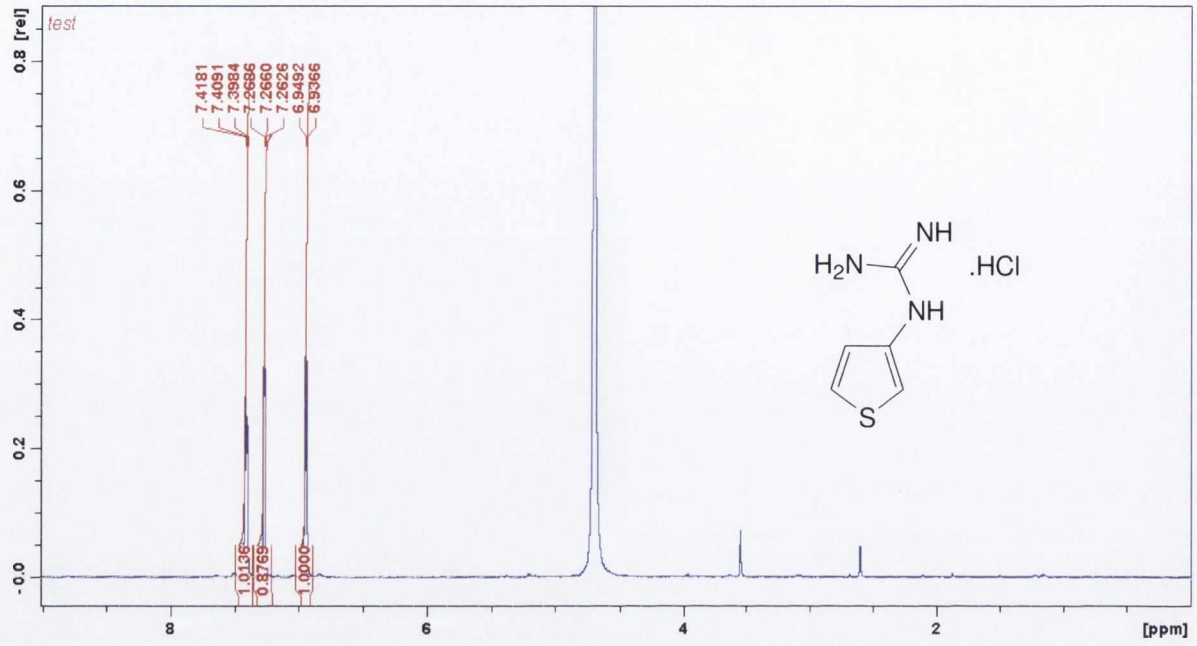


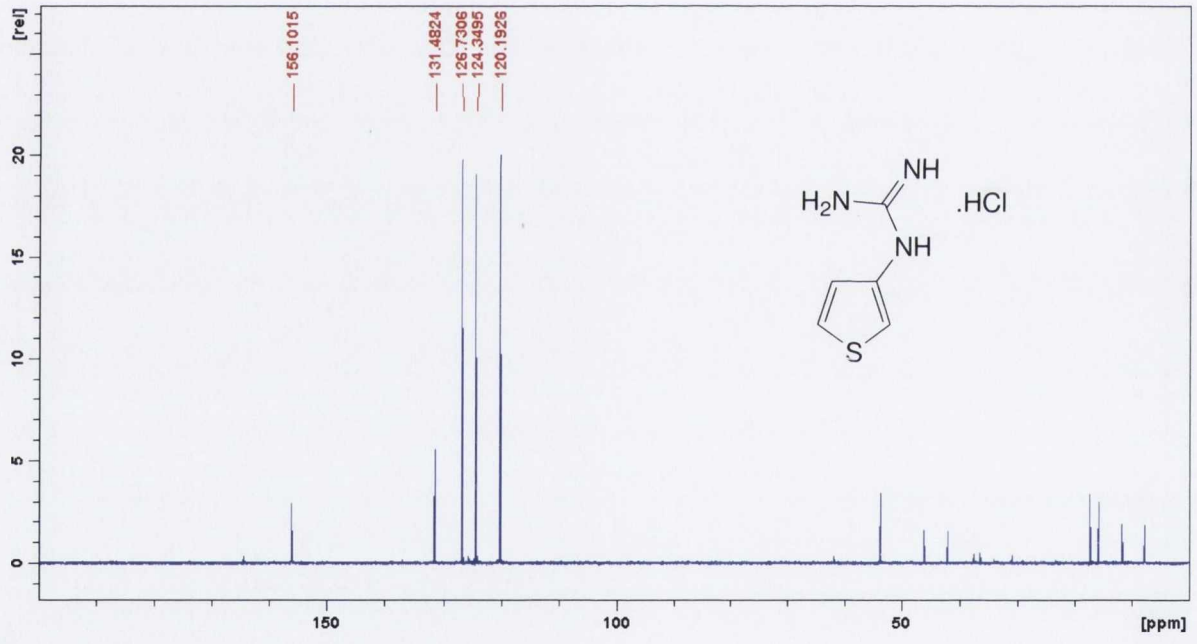
1-[Thien-2-yl]methylguanidine hydrochloride (143)



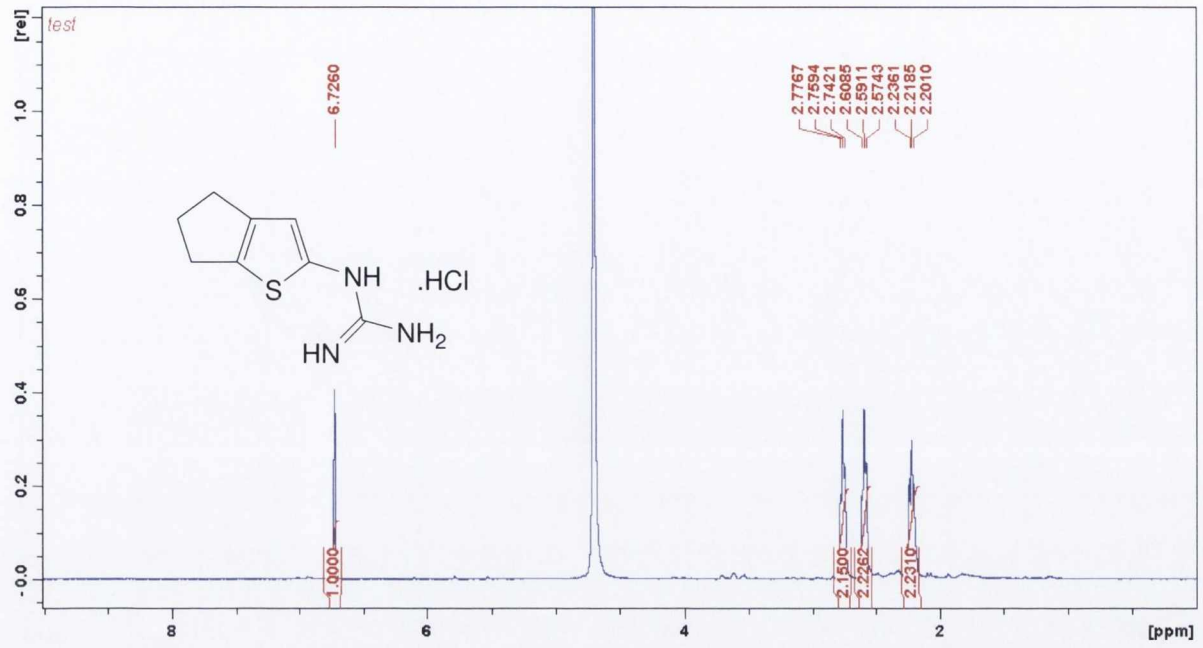


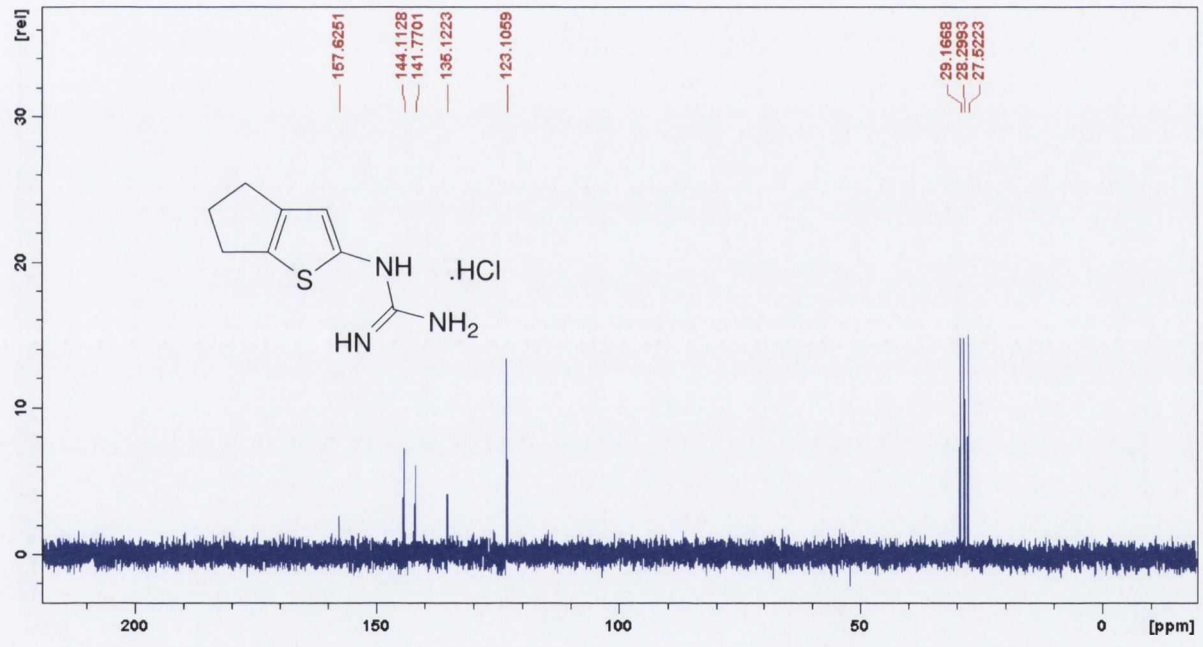
_N_-(Thien-3-yl)guanidine hydrochloride (147)



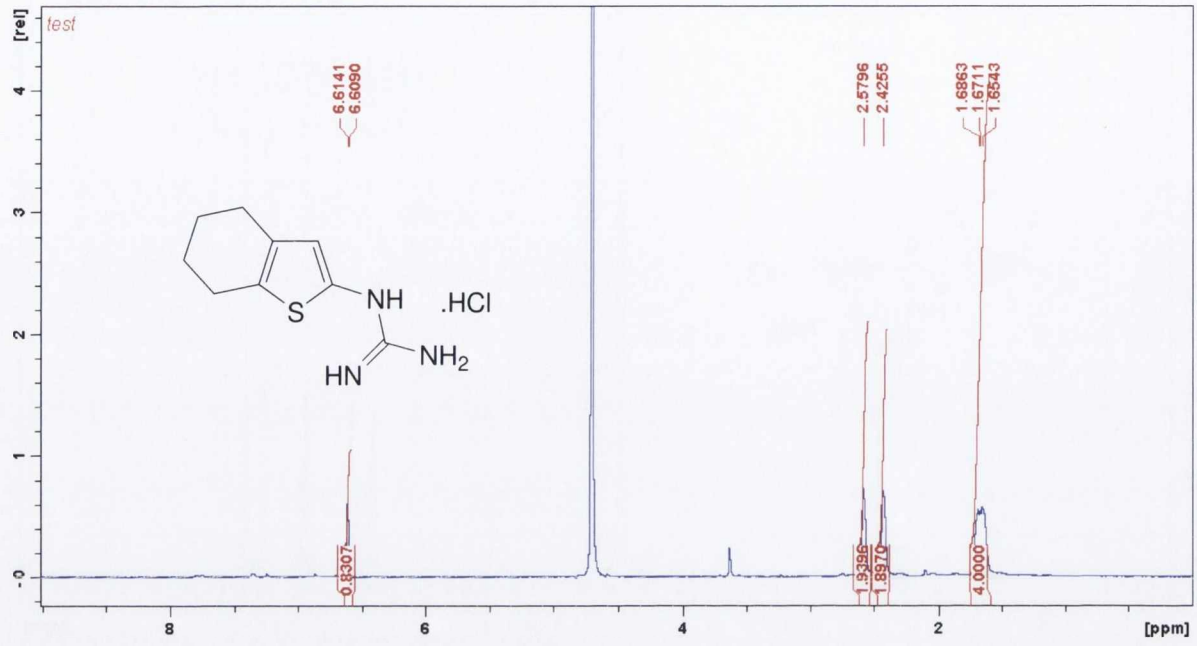


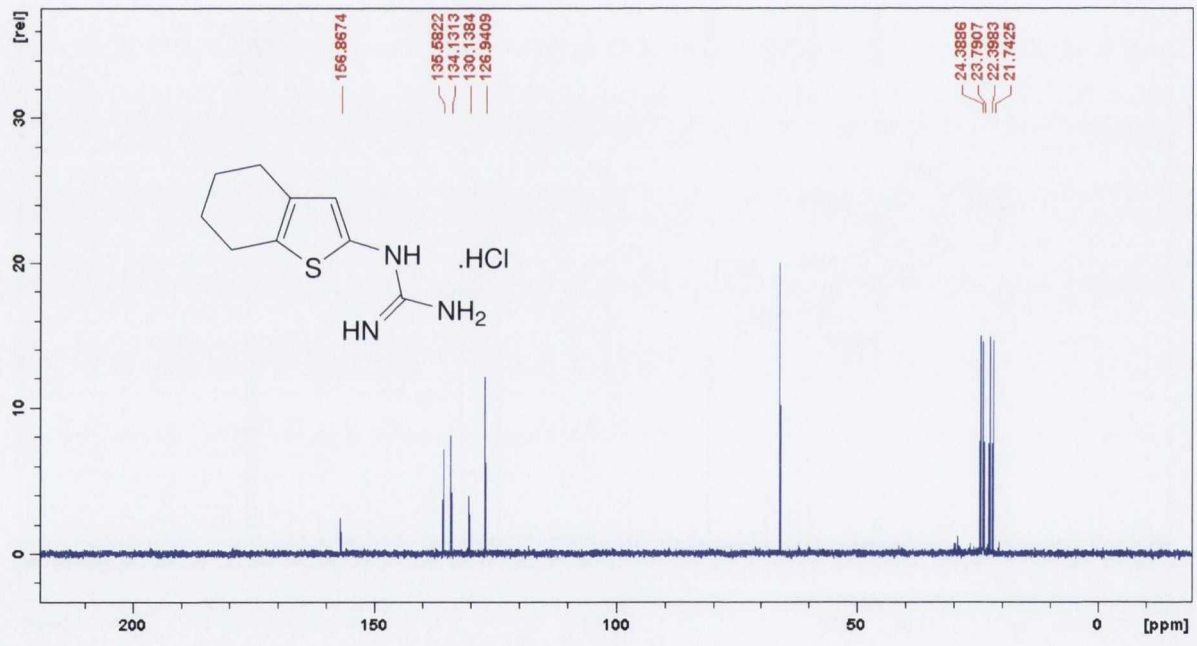
_N_-(5,6-Dihydro-4_H_-cyclopenta[_b_]thien-2-yl)guanidine hydrochloride (152)



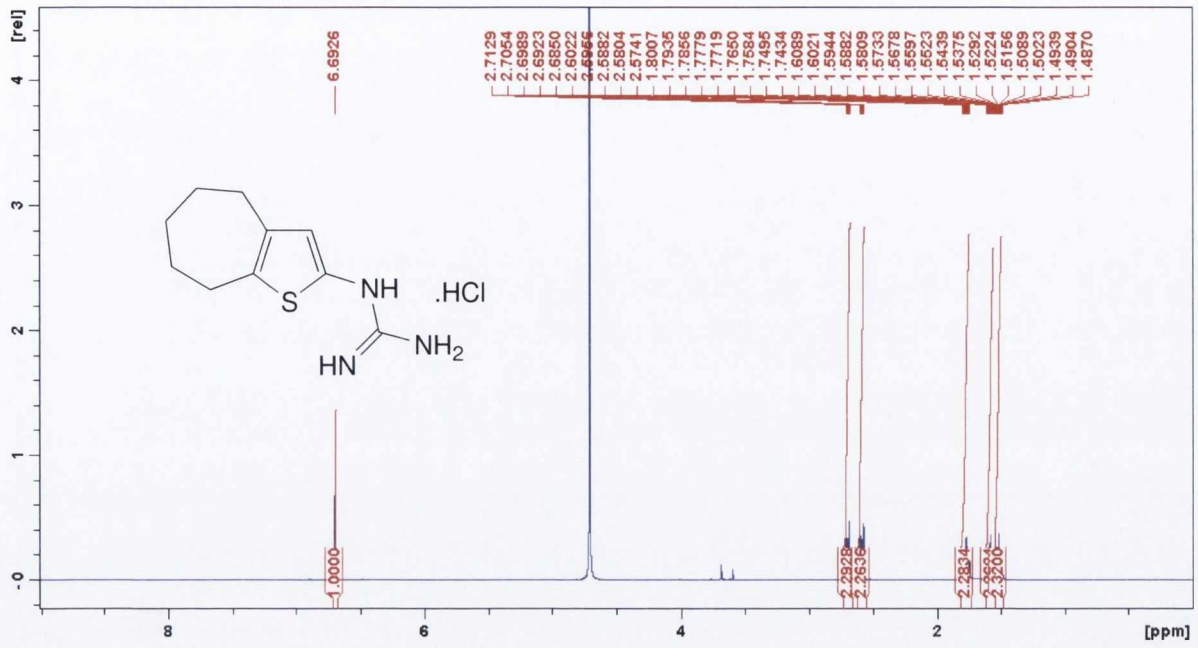


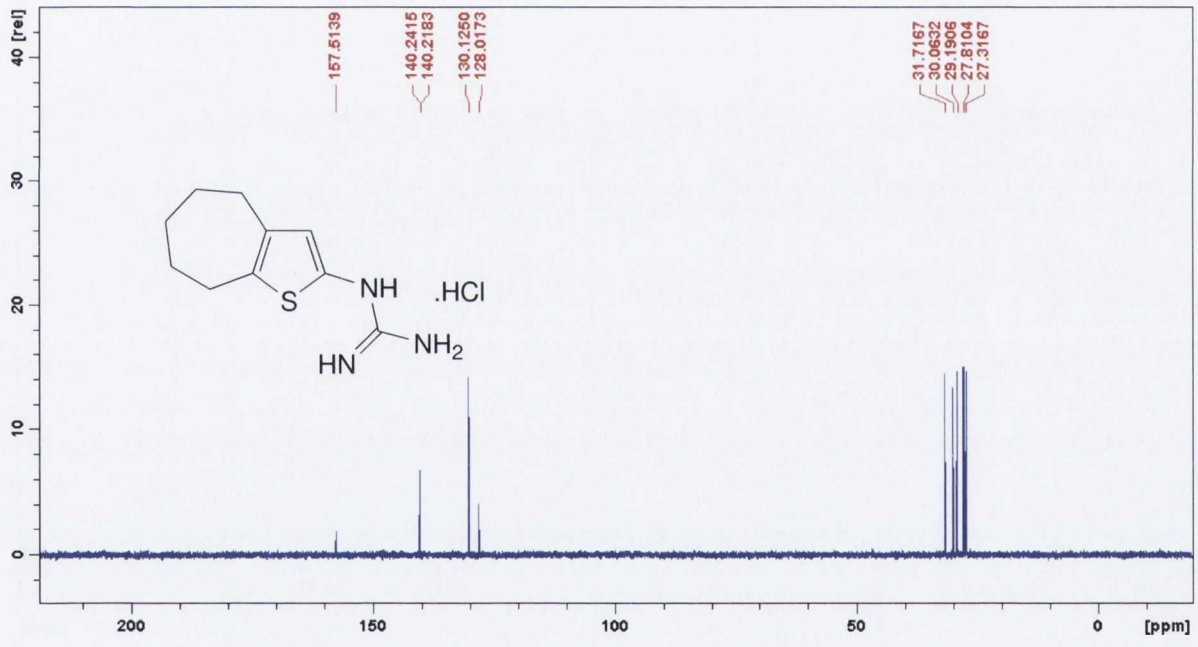
_N_-(4,5,6,7-4_H_-Tetrahydrobenzo[_b_]thien-2-yl)guanidine hydrochloride (151)



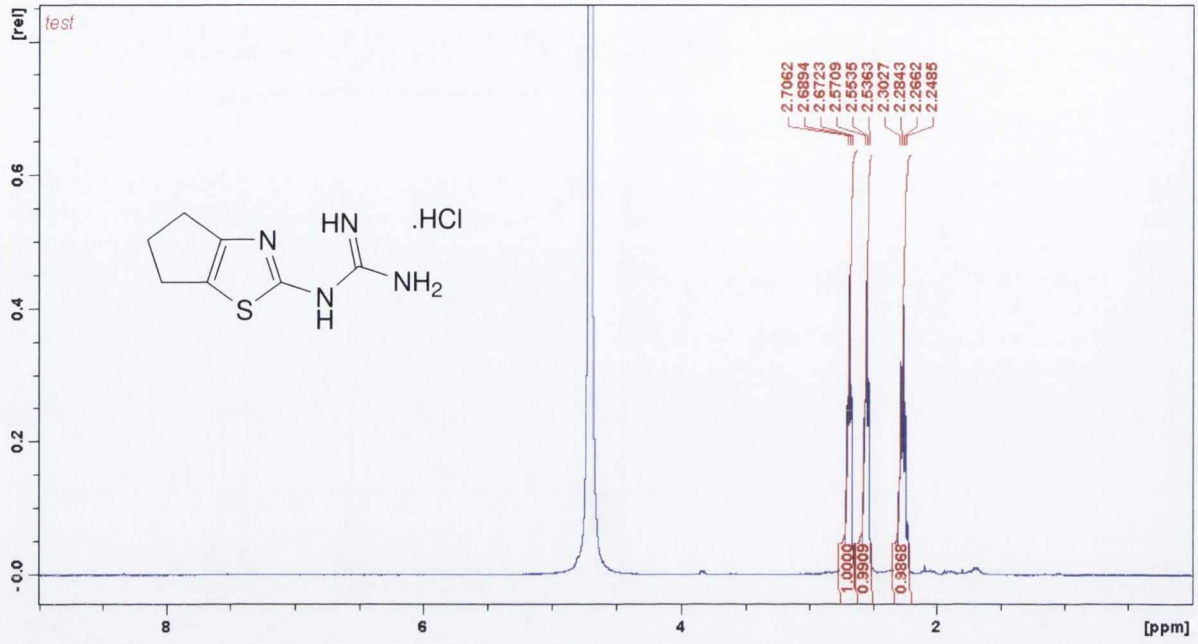


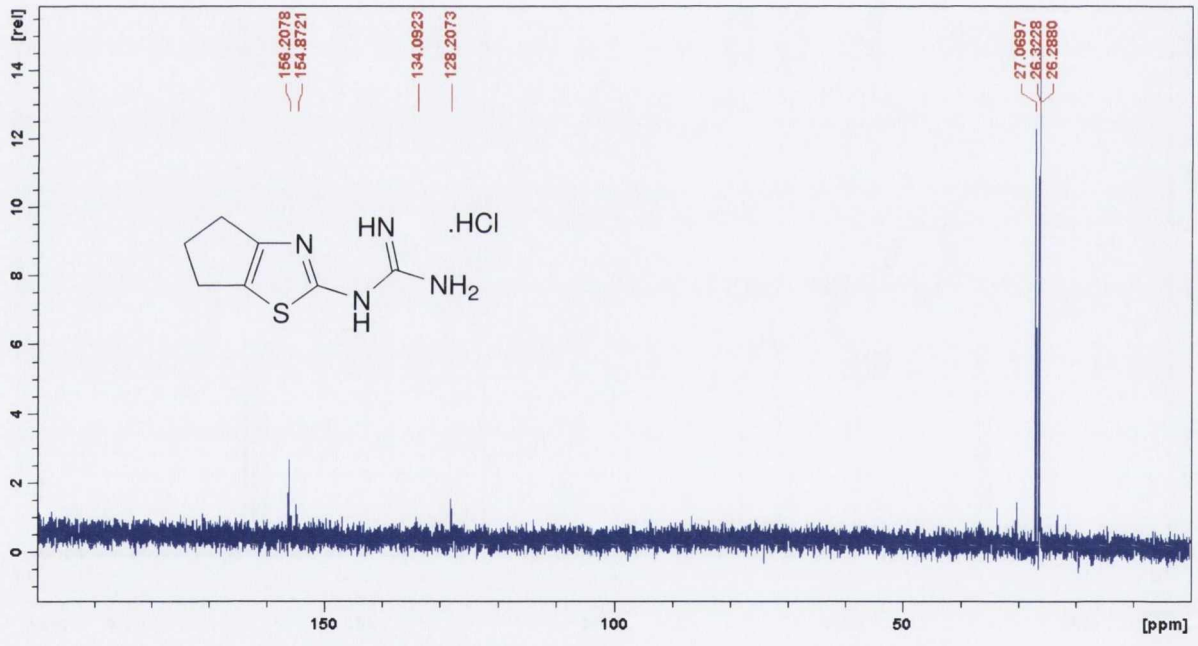
_N_-(5,6,7,8-Tetrahydro-4_H_-cyclohepta[_b_]thien-2-yl)guanidine hydrochloride (153) CHN



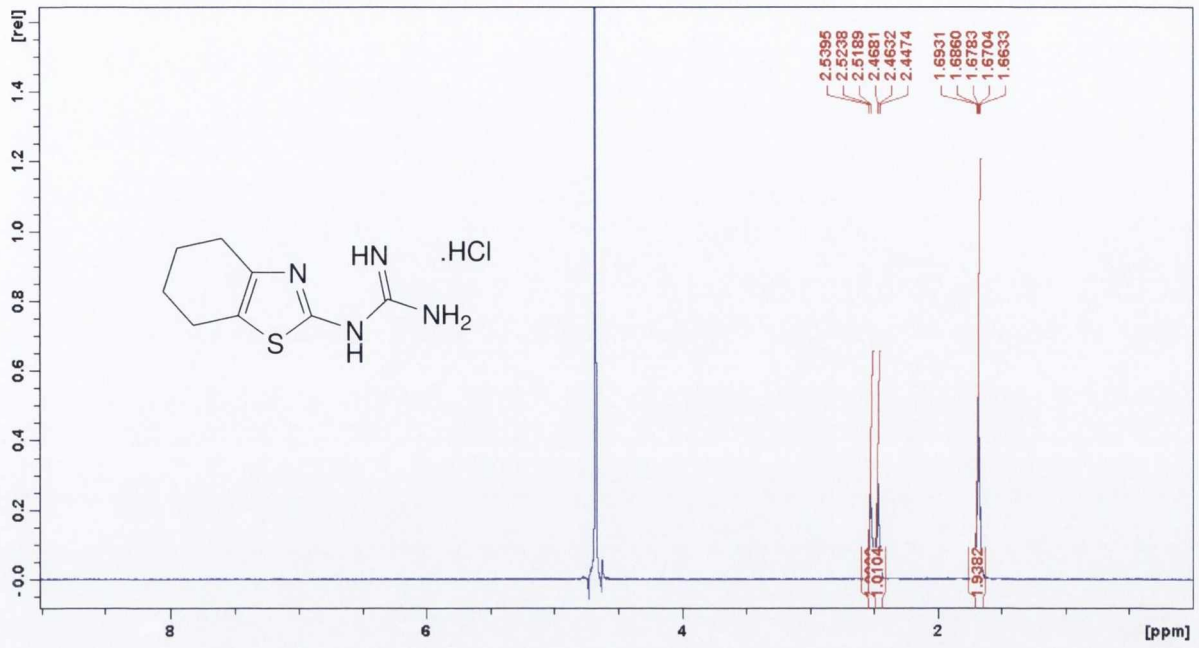


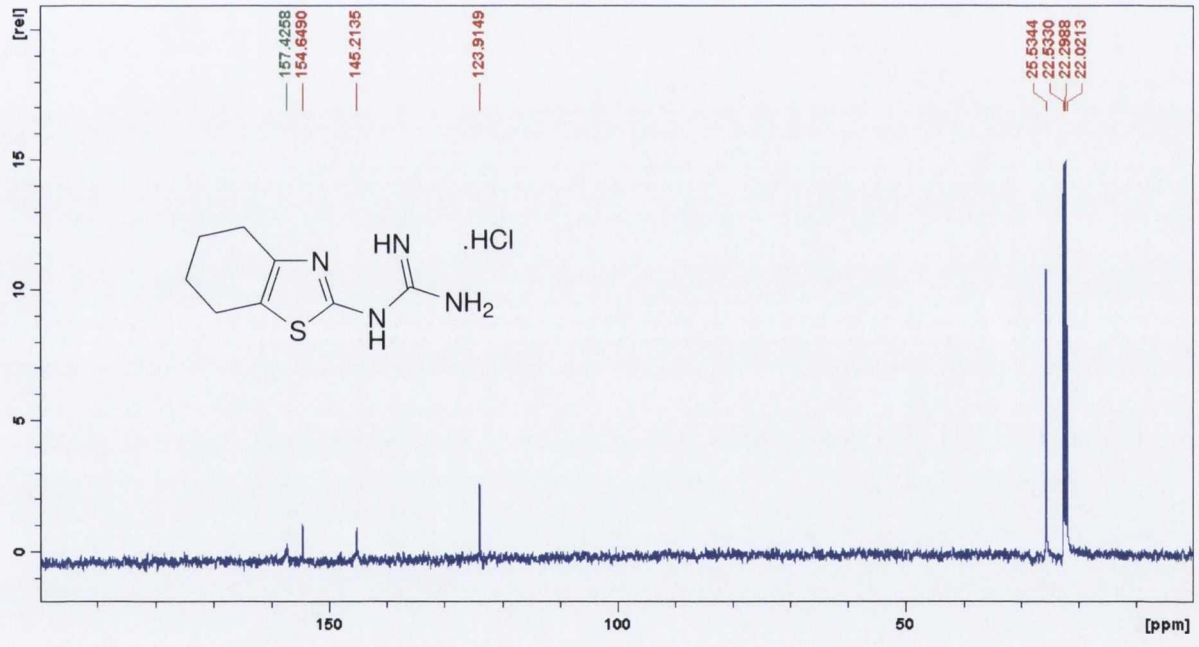
_N_-[5,6-Dihydro-4_H_-cyclopenta-[1,3]-thiazol-2-yl]guanidine hydrochloride (60) HPLC





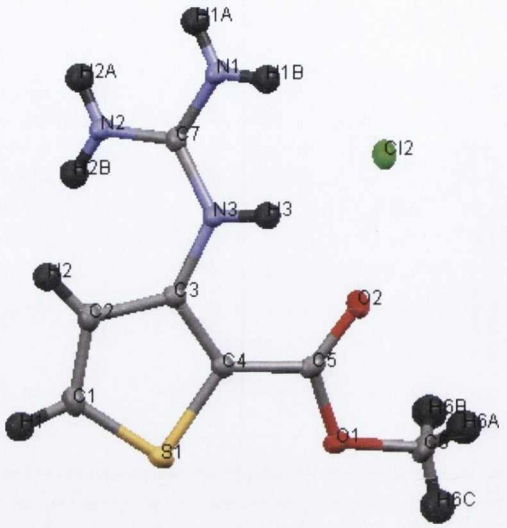
_N_-[4,5,6,7-Tetrahydrobenzo-[1,3]-thiazol-2-yl]guanidine hydrochloride (61) HPLC





_N_-(2-Methoxycarbonylthien-3-yl)guanidine hydrochloride (140)

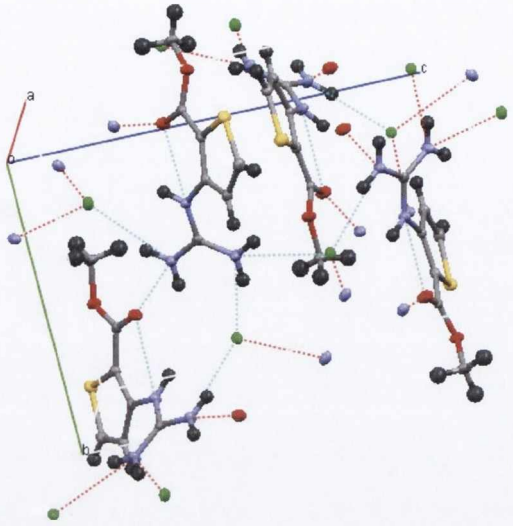
Crystal structure and atom numbering



Bond lengths

Number	Atom1	Atom2	Length	Number	Atom1	Atom2	Length
1	N1	H1A	0.8798	**13**	C3	C4	1.3843
2	N1	H1B	0.8804	**14**	O1	C5	1.3333
3	N2	H2A	0.8802	**15**	O2	C5	1.2159
4	H2B	N2	0.8798	**16**	C4	C5	1.4554
5	N3	H3	0.8800	**17**	O1	C6	1.4554
6	S1	C1	1.7023	**18**	C6	H6A	0.9796
7	C1	H1	0.9497	**19**	C6	H6B	0.9798
8	C1	C2	1.3579	**20**	C6	H6C	0.9792
9	C2	H2	0.9488	**21**	N1	C7	1.3227
10	N3	C3	1.4069	**22**	N2	C7	1.3301
11	C2	C3	1.4120	**23**	N3	C7	1.3407
12	S1	C4	1.7226				

Crystal packing structure including hydrogen bonding interactions

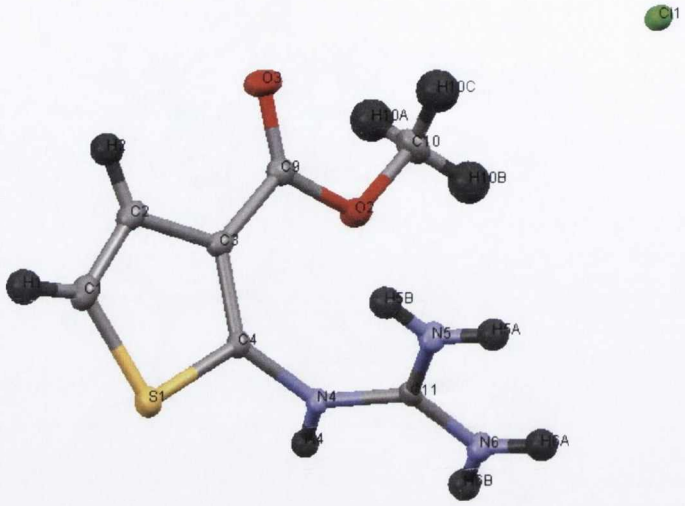


Crystal packing parameters

Space Group	Cell Lengths	Cell Angles	Cell Volume	Z, Z'	R-Factor (%)
P 2_1 2_1 2_1	a 7.361 Å	α 90°	1018.89 Å^3	Z: 1	2.27
	b 10.015 Å	β 90°		Z': 0	
	c 13.821 Å	γ 90°			

_N_-(3-Methoxycarbonylthien-2-yl)guanidine hydrochloride (141)

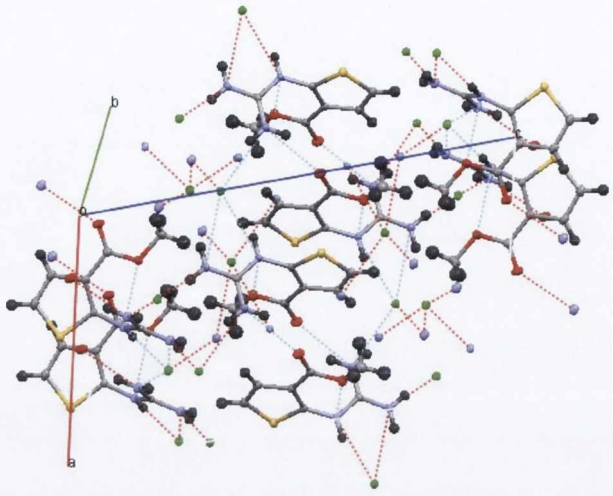
Crystal structure and atom numbering



Bond lengths

Number	Atom1	Atom2	Length	Number	Atom1	Atom2	Length
1	S1	C1	1.719(2)	**13**	N6	H6B	0.880(2)
2	S1	C4	1.723(2)	**14**	N6	C11	1.327(2)
3	O2	C9	1.336(2)	**15**	C1	H1	0.951(2)
4	O2	C10	1.444(3)	**16**	C1	C2	1.358(4)
5	O3	C9	1.216(2)	**17**	C2	H2	0.950(2)
6	N4	H4	0.880(2)	**18**	C2	C3	1.427(3)
7	N4	C4	1.405(2)	**19**	C3	C4	1.380(3)
8	N4	C11	1.350(3)	**20**	C3	C9	1.474(3)
9	N5	H5A	0.880(2)	**21**	C10	H10A	0.980(2)
10	N5	H5B	0.880(2)	**22**	C10	H10B	0.980(2)
11	N5	C11	1.323(2)	**23**	C10	H10C	0.981(2)
12	N6	H6A	0.880(2)				

Crystal packing structure including hydrogen bonding interactions

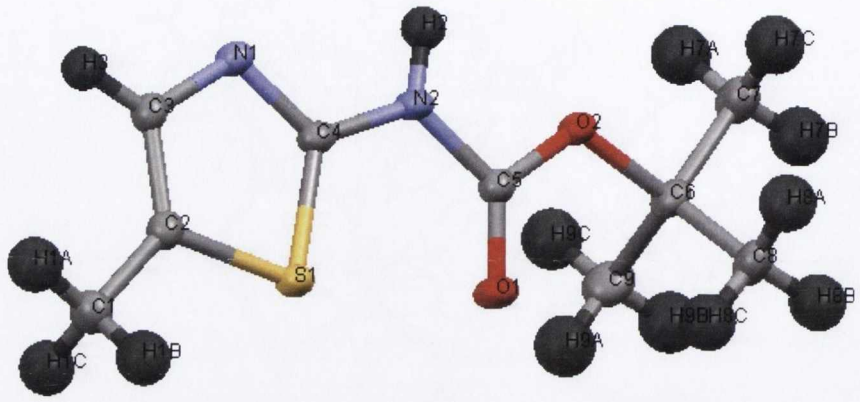


Crystal packing parameters

Space Group	Cell Length	Cell Angles	Cell Volume	Z, Z'	R-Factor (%)
C 2/c	a 11.739(4) Å	α 90.00°	2035.71 Å^3	Z: 1	3.37
	b 9.691(3) Å	β 107.522° (4)		Z': 0	
	c 18.765(6) Å	γ 90.00°			

tert-Butyl _N_-(5-methyl-thiazol-2-yl)carbamate (161)

Crystal structure and atom numbering

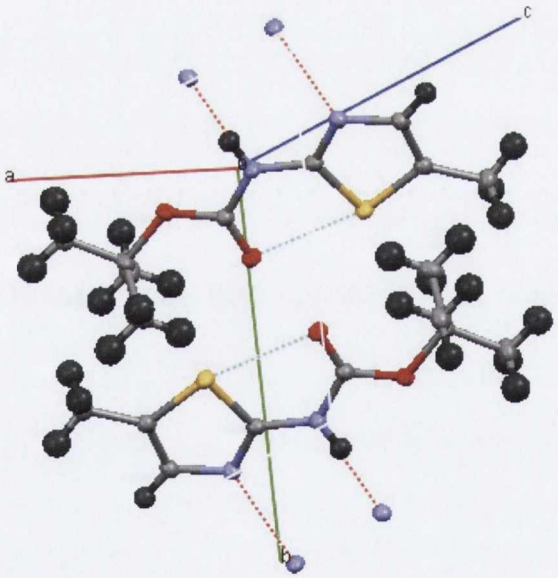


Bond lengths

Number	Atom1	Atom2	Length	Number	Atom1	Atom2	Length
1	C1	H1A	0.9799	**15**	N2	C5	1.3649
2	C1	H1B	0.9805	**16**	O2	C6	1.4868
3	C1	H1C	0.9799	**17**	C6	C7	1.5176
4	S1	C2	1.7387	**18**	C7	H7A	0.9796
5	C1	C2	1.4947	**19**	C7	H7B	0.9806
6	N1	C3	1.3830	**20**	C7	H7C	0.9801
7	C2	C3	1.3464	**21**	C6	C8	1.5217
8	C3	H3	0.9496	**22**	C8	H8A	0.9795
9	S1	C4	1.7335	**23**	C8	H8B	0.9802
10	N1	C4	1.3064	**24**	C8	H8C	0.9800
11	C4	N2	1.3811	**25**	C6	C9	1.5183
12	N2	H2	0.8798	**26**	C9	H9A	0.9799
13	O2	C5	1.3435	**27**	C9	H9B	0.9804
14	O1	C5	1.2130	**28**	C9	H9C	0.9799
Number	Atom1	Atom2	Length	Number	Atom1	Atom2	Length

1	C1	H1A	0.9799	**15**	N2	C5	1.3649
2	C1	H1B	0.9805	**16**	O2	C6	1.4868
3	C1	H1C	0.9799	**17**	C6	C7	1.5176
4	S1	C2	1.7387	**18**	C7	H7A	0.9796
5	C1	C2	1.4947	**19**	C7	H7B	0.9806
6	N1	C3	1.3830	**20**	C7	H7C	0.9801
7	C2	C3	1.3464	**21**	C6	C8	1.5217
8	C3	H3	0.9496	**22**	C8	H8A	0.9795
9	S1	C4	1.7335	**23**	C8	H8B	0.9802
10	N1	C4	1.3064	**24**	C8	H8C	0.9800
11	C4	N2	1.3811	**25**	C6	C9	1.5183
12	N2	H2	0.8798	**26**	C9	H9A	0.9799
13	O2	C5	1.3435	**27**	C9	H9B	0.9804
14	O1	C5	1.2130	**28**	C9	H9C	0.9799

Crystal packing structure showing hydrogen bond interactions

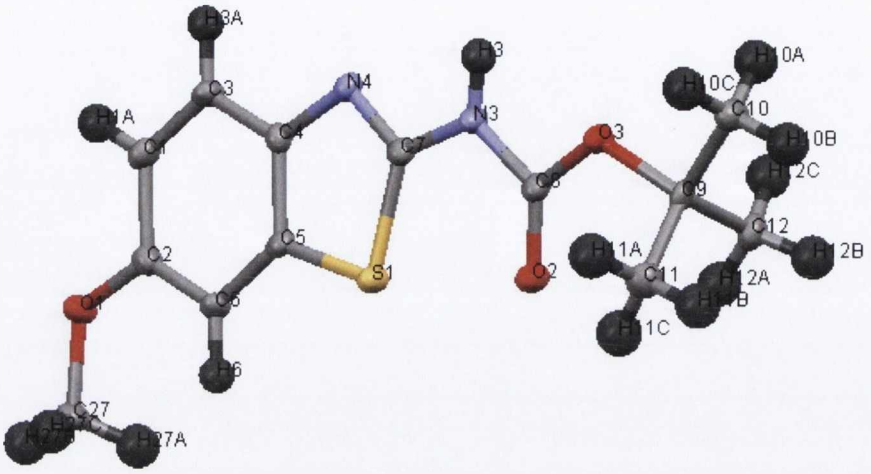


Crystal packing parameters

Space Group	Cell Lengths	Cell Angles	Cell Volume	Z, Z'	R-Factor (%)
P-1	a 6.618 Å	α 104.26°	552.27 Å^3	Z: 1	3.2
	b 8.796 Å	β 95.41°		Z': 0	
	c 9.842 Å	γ 91.08°			

tert-Butyl _N_-(6-methoxy-2-thiazol-2-yl)carbamate (162)

Crystal structure and atom numbering

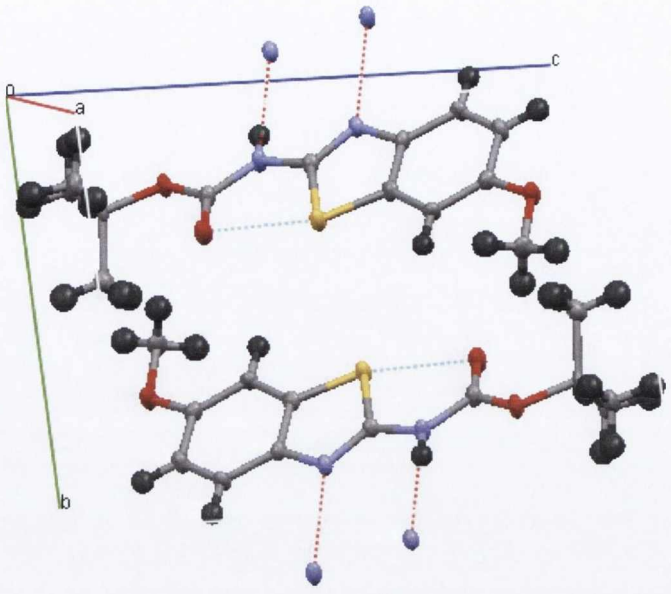


Bond lengths

Number	Atom1	Atom2	Length	Number	Atom1	Atom2	Length
1	N3	H3	0.8606	**37**	N3	H3	0.8606
2	C1	H1A	0.9311	**38**	C1	H1A	0.9311
3	O1	C2	1.3504	**39**	O1	C2	1.3504
4	C2	C1	1.4168	**40**	C2	C1	1.4168
5	C1	C3	1.3617	**41**	C1	C3	1.3617
6	C3	H3A	0.9291	**42**	C3	H3A	0.9291
7	N4	C4	1.3961	**43**	N4	C4	1.3961
8	C3	C4	1.3976	**44**	C3	C4	1.3976
9	S1	C5	1.7351	**45**	S1	C5	1.7351
10	C4	C5	1.3989	**46**	C4	C5	1.3989
11	C6	C2	1.3889	**47**	C6	C2	1.3889
12	C6	C5	1.3910	**48**	C6	C5	1.3910
13	C6	H6	0.9303	**49**	C6	H6	0.9303
14	S1	C7	1.7530	**50**	S1	C7	1.7530

Number	Atom1	Atom2	Length	Number	Atom1	Atom2	Length
15	N3	C7	1.3864	**51**	N3	C7	1.3864
16	N4	C7	1.2946	**52**	N4	C7	1.2946
17	O3	C8	1.3289	**53**	O3	C8	1.3289
18	O2	C8	1.2182	**54**	O2	C8	1.2182
19	N3	C8	1.3757	**55**	N3	C8	1.3757
20	O3	C9	1.4927	**56**	O3	C9	1.4927
21	C9	C10	1.5097	**57**	C9	C10	1.5097
22	C10	H10A	0.9612	**58**	C10	H10A	0.9612
23	C10	H10B	0.9601	**59**	C10	H10B	0.9601
24	C10	H10C	0.9590	**60**	C10	H10C	0.9590
25	C9	C11	1.5140	**61**	C9	C11	1.5140
26	C11	H11A	0.9605	**62**	C11	H11A	0.9605
27	C11	H11B	0.9589	**63**	C11	H11B	0.9589
28	C11	H11C	0.9606	**64**	C11	H11C	0.9606
29	C9	C12	1.5150	**65**	C9	C12	1.5150
30	C12	H12A	0.9602	**66**	C12	H12A	0.9602
31	C12	H12B	0.9597	**67**	C12	H12B	0.9597
32	C12	H12C	0.9599	**68**	C12	H12C	0.9599
33	O1	C27	1.4311	**69**	O1	C27	1.4311
34	C27	H27A	0.9602	**70**	C27	H27A	0.9602
35	C27	H27B	0.9608	**71**	C27	H27B	0.9608
36	C27	H27C	0.9599	**72**	C27	H27C	0.9599

Crystal packing structure showing hydrogen bonding interactions

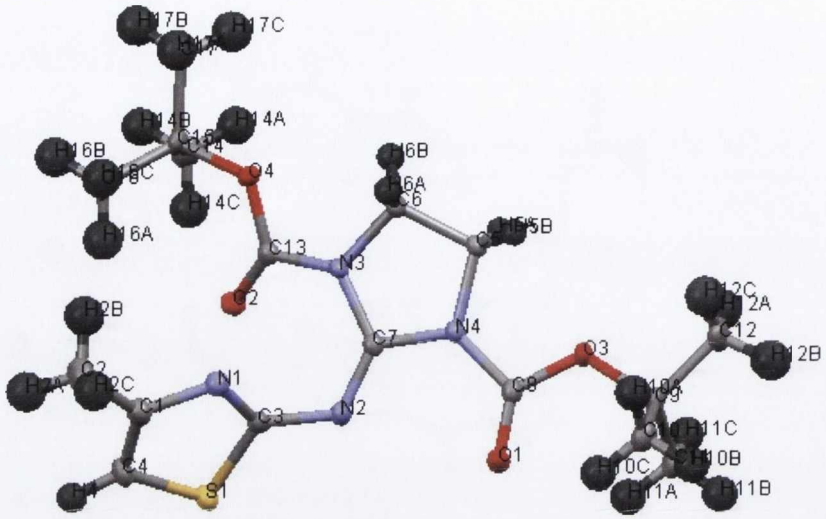


Crystal packing parameters

Space Group	Cell Lengths	Cell Angles	Cell Volume	Z, Z'	R-Factor (%)
P -1	a 6.388 Å	α 87.42°	667.961 Å^3	Z: 1	6.28
	b 9.354 Å	β 82.34°		Z': 0	
	c 11.948 Å	γ 70.74°			

[1,3-Di-*tert*-butoxycarbonyl]-2-(4-methylthiazol-2-ylimino)imidazolidine (176)

Crystal structure and atom numbering



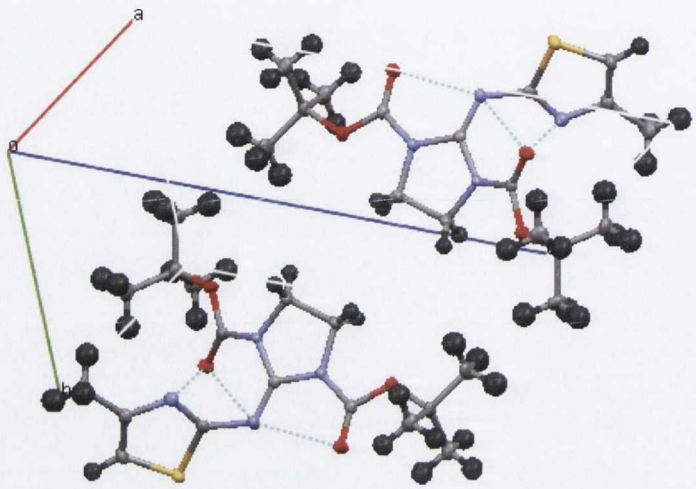
Bond lengths

Number	Atom1	Atom2	Length	Number	Atom1	Atom2	Length
1	S1	C3	1.734(1)	**54**	S1	C3	1.734(1)
2	S1	C4	1.717(2)	**55**	S1	C4	1.717(2)
3	O1	C8	1.201(2)	**56**	O1	C8	1.201(2)
4	O2	C13	1.204(2)	**57**	O2	C13	1.204(2)
5	O3	C8	1.338(2)	**58**	O3	C8	1.338(2)
6	O3	C9	1.482(2)	**59**	O3	C9	1.482(2)
7	O4	C13	1.337(2)	**60**	O4	C13	1.337(2)
8	O4	C15	1.490(2)	**61**	O4	C15	1.490(2)
9	N1	C1	1.385(2)	**62**	N1	C1	1.385(2)
10	N1	C3	1.304(2)	**63**	N1	C3	1.304(2)
11	N2	C3	1.384(2)	**64**	N2	C3	1.384(2)
12	N2	C7	1.276(2)	**65**	N2	C7	1.276(2)
13	N3	C6	1.469(2)	**66**	N3	C6	1.469(2)
14	N3	C7	1.401(2)	**67**	N3	C7	1.401(2)
15	N3	C13	1.390(2)	**68**	N3	C13	1.390(2)

16	N4	C5	1.473(2)	**69**	N4	C5	1.473(2)
17	N4	C7	1.394(2)	**70**	N4	C7	1.394(2)
18	N4	C8	1.396(2)	**71**	N4	C8	1.396(2)
19	C1	C2	1.497(2)	**72**	C1	C2	1.497(2)
20	C1	C4	1.349(2)	**73**	C1	C4	1.349(2)
21	C2	H2A	0.981(2)	**74**	C2	H2A	0.981(2)
22	C2	H2B	0.981(2)	**75**	C2	H2B	0.981(2)
23	C2	H2C	0.980(2)	**76**	C2	H2C	0.980(2)
24	C4	H4	0.950(2)	**77**	C4	H4	0.950(2)
25	C5	H5A	0.990(1)	**78**	C5	H5A	0.990(1)
26	C5	H5B	0.990(1)	**79**	C5	H5B	0.990(1)
27	C5	C6	1.521(2)	**80**	C5	C6	1.521(2)
28	C6	H6A	0.990(1)	**81**	C6	H6A	0.990(1)
29	C6	H6B	0.990(2)	**82**	C6	H6B	0.990(2)
30	C9	C10	1.517(2)	**83**	C9	C10	1.517(2)
31	C9	C11	1.513(2)	**84**	C9	C11	1.513(2)
32	C9	C12	1.515(2)	**85**	C9	C12	1.515(2)
33	C10	H10A	0.980(2)	**86**	C10	H10A	0.980(2)
34	C10	H10B	0.980(2)	**87**	C10	H10B	0.980(2)
35	C10	H10C	0.980(2)	**88**	C10	H10C	0.980(2)
36	C11	H11A	0.980(2)	**89**	C11	H11A	0.980(2)
37	C11	H11B	0.980(2)	**90**	C11	H11B	0.980(2)
38	C11	H11C	0.980(2)	**91**	C11	H11C	0.980(2)
39	C12	H12A	0.980(2)	**92**	C12	H12A	0.980(2)
40	C12	H12B	0.980(2)	**93**	C12	H12B	0.980(2)
41	C12	H12C	0.980(2)	**94**	C12	H12C	0.980(2)
42	C14	H14A	0.981(2)	**95**	C14	H14A	0.981(2)
43	C14	H14B	0.980(2)	**96**	C14	H14B	0.980(2)
44	C14	H14C	0.980(2)	**97**	C14	H14C	0.980(2)
45	C14	C15	1.513(2)	**98**	C14	C15	1.513(2)
46	C15	C16	1.519(2)	**99**	C15	C16	1.519(2)
47	C15	C17	1.513(2)	**100**	C15	C17	1.513(2)
48	C16	H16A	0.980(2)	**101**	C16	H16A	0.980(2)

49	C16	H16B	0.980(2)	**102**	C16	H16B	0.980(2)
50	C16	H16C	0.980(2)	**103**	C16	H16C	0.980(2)
51	C17	H17A	0.979(2)	**104**	C17	H17A	0.979(2)
52	C17	H17B	0.979(2)	**105**	C17	H17B	0.979(2)
53	C17	H17C	0.979(2)	**106**	C17	H17C	0.979(2)

Crystal packing structure including hydrogen bonding interactions



Crystal packing parameters

Space Group	Cell Lengths	Cell Angles	Cell Volume	Z, Z'	R-Factor (%)
P -1	a 6.7135 Å (16)	α 85.939° (7)	984.601 Å^3	Z: 1	3.43
	b 9.424 Å (3)	β 79.285° (7)		Z': 0	
	c 15.995 Å (4)	γ 82.456° (8)			