Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

THE SYNTHESIS AND BIOLOGICAL EVALUATION OF A NOVEL CLASS OF BUTYRYLCHOLINESTERASE INHIBITORS USING ISOSORBIDE AS A BUILDING BLOCK

Gerald Dillon M.Sc.

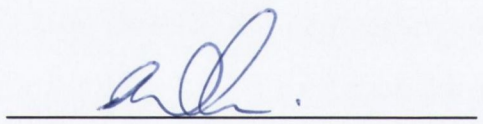
A thesis presented to the University of Dublin
for the degree of Doctor of Philosophy

Based on research carried out under the supervision of
John Gilmer
B.A. (Mod.), Ph.D
at the
School of Pharmacy and Pharmaceutical Sciences
Trinity College Dublin

2007

This thesis has not been presented as an exercise
for a degree at any other university.
The work described, except where duly acknowledged,
was carried out by me entirely.

I agree that the library may lend or copy this thesis upon request.



ABSTRACT

The aim of this project was to synthesise and optimise a novel class of cholinesterase inhibitors using the simple sugar, isosorbide, as a building block and to assess the inhibitory activity of these compounds *in vitro*. Cholinesterases are enzymes that can hydrolyse choline esters into their respective acid and choline portions. There are two types of cholinesterases, namely, acetylcholinesterase (AChE) [E.C. 3.1.1.7] and butyrylcholinesterase (BuChE) [E.C. 3.1.1.8]. While it is known that AChE has a specific biological role in the body, the biological function of BuChE is, as yet, unclear. In the last number of years, several lines of medical research have independently reported upon a link associating BuChE with the onset of Alzheimer's disease (AD). Inhibitors of this enzyme are therefore highly desirable for the elucidation of the biological role of BuChE but also, as potential therapeutics for the treatment of AD.

The design of huBuChE (human butyrylcholinesterase) inhibitors was based on the discovery that 2-ester derivatives of isosorbide are hydrolysed by BuChE with exceptional rapidity, demonstrating a high level of affinity and molecular recognition of these compounds for BuChE. It was therefore proposed that highly potent and selective inhibitors of BuChE could be synthesised by replacing the vulnerable 2-ester group with a carbamate group, which is understood to be capable of interacting with BuChE and causing the inhibition of the enzyme. Two initial families of compounds were synthesised, referred to as group 1 and group 2 compounds, which possessed either a carbamate or 'reversed carbamate' group at position-2 of isosorbide.

Chapter 2 focuses on the development of group 2 'reversed carbamate' compounds. This chapter describes the synthesis and evaluation of 17 test compounds, which were completely novel in structure and which transpired, in some cases, to be moderate inhibitors of huBuChE, with the lead compound **106** having an IC_{50} of 1.5 μM for huBuChE. Chapter 3 focuses on the synthesis and evaluation of group 1 carbamates, with particular emphasis in optimising the type of substitution at position-5 of isosorbide, as it was found that a benzyl carbamate was the optimum group for inhibition in position-2. The work in this chapter produced a total of 29 test compounds, which includes a range of highly potent and selective inhibitors of huBuChE with the lead compounds **121** and **124** having IC_{50} values for huBuChE of 5.77 and 12.33 nM respectively.

Chapter 4 describes the synthesis of substituted aryl-ester analogues of isosorbide at position-5 with retention of the benzyl carbamate group at position-2. This work was prompted by the discovery that the 2-aspirinate 5-salicylate ester of isosorbide **161** is the most successful aspirin prodrug discovered and therefore it was hoped that compound **162**, bearing a benzyl carbamate at position-2 with a salicylate group at position-5, would emerge as a highly potent inhibitor of huBuChE. All compounds described in this chapter offer high levels of inhibition of huBuChE, however, it was the development of **162** which represents the landmark achievement in this thesis, in the pursuit of potent inhibitors of huBuChE. This compound is 24-fold more potent than the previous lead isosorbide-based inhibitor **110** and 17-fold more potent than bambuterol, which hitherto was the most potent huBuChE inhibitor reported in the scientific literature. With an IC_{50} of 0.18 nM and a selectivity of approximately 60,000 for huBuChE over AChE, this inhibitor is the most potent and selective inhibitor of huBuChE ever reported. Finally, Chapter 5 examined the role of stereochemistry of isosorbide-based inhibitors and describes the synthesis and analysis of the stereoisomers of compound **110**.

Work is on going in this research group with compound **162** and other potent isosorbide-based inhibitors to assess the efficiency and applicability of these compounds *in vivo*.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Dr. John Gilmer for giving me the opportunity to undertake this project, for his continuous guidance, support and advice throughout, and, most of all, for his trusted and invaluable friendship over the last four to five years.

I would like to thank fellow researchers Dr. Joanne Gaynor and Dr. Sean Reidy, from the Athlone Institute of Technology, for your assistance and co-operation and whose hard work became integral to the development of my side of the project.

I would especially like to thank my parents, Margaret and Oliver and my sisters, Therese and Fiona, for everything you have given me, not just for the last four years, but for the last 30! I can safely say that I could not have done this without your constant love, support, advice and encouragement, not to mention a few quid thrown in as well!

I would like to thank Dr. John O Brien for his incredible enthusiasm and zest in providing excellent and efficient NMR data. I would like to thank Dr. Martin Feeney, of the Department of Chemistry, for performing HRMS analyses and Ann Connolly, of the Microanalytical Lab in UCD, for providing elemental analyses results. I would also like to express my gratitude to Ciaran Carolan for generating some splendid molecular modelling pictures and for proof reading with extraordinary attention to detail!

I would like to thank the technical staff in the teaching lab, including Ray, Rhona, Ann and Irene for all their assistance and support and I would also like to extend a special word of thanks to all the postgrads I have worked with over the last few years including lab partners; Miriam, Juan, Ana, Patrick, Cormac, Gabor and Denise, chemistry heads; Mary O, Stephen and Irene and, of course, Kennedy's stalwarts; Fiona, Jason, Niall and Tom. Last, but by no means least, I would like to thank all my many friends from the Town and also the gang from Coleraine Uni, who were always there to support me emotionally and to distract me when necessary during the writing up process!

TABLE OF CONTENTS

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

TABLE OF FIGURES

TABLE OF TABLES

ABBREVIATIONS

AChE	Acetylcholinesterase
Ac_2O	Acetic anhydride
AcCN	Acetonitrile
ACh	Acetylcholine
AChEI	Acetylcholinesterase inhibitors
AD	Alzheimer's disease
AIBN	Azobisisobutyronitrile
Ala	Alanine
APP	Amyloid precursor protein
Asp	Aspartic acid
ATCI	Acetylthiocholine Iodide
BCh	Butyrylcholine
BOC	*tert*-Butyl carbonyl
BOC_2O	Di-*tert*-butyl dicarbonate
BTCI	Butyrylthiocholine Iodide
BuChE	Butyrylcholinesterase
BuChEI	Butyrylcholinesterase inhibitor
Bz_2O	Dibenzyl ether
BzBr	Benzyl bromide
BzCl	Benzyl chloride
CNS	Central nervous system
COSY	Correlation spectroscopy
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DEAD	Diethyl diazenedicarboxylate
DEPT 135	Distortionless enhancement by polarization transfer
DIBAL	Diisobutylaluminium hydride
DMA	Dimethylacetamide

DMAP	N, N-Dimethyl 4-aminopyridine
DMF	N,N-dimethylformamide
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	Dimethyl sulfoxide
DTNB	5-5'-Dithiobis(2-nitrobenzoic) acid
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimidehydrochloride
Et_2N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
Glu	Glutamic acid
Gly	Glycine
GTN	Glyceryl trinitrate
His	Histidine
HIV	Human immunodeficiency virus
HOBt	Hydroxybenzotriazole
HRMS	High resolution mass spectroscopy
huBuChE	Human butyrylcholinesterase
IIDN	Isoiodide dinitrate
IMDN	Isomannide dinitrate
IR	Infrared spectroscopy
IS-5-MN	Isosorbide-5-mononitrate
ISDN	Isosorbide dinitrate
***iso*-OMPA**	Tetra(monoisopropyl) pyrophosphoramide
K_M	Measure of enzyme affinity
Leu	Leucine
LiHMDS	Lithium bis(trimethylsilyl)amide
M.pt.	Melting point
***m*CPBA**	*meta*-Chloroperoxybenzoic acid
MeOH	Methanol
Mol. wt.	Molecular weight
MS	Mass spectroscopy

NaOAc	Sodium acetate
NFT	Neurofibrillary tangles
NIS	N-iodosuccinimide
NMR	Nuclear magnetic spectroscopy
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
Pd/C	Palladium on activated carbon
Phe	Phenylalanine
[S]	Substrate concentration
SAR	Structure activity relationship
Ser	Serine
TBAF	Tetra-*n*-butylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TBDMSCl	*tert*-Butyldimethylsilyl chloride
t-Bu	tert-butyl
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TMSCl	Tetramethylsilyl chloride
Trp	Tryptophan
Ts	Tosyl group
TsCl	*para*-Toluenesulfonyl chloride
Tyr	Tyrosine
UV	Ultraviolet Spectroscopy
Val	Valine

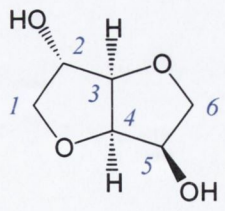
CHAPTER 1

AN INTRODUCTION TO CHOLINESTERASES AND THE RATIONALE FOR THE DESIGN OF INHIBITORS OF HUMAN BUTYRYLCHOLINESTERASE (huBuChE) USING ISOSORBIDE AS A BUILDING BLOCK

1.1. INTRODUCTION

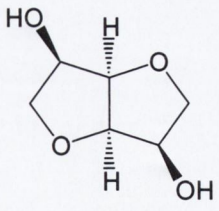
The aim of this project was to synthesise and optimise inhibitor compounds of the enzyme butyrylcholinesterase (BuChE, E.C. 3.1.1.8). This chapter gives an introduction to the characteristics of BuChE and its suspected biological roles in mammals. It also provides an insight into the significance of existing BuChE inhibitors (BuChEIs) and the rationale behind the further development of more potent and selective inhibitors of this enzyme. This includes background information on the basis of the design and synthesis of BuChEIs using the simple sugar isosorbide as a building block.

The introduction includes a comprehensive review of the chemistry and synthetic applications of a family of sugars called 1,4:3,6 dianhydrohexitols, which includes isosorbide **1**, isomannide **2** and isoiodide **3**. Whilst numerous publications exist involving chemical synthetic manipulations of 1,4:3,6 dianhydrohexitols, there has, up until now, been no concise compilation of the chemistry of these compounds. This review was undertaken firstly, to facilitate the efficient chemical synthesis of isosorbide derivatives for this project by drawing on the available chemical synthetic information of this family of compounds, but also to highlight the intrinsic novelty of the application of 1,4:3,6 dianhydrohexitols in this project and specifically to emphasise the novelty of the chemical structures of the compounds generated in the course of the project.



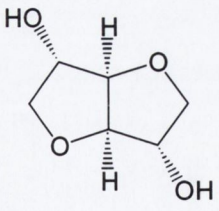
(1)

Isosorbide



(2)

Isomannide



(3)

Isoiodide

Figure 1.1: The chemical structures of 1,4:3,6 dianhydrohexitols

1.2. CHOLINESTERASES

Esterases belong to a class of enzymes called hydrolases, which are found in nearly all mammalian tissue types including blood, brain, lungs, liver and intestines. They are capable of hydrolysing a variety of substrates including esters, halides, amines and peptides[1]. Esterases may have substrates, which are either endogenous or exogenous[2]. The biological role of esterases may be highly specific with respect to a given substrate[2] or may be less specific, where they are capable of hydrolysing a range of substrates, both endogenous and exogenous[3]. Esterases play an important role in the human body in the metabolism of certain drugs such as ester prodrugs[4] and anaesthetics[5].

Cholinesterases are a category of esterases that can hydrolyse choline esters into the respective acid and choline[6]. There are two types of cholinesterases present in a wide variety of tissues in many species, including humans, namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)[7]. The existence of an enzyme capable of hydrolysing the neurotransmitter, acetylcholine (ACh) was first postulated by Dale[8] in 1914 and was demonstrated by Loewi and Navratil in 1926[9]. At this time it was believed that there was only one cholinesterase present in mammalian tissue[10]. However, the existence of a second cholinesterase was then evidenced by Steadman in 1932[11]. It was demonstrated that one of the enzymes exhibited specificity towards ACh and therefore was given the name acetylcholinesterase[12]. The other enzyme catalysed the hydrolysis of a wide variety of choline and non-choline esters and was given the namely pseudocholinesterase[13]. Later this became more commonly known as butyrylcholinesterase, due to the high specificity demonstrated in hydrolysing the synthetic substrate butyrylcholine (BCh)[14].

AChE is found in the CNS, red blood cells and striate muscles[15]. In the case of the CNS, most research on brain cholinesterases has been devoted to AChE, particularly in relation to its primary function in cholinergic neurotransmission[16]. It is now widely understood that ACh is an endogenous substrate for AChE and the capacity of AChE to hydrolyse ACh is an important physiological function of this enzyme in the termination of the

effects of this neurotransmitter at the cholinergic synapse[6, 7, 17]. AChE is the prime cholinesterase in the mammalian brain where it is present in excess concentration[18, 19]. In rat brain, approximately 80 % of cholinesterase activity is due to AChE with 20 % a result of BuChE activity[20]. Total AChE activity varies extensively in human brain, with the highest concentration in the telencephalic subcortical structures. There is intermediate concentration in the substantia nigra, cerebellum and spinal cord and low concentration in the fornix and cortical regions, including the hippocampus and temporal and parietal cortex[7].

BuChE is found in nearly all tissue types including the circulatory system, the liver, lungs and CNS[2]. It is an intriguing enzyme as its biological function is unknown and it appears to have no endogenous substrate[21]. However, it is known to hydrolyse drugs including heroin[22], cocaine[23] and aspirin[24] and it is important in the metabolism of anaesthetics such as amethocaine[25] and succinylcholine[26] suggesting that the main role of BuChE in the body is to act as a primary scavenger for xenobiotic esters[27] and to detoxify foreign compounds[28, 29]. The highest levels of BuChE are found in the liver and lungs, which are the main detoxification sites in humans[30].

People with variant forms of BuChE are unable to clear foreign esters as readily as people with common BuChE[31]. This can be problematic when anaesthetics like succinylcholine are used to treat patients during surgery. People with variant forms of BuChE who cannot metabolise succinylcholine experience prolonged severe apnoea and muscle paralysis and therefore patients must be screened for variant BuChE prior to surgery to avoid any potential adverse side effects[32, 33].

Over 40 variants of BuChE have been identified and generally these different enzymes are associated with different levels of catalytic activity[34, 35]. Some common variants are the K variant'[36] which has 33 % less activity than normal, the 'J variant'[37] which has 66 % lower activity than normal and the 'H variant'[38, 39] which exhibits approximately 90 % lower activity than normal. Some variants are called 'silent' variants[40], as they seem to display no esterases activity at all. BuChE is synthesised in the liver and secreted into the

plasma where it is present in high concentrations[9]. In hepatic disorders, BuChE is depressed and therefore measurement of BuChE levels can give an indication of diseases such as hepatitis, malaria and liver carcinomas[41]. In the brain, BuChE is present at lower levels than AChE but its distribution throughout the brain is more evenly spread[7].

Traditionally, most research has been on AChE and its relationship with ACh. In this regard, BuChE has been seen by many as a non-essential enzyme that interferes with studies on AChE[10]. This belief was substantiated by the high levels of the enzyme outside the CNS and also by the fact, that many seemingly healthy people possess an inactive, sometimes called 'silent', variant of the enzyme or indeed possess no BuChE at all without demonstrating any degree of ill health or any deleterious effects[42, 43].

Conversely, other researchers have expressed the view that if BuChE is an enzyme of no significance and plays no role in the body why would it be retained in the human body or any other evolved species[10, 44]. In this context it is important to point out that the term 'silent' in relation to the activity of the enzyme only refers to its ability to hydrolyse cholinesters[21]. It is possible therefore that the ability of BuChE to hydrolyse cholinesters could be co-incidental and that an as yet unidentified endogenous substrate may be structurally unrelated to cholinesters[45]. Also, the fact that AChE and BuChE are products of two different chromosomes, namely chromosomes 3 and 7 respectively, suggests that they may be generated for separate roles. [46]

1.3. STRUCTURE OF BuChE

BuChE and AChE belong to a large family of proteins that have a common α/β hydrolase fold. This family of proteins includes lipases, peptidases, dehalogenases and adhesion proteins[47]. The molecular structures of BuChE and AChE are very closely related and the alignment of their amino acid sequences is 54 % identical[47]. The structure of the active site within the enzymes is also very similar and prior to the elucidation of the crystal structure of BuChE, all structural studies of BuChE relied on homology models based on the crystal structure of AChE[46].

AChE was first crystallized in 1991 by Sussman, which represented a major breakthrough understanding the structure and chemical processes of cholinesterases[48]. The structure revealed some interesting and previously unknown structural features of the enzyme, primarily the fact that the catalytic active site, which comprises of three amino acid residues, was located at the bottom of a deep and narrow gorge, which was lined by aromatic amino acid residues[48]. The depth of the gorge is approximately 20 Å. Previous to this it was thought that the active site of cholinesterases was at the surface of the enzyme due to the highly efficient cycle of these enzymes[48]. Also, the 3D structure showed that the carboxylic acid residue of the catalytic triad was a glutamate instead of an aspartate residue, as is the case in other serine proteases[48].

The crystal structure of BuChE was successfully generated by Nicolet in 2003[49]. Several attempts had been made to crystallise BuChE, however, due to the heavy glycosylation of the enzyme, this process proved difficult. The crystallisation of BuChE was finally successfully achieved using a recombinant enzyme, which was lacking 4 out of the 10 glycosylation sites[50].

Figure 1.2 shows the structure of BuChE with the entrance to the active site gorge (highlighted with yellow arrow). The active site gorge of BuChE and AChE differ by the type of amino acid residues that line the gorge. There are 14 aromatic residues lining the gorge in AChE[51]. In BuChE, six of these aromatic residues are substituted by smaller aliphatic residues, thus giving a wider active site gorge in the enzyme[51].

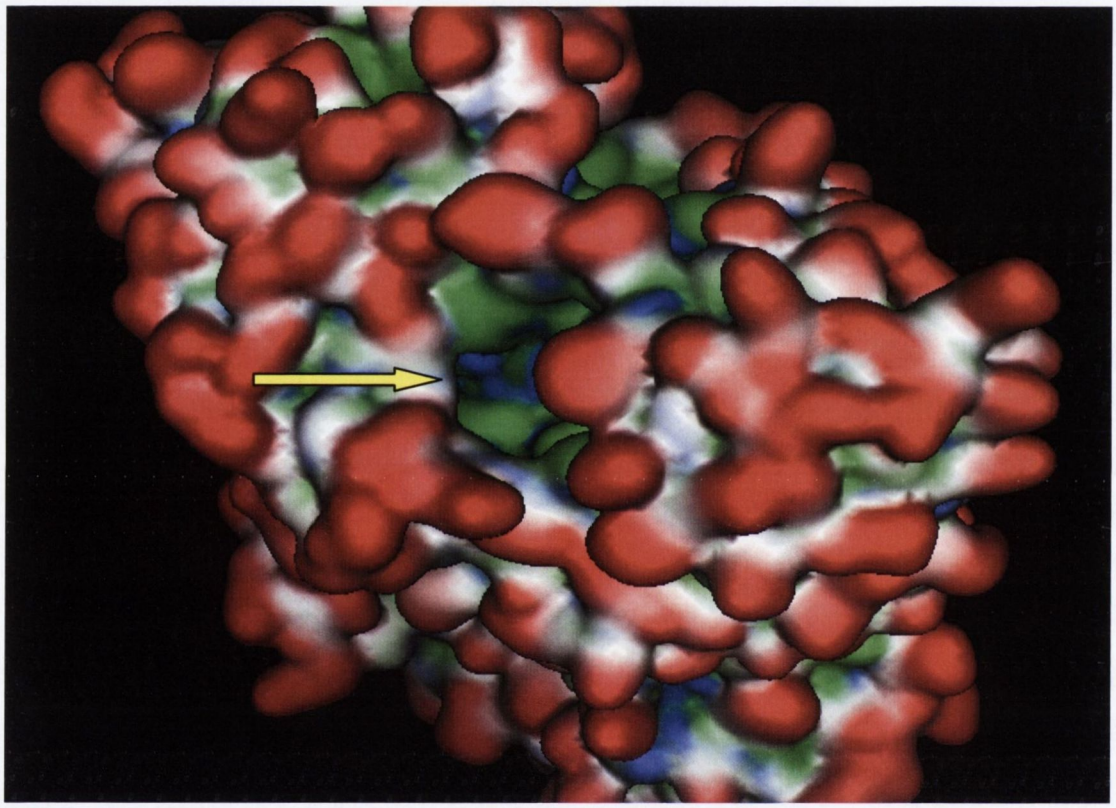


Figure 1.2: The structure of BuChE

Within the active site gorge of BuChE four sub-sites have been identified[50]:

- The acylation site (or active site), Ser 198, His 438 and Glu 325 with the oxyanion hole, Ala 199 Gly 116 and Gly117
- The acyl binding pocket, Leu 286 and Val 288
- The anionic site, Trp 82
- The peripheral site, Asp 70 and Tyr 332

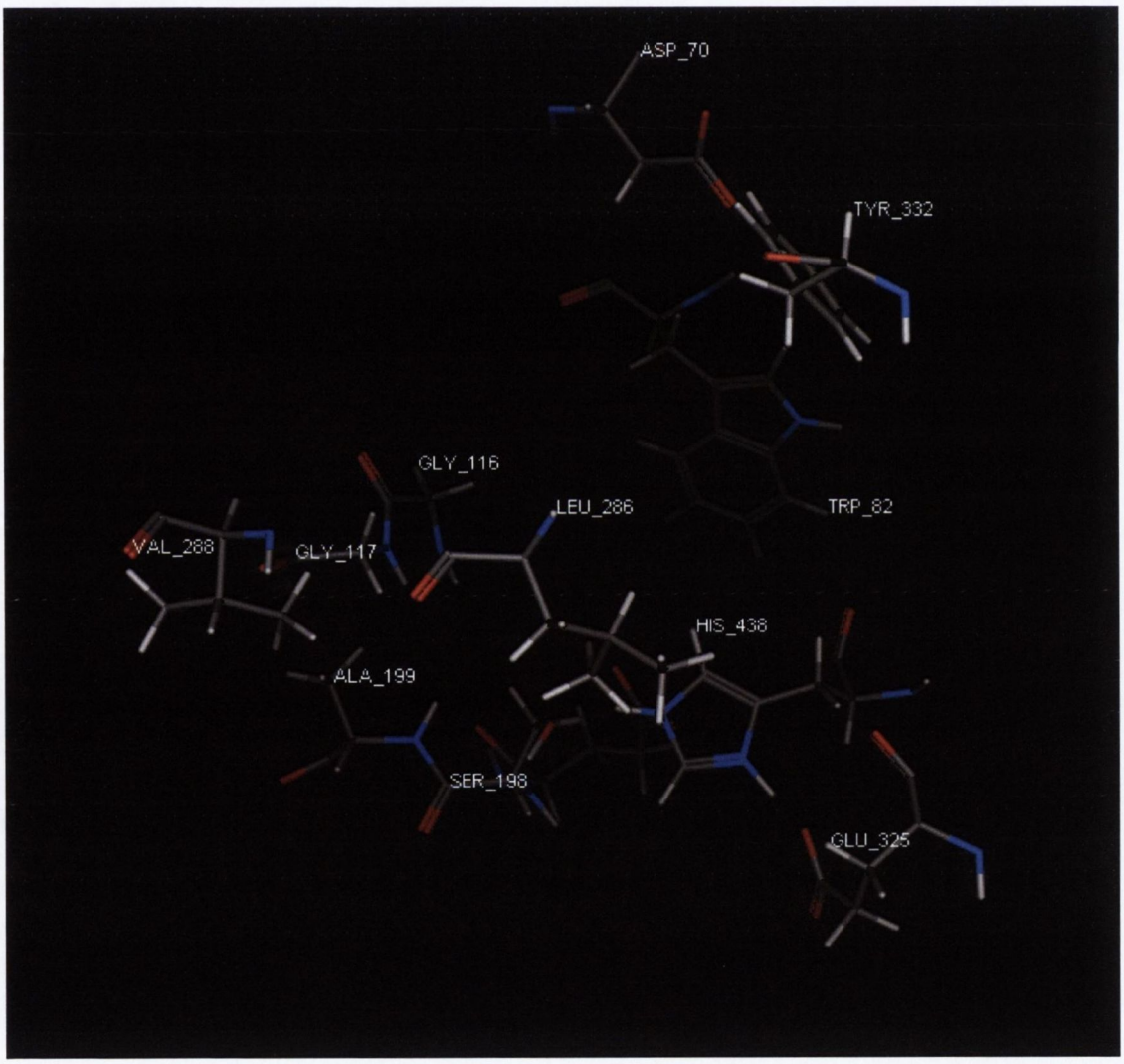


Figure 1.3: The active site gorge of BuChE

The acylation site is located at the very bottom of the active site gorge and it is here where the chemistry of ester hydrolysis takes place[50]. The acylation site consists of the catalytic triad of amino acids, serine 198, histidine 438 and glutamic acid 325. The hydrolysis of esters is demonstrated in Figure 1.4 by the reaction of butyrylcholine with the catalytic active site[47]. The interaction of the cholinester with serine 198 gives an intermediate acyl-enzyme product and releases the choline portion of the molecule. Nucleophilic attack by water on the acyl-enzyme intermediate evolves to give the free enzyme and the carboxylic acid product. Adjacent to the catalytic triad is what is known as the oxyanion hole[50]. This consists of alanine 199 and two glycine amino residues, 116 and 117. These residues contribute to the stabilization of the transition state of the substrates being hydrolysed by hydrogen bonding to the carbonyl oxygen of the substrate.

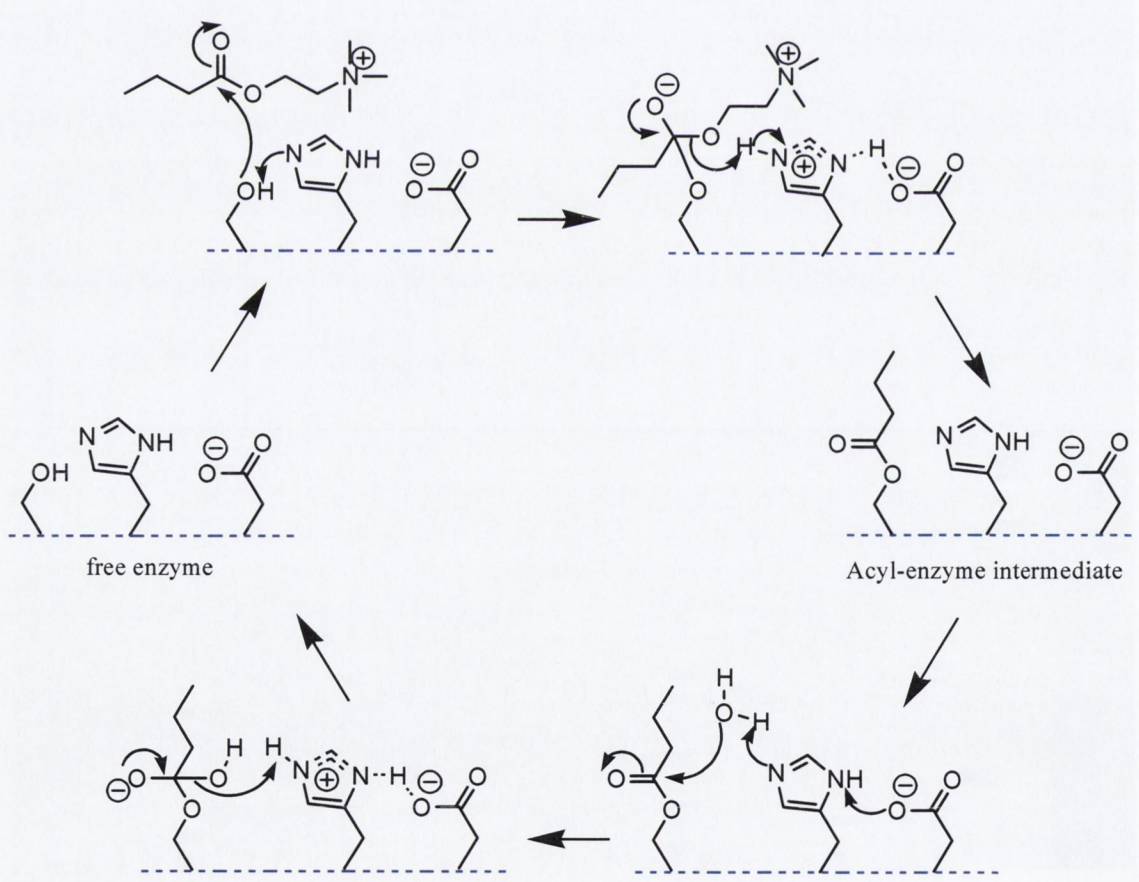


Figure 1.4: The reaction of butyrylcholine with the catalytic active site of BuChE

The second sub-site is called the acyl-binding pocket where the acyl group of ester substrates are held in place during catalysis[52]. In BuChE, two aliphatic residues line this pocket, namely leucine 286 and valine 288. These two amino acids are not large compared to the relatively bulkier phenylalanine amino acids, which line the pocket in AChE, and therefore BuChE can accommodate larger acyl groups and hence larger substrates than AChE[53].

The third site is the so-called anionic site, which consists of the amino acid, tryptophan 82[52]. The residue is capable of interacting with the quaternary nitrogen head of cholinesters. Finally, the peripheral site consists of the amino acid residues aspartic acid 70 and tyrosine 332 which are located at the lip of the active site gorge[54]. It is believed that these residues can interact with positively charged residues such as butyrylcholine, which are subsequently guided down the gorge towards the catalytic triad of the active site.

Both BuChE and AChE exist in a number of different molecular forms, which can either be globular or asymmetric[14, 55]. Globular forms can either be monomers or oligomers. The globular monomeric form of BuChE is called the G1 form. The G2 form is a dimeric form consisting of two globular monomers joined by a disulfide bridge and two dimeric forms can be held together to give the oligomeric G4 form. The asymmetric forms of BuChE exist as tetramers, can be attached to membranes by a protein or collagen tail-like anchors.

1.4. BuChE IN RESEARCH

1.4.1. BuChE AND THE HYDROLYSIS OF ACh

Whilst a specific biological role has yet to be assigned to BuChE, a number of different avenues of research demonstrate the potential of BuChE to function in a biological role in a range of alternative settings.

One particular role mooted for BuChE is that it may act as a co-regulator of ACh in the body and therefore ACh may actually be a natural substrate for the enzyme[16, 17, 56]. It is known that BuChE can actually hydrolyse ACh in vitro, but much less efficiently than AChE and therefore its role in the brain is uncertain[57]. However, it has been demonstrated that inhibition of BuChE in rats leads to dose dependent increases in levels of ACh in the brain[58, 59]. This was achieved by introducing the highly selective BuChE inhibitor, MF-8622 **4**, intracortically into rat brain and measuring ACh using a sensitive microdialysis method. It was demonstrated that ACh levels increased 15-fold and reached a level approaching inhibitory concentrations for AChE. ACh inhibition of AChE occurs at high concentrations, when excess ACh binds to the peripheral site of AChE causing a sequence of conformational changes in the active site gorge which blocks the entrance/exit of ACh to the catalytical active site[60]. It is possible that there are two functional pools of cholinesterases in the brain, one neuronal and AChE[61] dependent and one glial and mainly BuChE dependent acting in the event of decreased levels of AChE in the brain[62]. In this case, BuChE could operate in a compensatory role where it could hydrolyse ACh in the absence of AChE.

The possibility of BuChE acting in a compensatory role was further demonstrated when AChE deficient mice were specifically bred to examine their physiology in the absence of AChE[16]. These AChE deficient mice or 'knockout' mice exhibit several physiological abnormalities such as weak muscles, sexual dysfunction and pinpoint pupils in the eye[63]. However, the fact that the 'knockout' mice survived to adulthood was itself surprising and demonstrated that AChE is not indispensable in cholinergic transmission. It has

therefore been hypothesized that BuChE hydrolyses ACh after it diffuses out of the synapses[64]. If BuChE does compensate for AChE it can be said that it is an inadequate substitute for AChE, which can be attributed to its low abundance in the brain, the slower rate of catalysis and the fact that BuChE is not located sufficiently at the specific location in the brain for ACh transmission. Nonetheless, it has also been shown that BuChE inhibition in 'knockout' mice proves to be lethal strongly indicating its biological importance in this context[65].

1.4.2. BuChE AND ALZHEIMER'S DISEASE (AD)

In the last 25 years several independent researchers have reported on a suspected link between cholinesterases and Alzheimer's disease (AD). AD, or senile dementia as its also known, is a neurodegenerative disease and is associated with the loss of cognition and functional ability and changes in behavioural characteristics, eventually leading to death[66, 67]. AD is characterised biologically by the depletion of cholinergic neurons, which would normally exhibit high levels of AChE and ACh[68]. Curiously, there is a remarkable increase in the expression of BuChE in the brain[69]. Several figures have been published on the decrease of AChE activity in the brain with figures of between 45 %[70] and 70 %[71] being reported, going as low as 90 % in some severe cases of AD. Increases of between 120[72] – 165 %[71] have been reported for BuChE. More importantly however is that very high levels of both AChE and BuChE are found in amyloid plaques and neurofibrillary tangles (NFTs), which are the neuropathological hallmarks of AD[73].

Amyloid plaques are complex protein structures, which are formed and deposited in the aged brain[74]. They originate from a larger amyloid precursor protein (APP), which is approximately 700 amino acids long. APP breaks down to give smaller amyloid proteins consisting of approximately 40 amino acids, which accumulate to form plaques. They can be present in demented and non-demented individuals. However, plaques in non-demented individuals tend to be diffuse, while a more compact form of plaque tends to be present in AD patients[75]. The plaques are believed to be responsible for neurodegeneration as they cause cell damage and organ dysfunction leading to death.

NFTs are also found in the brains of victims of AD and are also in patients with other neurological disorders[76]. NFTs are made from collapsed microtubules, which are small structures in brain cells associated with transporting substances in and out of healthy individuals[77].

The cholinesterases found in amyloid plaques and NFTs exhibit enzymatic properties different to those cholinesterases found in neurons, such as requiring a different pH for enzyme expression in histochemical procedures[70, 77] but mainly in their response to cholinesterase inhibitors, where they seem to be more resistant to conventional cholinesterase inhibitors but more sensitive to indolamine inhibitors[78]. These enzymatic properties are more similar to cholinesterases in glial cells indicating that glial cholinesterases are the source for the over expression of cholinesterases in neuritic plaques and tangles[73]. This has been substantiated by reports that the amount of BuChE found in plaques and tangles greatly exceeds the small quantity of neuronal BuChE in the normal brain[73].

In addition, BuChE activity is found primarily in compact or maturing plaques as opposed to the more diffuse type of plaque suggesting that BuChE might be responsible in part in the maturing process from the diffuse to the compact neurodegenerative form of the plaque[79]. Furthermore, it has been demonstrated that the addition of BuChE to amyloid plaques increases the neurotoxicity of the plaque *in vitro*[75].

It has also been reported that the predominant molecular form of BuChE in amyloid plaques is the G1 form[80]. The G1 form of BuChE is found in embryonic development and the G4 form is the primary form found in the mature brain[81]. Therefore, it has been postulated that reversion to the G1 form may be culpable in the neurodegenerative process. Several reports have also shown a link between AD and variant strains of BuChE, where people carrying the 'K variant' form of BuChE are at a greater risk of developing AD[82, 83, 84]. However, research in this area has proved inconclusive with some researchers seeming to confirm these reports whilst others have refuted the findings[85, 86].

Other non-esterase functional roles have also been suggested for BuChE. These include the ability of BuChE to exhibit peptidase activity[87] and protein-protein interactions[88]. The ability of BuChE to interact with other protein may help to explain its association in a number of biological processes such as the development of the nervous system and neurodegeneration.

Transient high levels of BuChE have been exhibited in the embryonic nervous system and cell growth in postnatal brain of several species suggesting a role for the enzyme in the development of the nervous system by its ability to regulate other proteins[89, 90, 91]. This claim is supported by the fact that cholinesterases share similar homology with adhesion proteins and that BuChE[92] has been shown to interact and cause the enhancement of the enzymatic activity of the protease, trypsin[88]. In addition, abnormal cholinesterase expression has been observed in several types of tumour cells including myeloma[93], brain tumours[94], and ovarian tumours[95] and in the sera of patients with different types of primary carcinomas[96]. It is possible that if BuChE is responsible for cell growth in early development, the amplified levels of cholinesterases may influence the ability of tumour cells to proliferate more rapidly[97]. The assertion that BuChE exhibits peptidase activity is also very significant with respect to its potential role in the onset of AD. Among other proteins, it has been shown that BuChE can cleave the APP *in vitro*[98], emphasising the link between BuChE and the formation of amyloid plaques. Furthermore, it has been reported that trypsin and trypsin-like serine proteases might be involved in amyloid precursor processing[99]. These findings go hand in hand with the previous reports of the effects of BuChE on APP and BuChE on trypsin activity.

1.5. CHOLINESTERASE INHIBITORS.

From the wealth of research and evidence linking cholinesterases with AD, it is clear that inhibitors of cholinesterases are highly desirable for use as potential therapeutic agents. In the case of BuChE, potent and selective inhibitors of this enzyme could also be useful in the elucidation of its biological function.

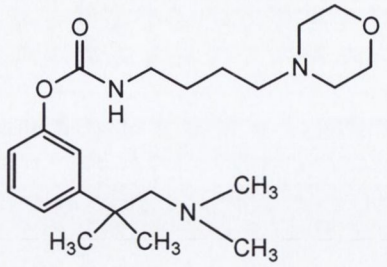
In a therapeutic sense, cholinesterase inhibitors can be used to inhibit cholinesterases in the brain thus maintaining or increasing the levels of ACh for neurotransmission and therefore improving the cognitive processes such as memory and attention[100]. As AChE is the primary hydrolysing enzyme of ACh, most of the work to date has related to the development of AChE inhibitors. Advances in AChE development are such that several AChE inhibitors are currently used as therapeutics in the treatment of AD[57]. These drugs have been found to moderately improve cognitive functions[101]. AChE inhibitors include tacrine **5**, BW284c51 **6** and the naturally occurring physostigmine (eserine) **7**. This is the primary and best-known cholinesterase inhibitor. It is isolated from the Calabar bean, a dried ripe seed of the *Physostigma venenosum* vine grown in West Africa. Other alkaloids such as geneserine and physosvenine are also cholinesterases inhibitors isolated from the same plant. As physostigmine is a naturally occurring substance not designed for therapeutic use it suffers from a number of shortcomings when used as a therapeutic agent in the treatment of AD such as having low bioavailability and causing a large number of undesirable side effects like vomiting and nausea[102]. Tacrine is also no longer prescribed due to its unacceptable adverse side effects. The main AChE inhibitors in use today are the second-generation inhibitors donepezil **8** and rivastigmine **9**[103].

In recent times however, much interest has been generated in developing inhibitors of BuChE. This has occurred for a number of reasons; firstly, recent studies have shown that BuChE is present to a larger degree in neurons in the brain than previously thought with 10 –15 % of cholinergic neurons in the hippocampus and amygdala in the basal forebrain possessing BuChE, indicating that BuChE is involved in the hydrolysis of the neurotransmitter ACh[104]. The results of the experiments on the knockout mice and the

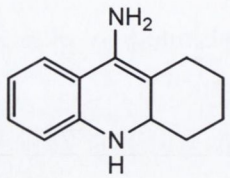
experiments on the inhibition of BuChE in the brain increasing ACh levels substantiate a more significant role of BuChE in the brain. BuChE is also inhibited by physostigmine and can be inhibited by other carbamates and organophosphates[105], however, selective inhibitors of BuChE are not as common as selective inhibitors of AChE. Until recently, *iso*-OMPA **10**, which is only modestly selective of BuChE over AChE, demonstrating 35-fold selectivity towards the enzyme[106], was the prototypical selective inhibitor of BuChE. However due to the highly toxic nature of this inhibitor[107], it cannot be used in a therapeutic setting.

The absence of an endogenous substrate for BuChE makes the design of inhibitors of BuChE more difficult than for AChE and generally the synthesis of selective inhibitors has involved building structural features into established AChE inhibitors that could distinguish between the two enzymes[108, 109]. This approach has been met with moderate success, although two inhibitors namely phenethylcymserine **11** and MF-8622 **4** prepared this way have demonstrated selectivity of approximately 5,000 and 10,000 for BuChE over AChE respectively[110].

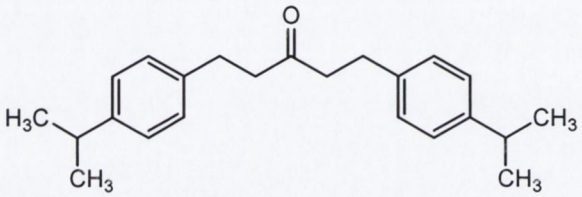
As well as the role of BuChE inhibitors in stabilising levels of ACh in the brain, they may also reduce levels of APP, the source of amyloid plaques and thus stem the onset of AD. The use of phenethylcymserine has been shown to reduce the levels of APP in cell cultures and in *in vivo* studies[57]. This also highlights the potential of dual inhibitors of cholinesterases, which could increase ACh levels in the brain whilst discouraging the growth of amyloid plaques and NFB. Rivastigmine is a dual inhibitor that is used in the treatment of AD. It has been shown to improve cognition in AD patients over a 12-month period of use[57].



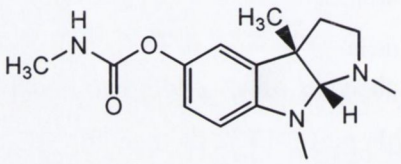
MF-8622 (**4**)



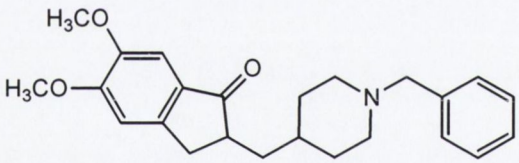
Tacrine (**5**)



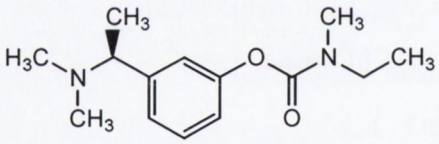
BW284c51 (**6**)



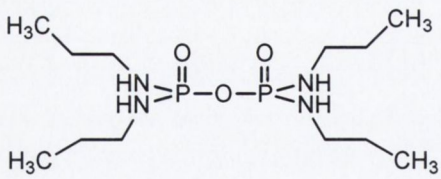
Physostigmine (**7**)



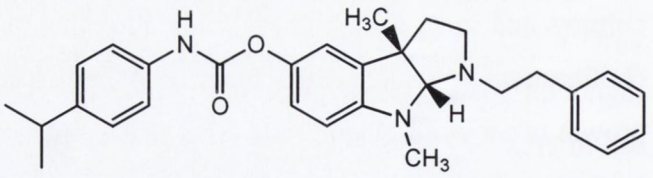
Donepezil (**8**)



Rivastigmine (**9**)



iso-OMPA (**10**)



Phenethylcymserine (**11**)

Figure 1.5: Established inhibitors of BuChE and AChE

1.6. THE BACKGROUND TO THE DESIGN OF BuChE INHIBITORS USING ISOSORBIDE AS A BUILDING BLOCK

In 2001, it was discovered by a research group at the School of Pharmacy in Trinity College Dublin that certain esters of the simple sugar isosorbide **1** are hydrolysed with exceptional rapidity by huBuChE in mammalian blood plasma and especially in human serum[4, 111]. Further investigation of this phenomenon revealed that the rate of hydrolysis increased significantly when a 2-benzoate isosorbide ester was also substituted at position-5 with a benzoate ester. The di-benzoate compound **12** undergoes hydrolysis in human serum at a rate approaching that of the prototypical substrate, BCh. Furthermore, the K_M value for the hydrolysis of esters of this type in human plasma is lower than that of BCh, demonstrating that these esters have a greater affinity for huBuChE than BCh itself.

This discovery presented an opportunistic breakthrough in designing inhibitors of huBuChE. Based on this discovery, it was proposed that it might be possible to construct the first non-choline based inhibitors for a cholinesterase by retaining the highly specific molecular recognition features of molecule **12,** while incorporating cholinesterase inhibitor functionality. This could be achieved by replacing the vulnerable ester group at position-2 with a carbamate function, which is known to exhibit cholinesterase inhibition.

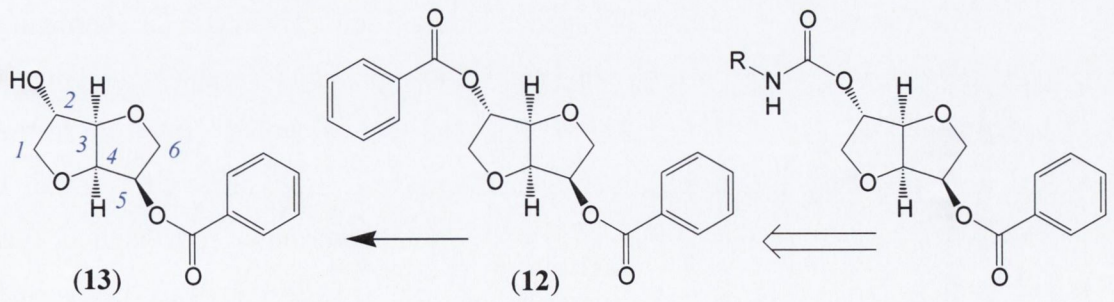


Figure 1.6

The proposed design of these inhibitors was based on entirely new templates, originating from a specific relationship between isosorbide and huBuChE and, unlike the design of other selective BuChE inhibitors, they were not based on existing AChE inhibitor templates. The initial design of the inhibitors of this type fell into two groups. These two groups represented the first generation of this type of inhibitor design. The structural design of group 1 and group 2 inhibitors are displayed in Figure 1.7.

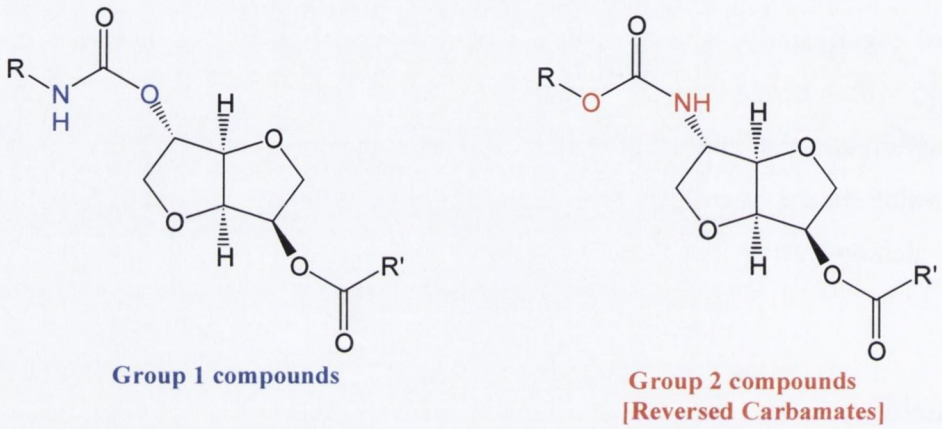


Figure 1.7

The design of group 1 compounds involved the direct replacement of the benzoate ester of compound **12** with a carbamate ester, as shown in Figure 1.6. Group 2 compounds were different to group 1 compounds, whereby the carbamate group in position-2 was 'reversed', putting the oxygen and nitrogen atoms of the carbamate in alternating positions, compared to their positions in group 1 compounds. Group 2 compounds became commonly known in this project as the 'reversed carbamates' referring to the chemical structure of the carbamate group, which was 'reversed' compared to group 1 compounds. The term 'reversed carbamates' will be used from now on throughout this thesis to describe group 2 compounds. The chemical synthesis and analysis of reversed carbamates compounds are described first in this thesis in Chapter 2. The initial investigative synthetic work of group 1 compounds was performed by our sister research group based at the Athlone Institute of Technology.

1.7. A COMPREHENSIVE REVIEW OF THE CHEMISTRY OF 1,4:3,6-DIANHYDROHEXITOLS

1.7.1. THE UNIQUE STRUCTURE AND STEREOCHEMISTRY OF 1,4:3,6 DIANHYDROHEXITOLS

1,4:3,6 dianhydrohexitols are a family of isomeric carbohydrates derived as by-products from the starch industry[112]. This family of compounds includes *isosorbide* **1**, also known as 1,4:3,6 dianhydro-D-gluticol, *isomannide* **2** (1,4:3,6 dianhydro-D-mannitol) and *isoiodide* **3** (1,4:3,6 dianhydro-L-iditol). The three isomers differ according to the positions of the two free hydroxyl groups located on the carbon atoms in position-2 and 5[113]. The 1,4:3,6 dianhydrohexitols have two cis-fused five member tetrahydrofuran rings and take the form of a V-shaped wedge at an angle of approximately 120°, with the hydroxyl groups situated at positions-2 and 5 assuming an orientation either inside or outside the V, as depicted in the 2D and 3D configurations in Figure 1.8[114].

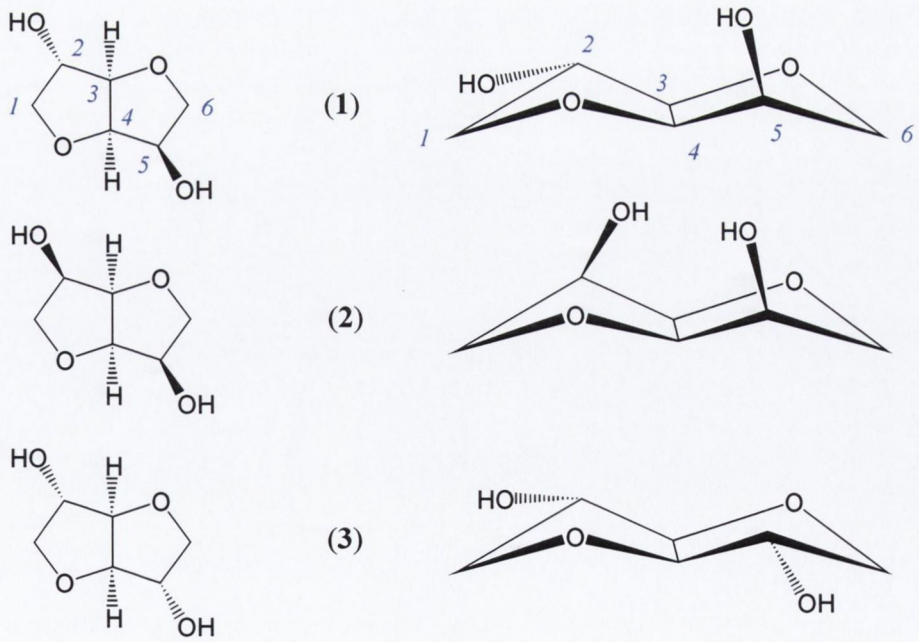
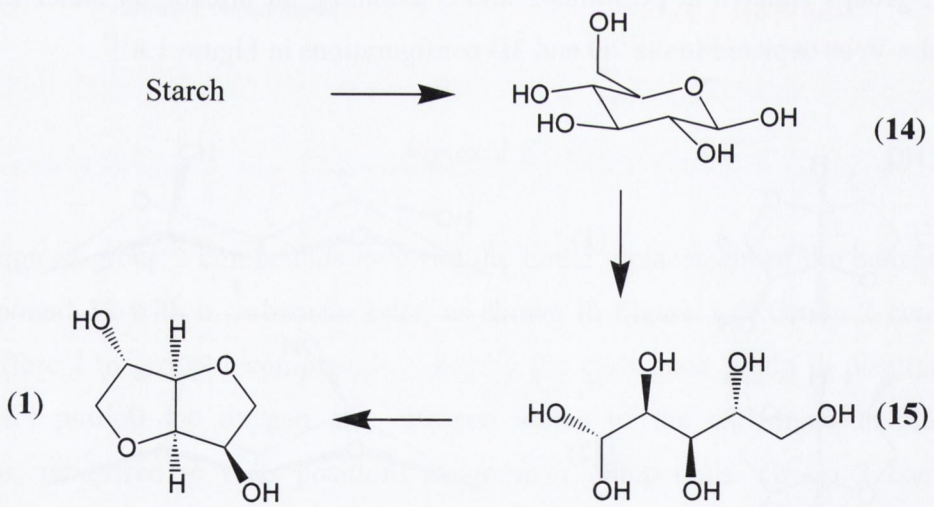


Figure 1.8: 3D-structures of 1,4:3,6 dianhydrohexitols

The configuration of a group inside the wedge may be designated as 'endo' whereas a group on the outside of the wedge is considered as 'exo'. Therefore, in isosorbide, the hydroxyl group at position-2 is exo and the hydroxyl group at position-5 is endo. With regards to isomannide, both hydroxyl groups are endo, whilst in isoiodide both hydroxyl groups take the exo position.

1.7.2. THE MANUFACTURE AND SYNTHESIS OF 1,4:3,6 DIANHYDROHEXITOLS

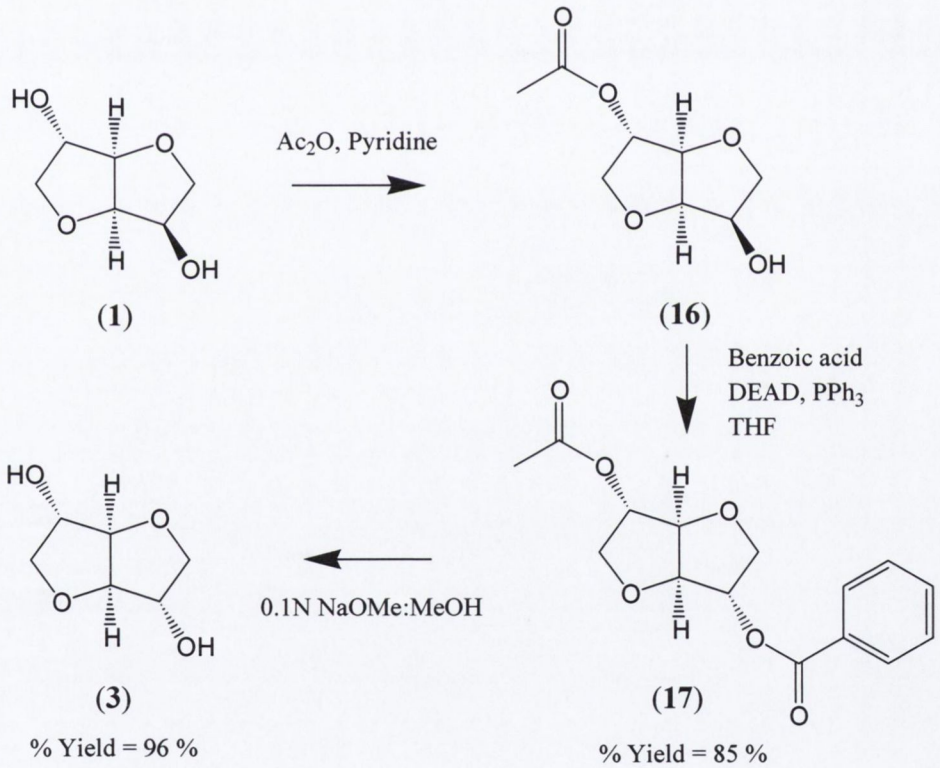
The bulk production of isosorbide begins with the enzymatic hydrolysis of starch to give the sugar D-glucose **14**. This is then converted via catalytic hydrogenation to give D-sorbitol **15**, which is also known as D-gluticol. Acid catalysed dehydration of D-sorbitol **15**, by the loss of two water molecules per molecule of D-sorbitol, yields the corresponding dianhydrate, i.e. isosorbide **1**[115].



Scheme 1.1

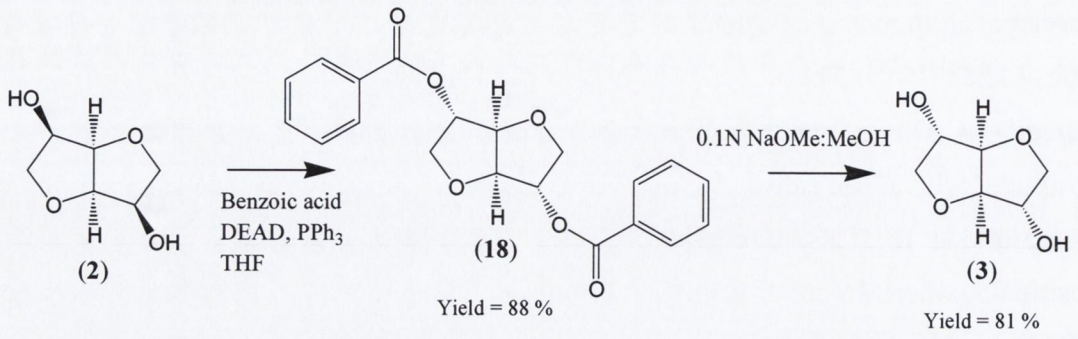
Isomannide and isoiodide are produced in the same manner from the sugars D-mannose and L-fructose respectively. However, in the case of isoiodide the relevant sugar L-fructose is quite rare and therefore isoiodide has been traditionally produced from D-glucose in a time consuming seven-step synthetic procedure. This has led to the

development of more convenient, economic and time efficient methods for the production of isoiodide[116, 117]. These methods involve the synthetic conversion of isosorbide **1** to isoiodide **3**. One method involves the selective acetylation of the hydroxyl group in position-2 with acetic anhydride in pyridine to give to the mono exo-acetylated derivative of isosorbide **16**. This is followed by Mitsunobu inversion of the hydroxyl group in position-5, where **16** and triphenylphosphine are added to a solution of benzoic acid and DEAD in anhydrous THF giving the exo-acetate exo-benzoate isoiodide derivative **17**. Hydrolysis of **17** in a solution of methanol and 0.1 N sodium methoxide gives isoiodide **3**.



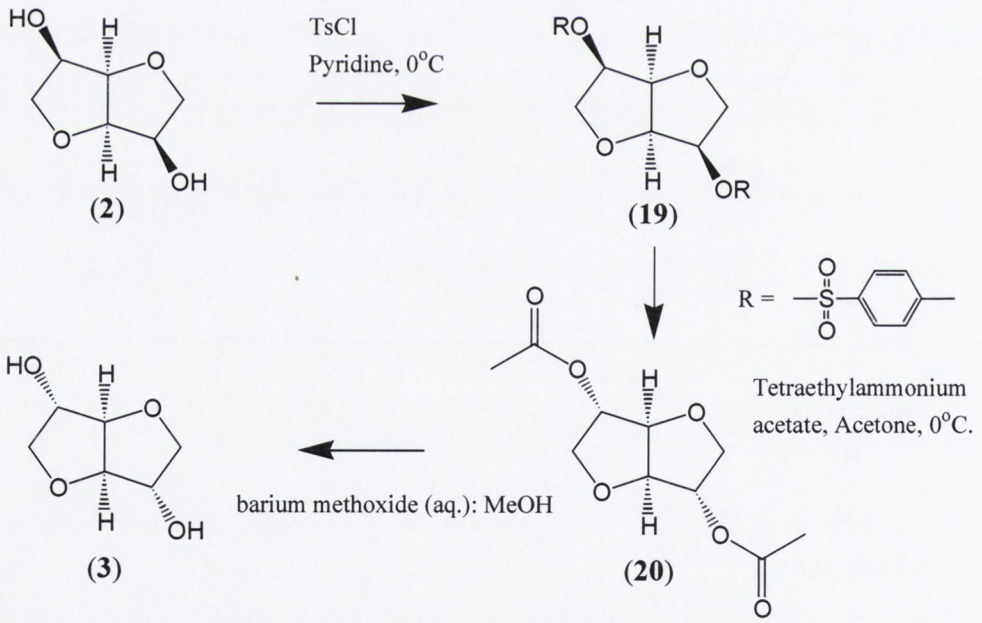
Scheme 1.2

Alternatively Mitsunobu conversion of isomannide to make the dibenzoate of isoiodide **18**, can then be followed by hydrolysis to give isoiodide **3**.



Scheme 1.3

Isoiodide can also be derived from isomannide without employing the Mitsunobu reaction[118]. This involves preparing the di-acetate of isoiodide **20** from the di-tosylate of isomannide **19**, followed by hydrolysis of the acetate esters to give isoiodide **3**.

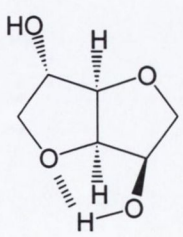


Scheme 1.4

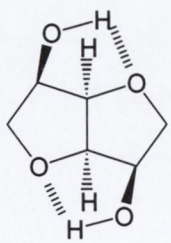
1.7.3. STEREOCHEMICAL REACTIVITY OF 1,4:3,6 DIANHYDROHEXITOLS

The two hydroxyl functional groups at positions-2 and 5 of isosorbide have been shown to possess different degrees of reactivity[119]. The difference in reactivity allows various mono substitutions of either functional group to be carried out depending on the experimental conditions. Specifically, the hydroxyl group in position-5 demonstrates greater reactivity compared to that in position-2. The hydroxyl group in position-5, being in an endo orientation, allows for the possibility of intramolecular hydrogen bonding between the hydrogen atom and the oxygen atom in the adjacent tetrahydrofuran ring, as shown in Figure 1.9. The oxygen-hydrogen bond in position-5 is therefore weaker than the corresponding oxygen-hydrogen bond in position-2 and is more prone to nucleophilic attack. Interestingly, the hydroxyl group in position-5, despite being more reactive, is actually more sterically hindered than the group in position-2.

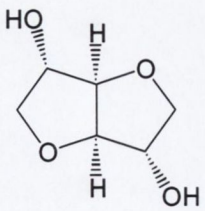
These characteristics can be exploited under certain experimental conditions to facilitate selective substitutions at either hydroxyl position. The hydroxyl groups of isomannide **2** and isoiodide **3** are stereochemically equivalent and therefore do not demonstrate any differences in reactivity. Both hydroxyl group participate in intramolecular hydrogen bonding in isomannide **2** and conversely this characteristic is absent in isoiodide **3**. It follows therefore, that the two molecules do exhibit different reactivity compared to each other.



Isosorbide (**1**)



Isomannide (**2**)

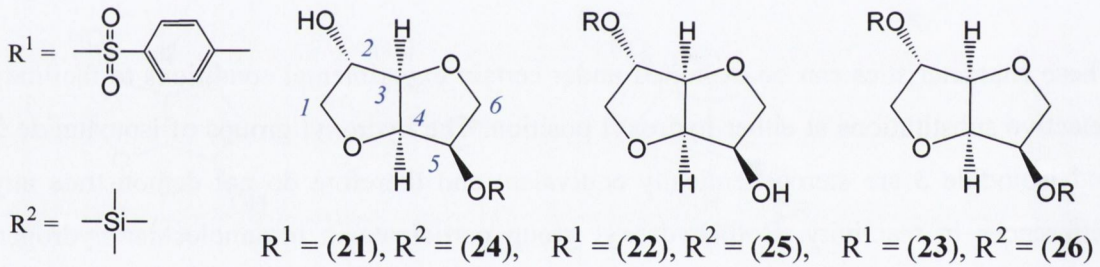


Isoiodide (**3**)

Figure 1.9

1.7.4. THE REGIOSELECTIVE SUBSTITUTION OF ISOSORBIDE

Several investigations to demonstrate the respective reactivity of the endo and exo hydroxyl groups of isosorbide have been published. These include the reaction of isosorbide with TsCl to the form mono and di tosylate derivatives of the compound[120]. In this study when one mol. eq. of isosorbide was reacted with one mol. eq. of TsCl, three products were obtained including the two mono-tosylates **21** and **22**, where the ratio between the two was 2:1 respectively in terms of the yield produced. Some of the di-tosyl compound **23** was also produced. It was also found that when *N,N*-diisopropylethylamine was included in the reaction mixture, only the mono-tosylate **21** was produced along with a small quantity of di-tosylate **23**. These results were consistent with those of other researchers[121].

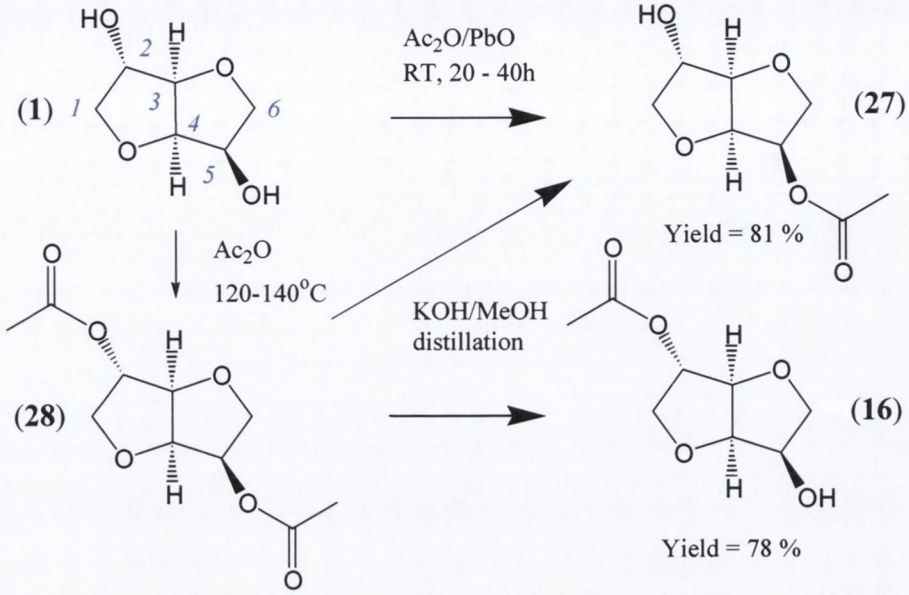


R^1 = (**21**), R^2 = (**24**), R^1 = (**22**), R^2 = (**25**), R^1 = (**23**), R^2 = (**26**)

Figure 1.10

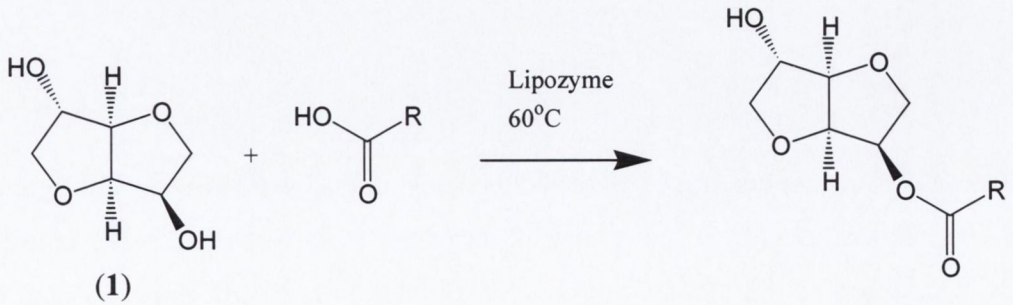
The greater reactivity of the five position has also been displayed in the reaction of isosorbide with TMSCl[122] where the mono protected and di protected compounds are obtained in a ratio of 2:1:1.25 for compounds **24**, **25** and **26** respectively.

The regioselective esterification of isosorbide in position-5 has also been achieved[123]. Acetylation of isosorbide with acetic anhydride in the presence of heavy metal salts, such as lead oxide, barium oxide or silver oxide, gives high yields of 5-acetyl isosorbide **27** with good regioselectivity where no significant quantity of 2-acetyl isosorbide **16** is produced.



Scheme 1.5

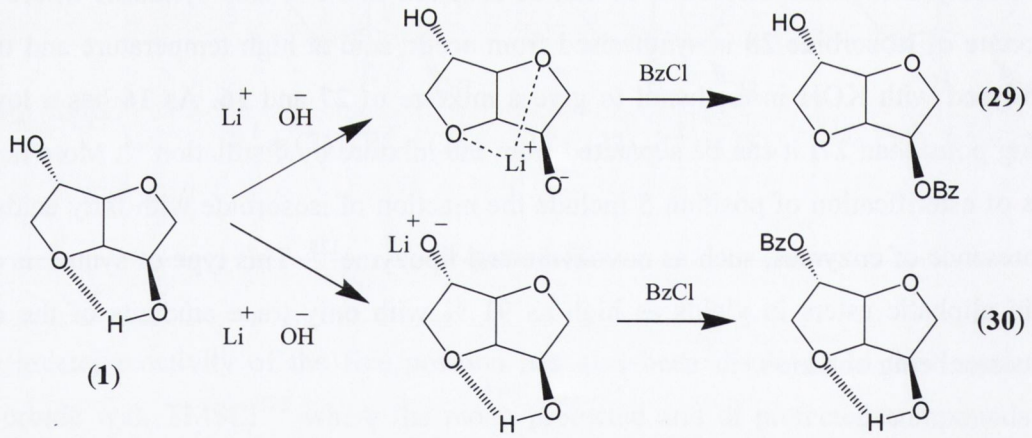
The 2-acetyl isosorbide derivative **16** can be acquired in a two-step synthesis where the di-acetate of isosorbide **28** is synthesised from acetic acid at high temperature and then hydrolysed with KOH in methanol to give a mixture of **27** and **16**. As **16** has a lower boiling point than **27**, it can be separated from the mixture by distillation[124]. More novel ways of esterification of position-5 include the reaction of isosorbide with fatty acids in the presence of enzymes, such as novozyme and lipozyme[125]. This type of synthesis can obtain aliphatic esters in yields as high as 91 % with only trace amounts of the exo monoester being obtained.



Scheme 1.6

Experimental conditions have also been developed for the selective benzylation of the endo hydroxyl group of isosorbide **1**[126]. The reaction procedure for synthesising benzyl ethers involves dissolving isosorbide **1** in an aprotic solvent such as DMF or DMSO in the presence of benzyl chloride and a base such as LiOH, NaOH, KOH, or CsOH. This synthetic route generally favours the formation of endo-substituted ethers although exo-substituted ethers are also obtained, albeit in a much smaller yields.

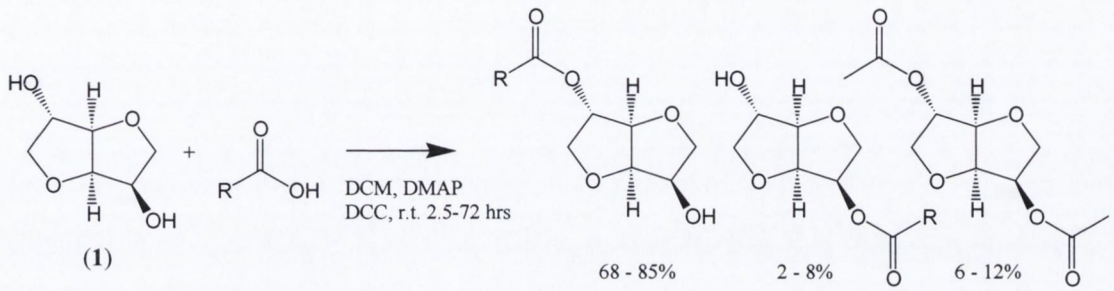
Regioselectivity increases with respect to the endo ether where smaller metallic cations are employed, i.e. Li^+, Na^+, K^+, Cs^+ from their respective bases. As well as the hydrogen bonding effect, which gives the endo position greater reactivity, chelation may also occur between the metallic cations and the oxygen atoms of the terahydrofuran rings, giving a more reactive intermediate than that obtained in the synthesis of the exo ether. This chelation effect increases with a decrease in the size of the cation employed. These effects are summarised in Scheme 1.7.



Scheme 1.7

However, experimental conditions have also been developed which favour the formation of the exo ether in isosorbide. To allow the formation of these ethers, water is used as solvent. This removes the intramolecular hydrogen bonding of isosorbide, causing both hydroxyl groups of isosorbide to be chemically equivalent and as position-5 is more sterically hindered, position-2 reacts more readily[127].

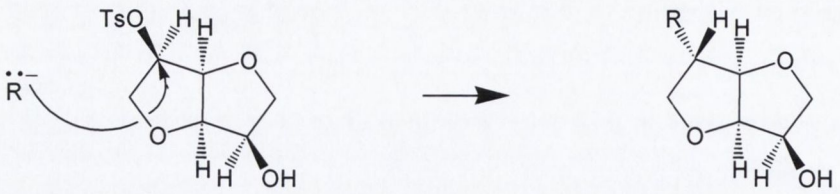
Regioselective esterification of the less reactive position-2 can alternatively be achieved by specifically using sterically voluminous acylating agents, such as DCC[128]. Preferential esterification takes place in position-2 because the approach of the acylating agent to the more sterically hindered position-5 is prevented.



Scheme 1.8

1.7.5. S_N2 REGIOSELECTIVE DISPLACEMENT OF 1,4:3,6 DIANHYDROHEXITOL TOSYL DERIVATIVES

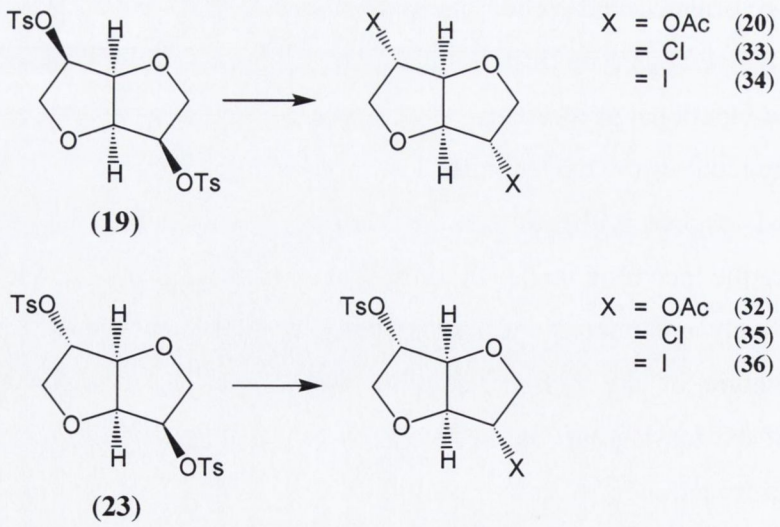
Several publications have reported on the reactivity of tosyl derivatives of 1,4:3,6 dianhydrohexitols. These publications illustrate the differences in reactivity when the tosyl groups are oriented in the endo or exo positions[118, 129]. The substitution mechanism of tosylates involves S_n2 type displacement, where the nucleophilic reagent approaches the back of the functional group. The route of access to the rear of a carbon atom bearing an exo-tosylate requires the nucleophilic agent to attack from inside the molecule[118]. The exo substituted tosylate is therefore more sterically hindered than an endo substituted tosylate, where the incoming nucleophile is allowed to attack from outside the molecule, which is relatively unhindered and subsequently, displacement can occur quite easily. Due to the nature of the S_n2 nucleophilic attack, the characteristic feature of the substitution of the tosyl group, is the inversion of the functional group attached to the carbon atom from an endo to an exo position or visa versa. This reaction mechanism is shown in Scheme 1.9. The greater degree of reactivity of the endo-substituted position over the exo-substituted position has been reflected in many experimental results.



(31)

Scheme 1.9

The reaction products of the di-tosylate of isomannide and the di-tosylate of isosorbide with several reagents are summarised in Scheme 1.10. The reaction with tetraethyl ammonium acetate in acetone or sodium acetate in DMSO converts the endo-endo tosylate of isomannide to the corresponding exo-exo acetate product **20**. In the di-tosylate derivative of isosorbide only the endo-tosylate is converted by S_n2 displacement giving an exo-tosylate exo-acetate product **32**. Similarly, the reaction with lithium chloride in ethanol or sodium iodide in acetone[130] gives the corresponding exo-exo chloride or iodide for the reaction with the di-tosylate of isomannide, **33** and **34**, while only the endo-tosylate of the di-tosylate of isosorbide is displaced to give the exo-tosylate exo halide products, **35** and **36**.

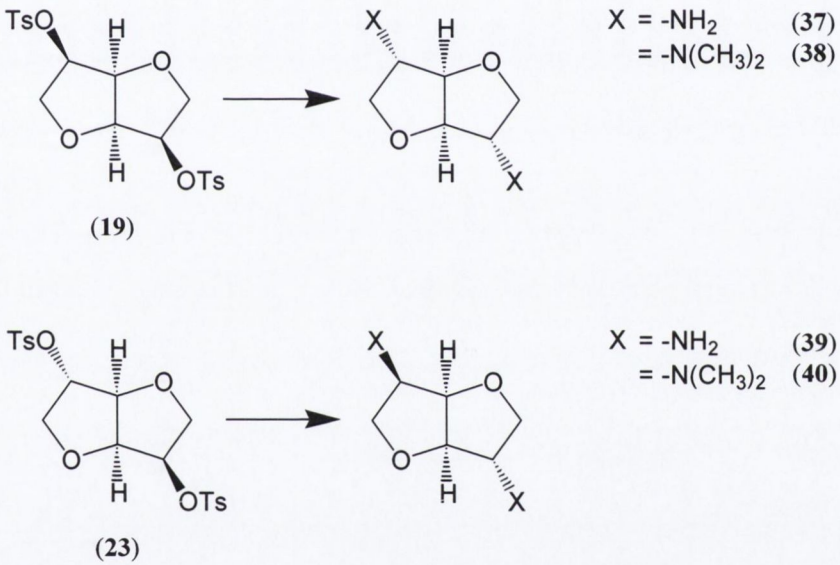


(19)

(23)

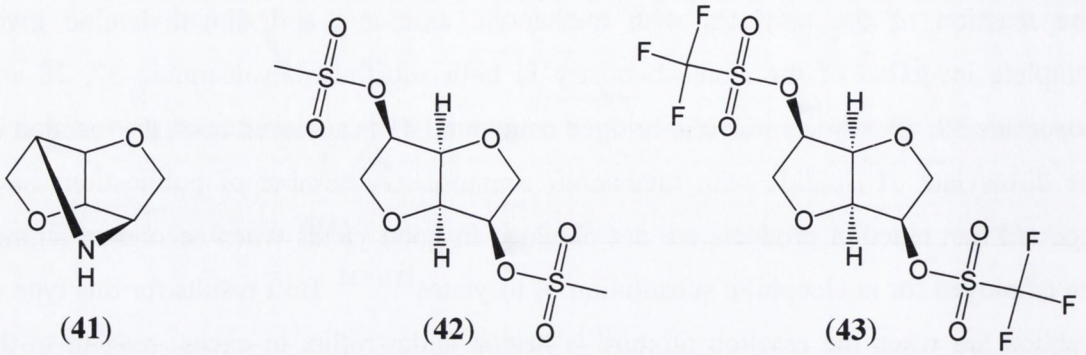
Scheme 1.10

The reaction of the tosylates with methanolic ammonia and dimethylamine gives complete inversion of the stereochemistry in both substituted isomannide **37**, **38** and isosorbide **39**, **40**, while a tricyclic-bridged compound **41** is achieved upon the reaction of the di-tosylate of isoidide with methanolic ammonia. A number of publications have reported that reaction products are not obtained in good yields when secondary amines are employed for nucleophilic substitution of tosylates[131, 132]. Best results for this type of reaction are when the reaction mixture is heated under reflux in excess reagent in the absence of solvent, under microwave conditions or heated to high temperature in steel tubing.



Scheme 1.11

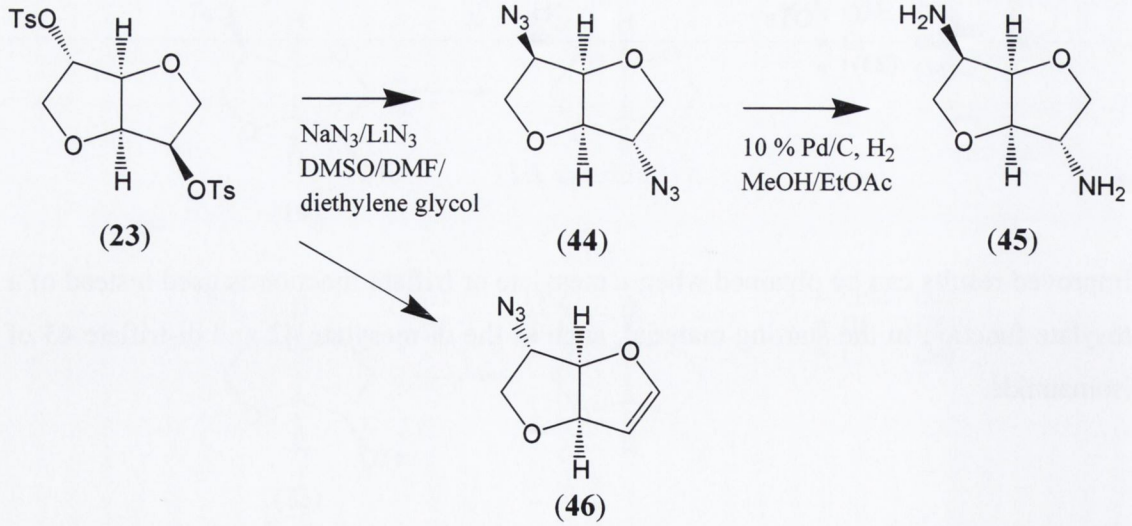
Improved results can be obtained when a mesylate or triflate function is used instead of a tosylate function in the starting material, such as the di-mesylate **42** and di-triflate **43** of isomannide.



(41) (42) (43)

Figure 1.11

Alternatively, an alcohol group of a 1,4:3,6 dianhydrohexitol can be converted and inverted to an amine by the reaction of the respective tosylate, mesylate or triflate with sodium azide in DMSO at 120°C, sodium azide in di-ethylene glycol at 135°C[133] or lithium azide in DMF[134]. The inverted azido product can then be reduced to the corresponding amine by palladium on activated carbon under an atmosphere of hydrogen gas. Scheme 1.12 shows the conversion of the di-tosylate of isosorbide **23** to the inverted di-amine **45** vis-à-vis the formation and subsequent reduction of the di-azido compound **44**.



(23) (44) (45)

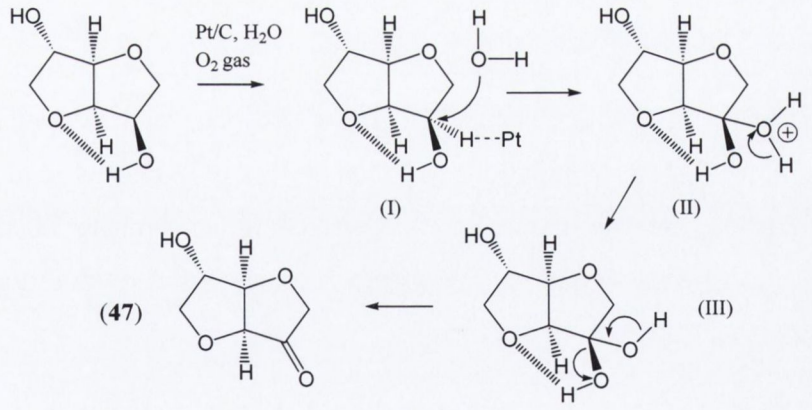
(46)

Scheme 1.12

S_n2 reactions involving 1,4:3,6 dianhydrohexitol sulfonyl derivatives sometimes also give an elimination product, such as **46** in Scheme 1.12. Elimination tends not to happen when the sulfonyl group is in the endo position. This position offers less steric hindrance to nucleophilic attack and therefore exhibits a greater tendency for substitution to occur rather than elimination. Experimental results have given a yield of 11 % of the elimination product **46** from the di-tosylate of isosorbide[116], a yield of 16 % from the reaction of sodium azide with the di-mesylate of isoiodide[135], while a yield of 26 % was obtained from an exo-triflate under reaction with lithium azide[134].

1.7.6. THE OXIDATION AND REDUCTION OF 1,4:3,6 DIANHYDROHEXITOL DERIVATIVES

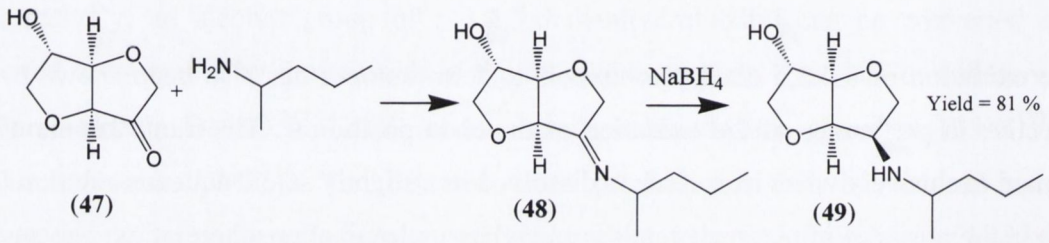
The oxidation of 1,4:3,6 dianhydrohexitols to form ketones has also been shown to be selective in preference of the oxidation of the endo position[136]. The mono ketone **47** is formed exclusively when isosorbide is dissolved in a slightly acidic aqueous solution (pH 4.5) in the presence of platinum catalyst on carbon under an atmosphere of oxygen gas.



Scheme 1.13

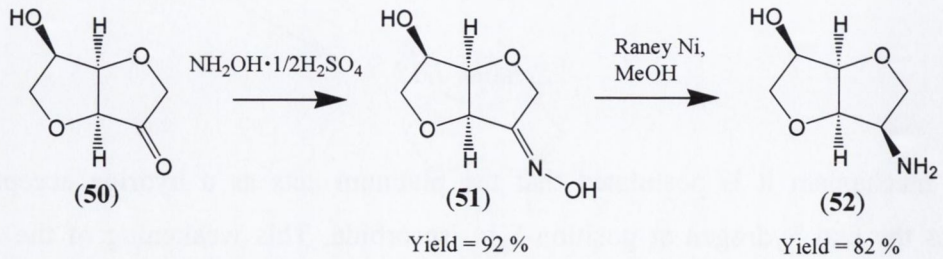
In this mechanism it is postulated that the platinum acts as a hydride acceptor and activates the exo hydrogen at position-5 in isosorbide. This weakening of the carbon-hydrogen bond allows the formation of the intermediates (I), (II) and (III) after nucleophilic attack by water. This gives the selective oxidation of position-5. No

oxidation is observed when other solvents, such as dioxane, THF, acetonitrile, acetone, acetic acid, benzene or heptane are used. No oxidation is observed in position-2 in water or any other solvents. An amine function can be introduced into the isosorbide moiety by the reaction of the mono-ketone of isosorbide with a relevant amine[137]. This mechanism first forms an imine, which can then be reduced to the amine by a base. In Scheme 1.14, the mono-ketone is reacted with 2-pentylamine to give the imine in a yield of 81 %. This was then reduced to the amine compound with sodium borohydride. Critically, the stereochemical endo orientation is maintained in this mechanism. These types of compounds are useful in their function as monomers in the production of polymers.



Scheme 1.14

The formation of the endo amine from a ketone has also been reported when an oxime is furnished from the ketone compound and then reduced[138]. In this example 1,4:3,6-dianhydro-D-fructose **50** was reacted with hydroxylamine sulphate in methanol and aqueous NaOH to give the oxime **51**. The oxime was then reduced with Raney Nickel in methanol at 40°C under 50 psi for eight hours.



Scheme 1.15

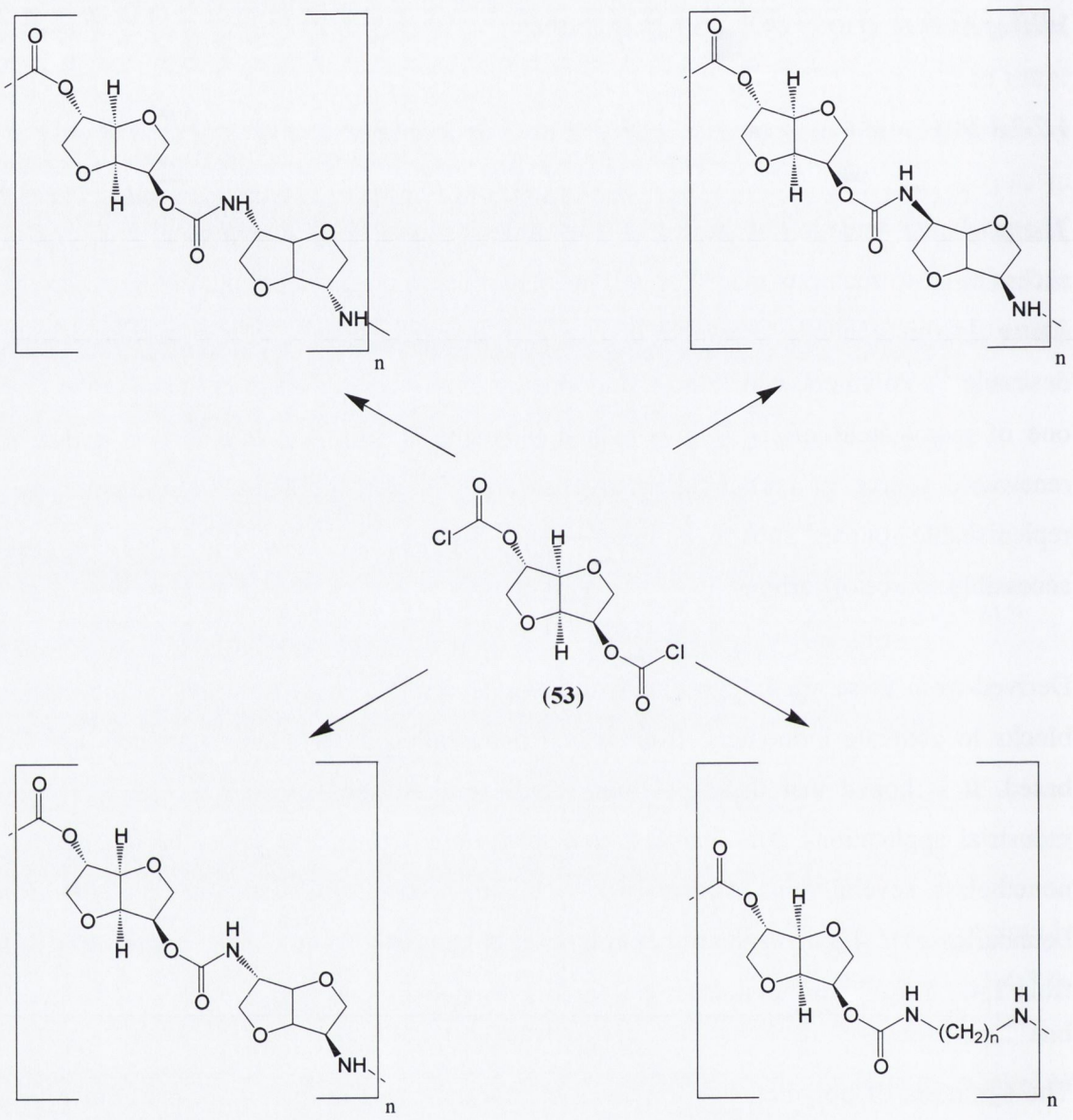
1.7.7. APPLICATIONS OF 1,4:3,6 DIANHYDROHEXITOLS

1.7.7.1. POLYMERS

The predicted depletion of the world's resources of fossil fuels dictates that commercial accessibility to such raw materials will become much more difficult and expensive in the future. In this context, renewable or re-grown sources of carbon have become highly desirable[137]. Polymers, which are predominantly manufactured from petrochemicals, are one of many areas of industry, which could benefit from the discovery of a viable renewable source of carbon alternatives. Carbohydrates produced from natural and replenishable sources, such as biomass provide a cheap, thermostable and continuously accessible source of carbon[139].

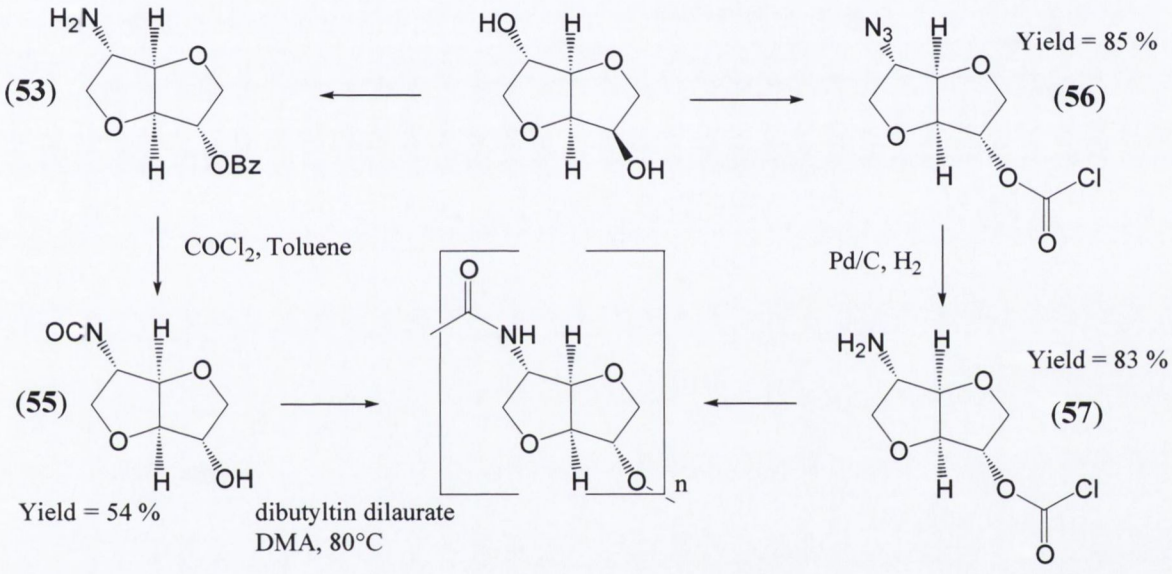
Derived from these are 1,4:3,6 dianhydrohexitols, which can be used as chiral building blocks to generate monomers, from which potentially biodegradable polymers can be based. It is hoped that these polymers could have various uses in a wide range of industrial applications. The manifestation of these polymers has yet to be understood, nonetheless, several veins of research have commenced in order to deduce the chemical boundaries of 1,4:3,6 dianhydrohexitols in this context. Among the polymers created from 1,4:3,6 dianhydrohexitols are polyurethanes, polyamides, polyesters and polyethers.

The synthesis of polyurethanes involves the formation of an isosorbide monomer that incorporates a chloroformate function at each of the free hydroxyl groups[116, 117]. The chiral differences in 1,4:3,6 dianhydrohexitols are then exploited in order to create a number of different polyurethanes with different backbones. The chloroformate compound **53** can be synthesised from isosorbide by reacting it with phosgene in a solution of DCM/DMF at 0°C in dry conditions. The chloroformate is then reacted with di-amino derivatives of isosorbide, isomannide and isoiodide to yield the respective polymers shown in Scheme 1.16.



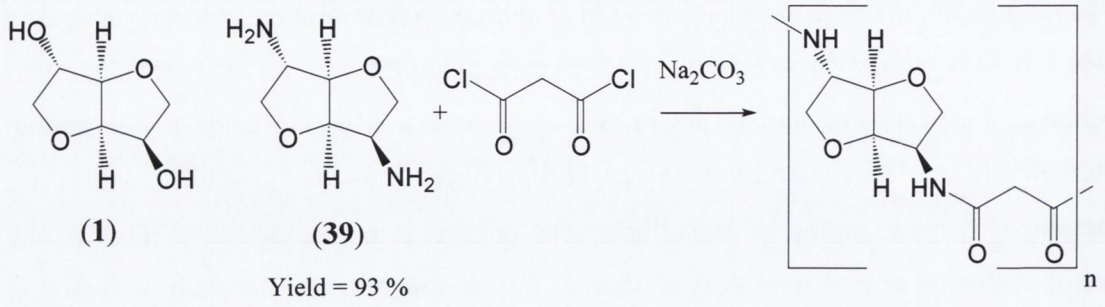
Scheme 1.16

A development of this concept is to build monomers incorporating chloroformate and azide functions as shown in Scheme 1.17, to give polymers with an alternative exo-exo backbone. Polycondensation occurs in situ upon reduction of the azide to the amine. Alternatively, an isocyanate function can be introduced into the isosorbide scaffold by reaction of a mono-amine of isosorbide with phosgene in toluene at -15°C with the careful exclusion of moisture. Polyaddition of this monomer takes place when a reaction is catalysed by dibutyltin dilaurate in DMA at 80°C[140].

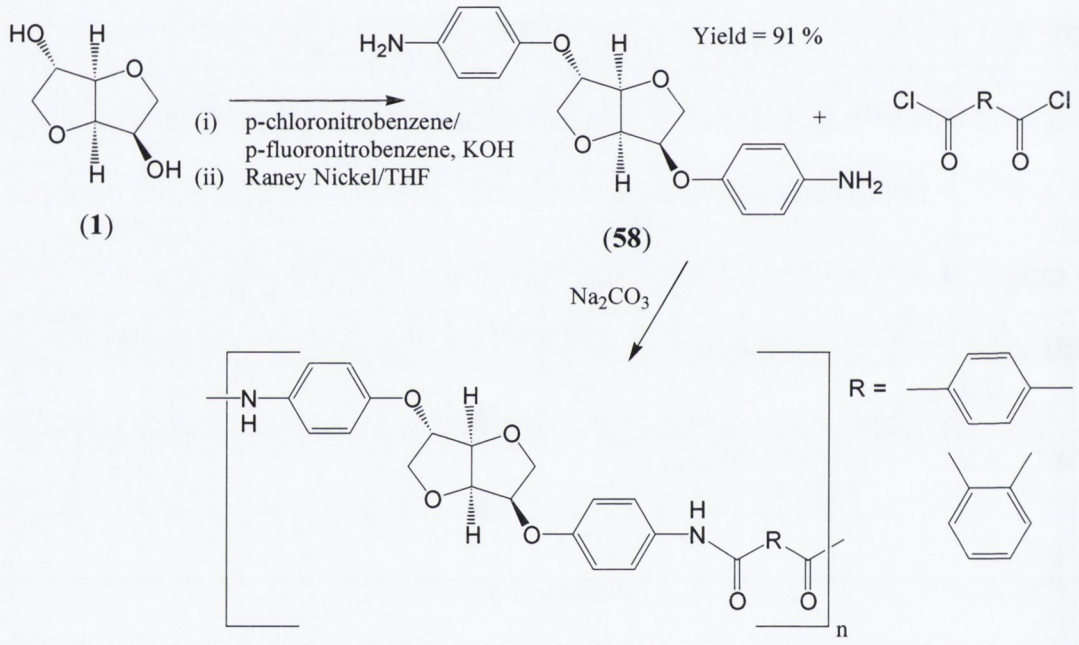


Scheme 1.17

Polyamides and polyesters can be prepared using similar methods. They can be converted to polymers by their reaction with dichlorides in an aqueous sodium carbonate solution[117] (Scheme 1.18). Alternative types of polyamides have been published where different functional groups are first introduced into the 1,4:3,6 dianhydrohexitol monomers and then the amides are synthesised as before with dichlorides[141]. In Scheme 1.19, a nitro benzene ether is first prepared from isosorbide using *p*-chloronitrobenzene or *p*-fluoronitrobenzene in an aqueous KOH solution. The reduction of the nitro group and subsequent reaction with dichlorides gives the relevant polyamide compounds.



Scheme 1.18



Scheme 1.19

Varying the structure of monomer units can help change the chemical properties of polymers produced. The polyester[139] and polyamide[142] monomers in Figure 1.12 are more hydrophilic and can be broken down more easily than the previous examples. It is hoped that the utilization of biodegradable polymers could be used to address environmental issues arising from waste and non-degradable plastics[143].

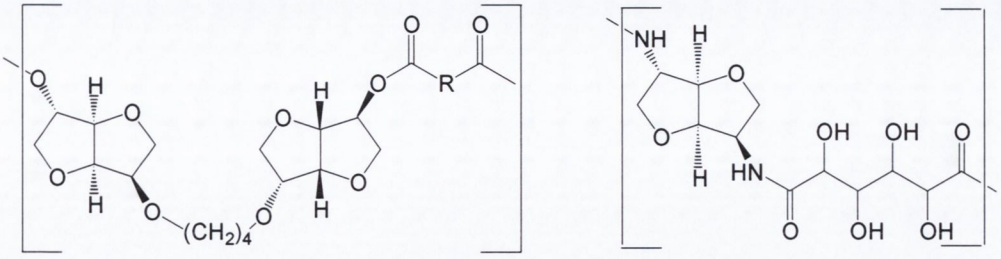
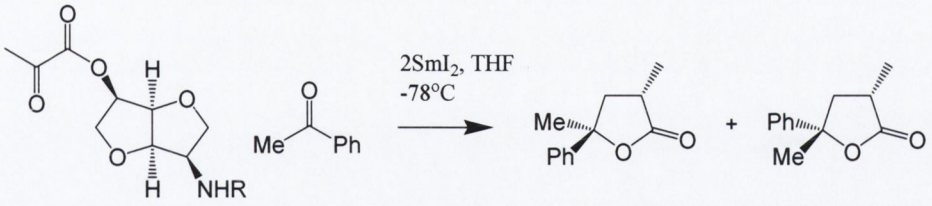


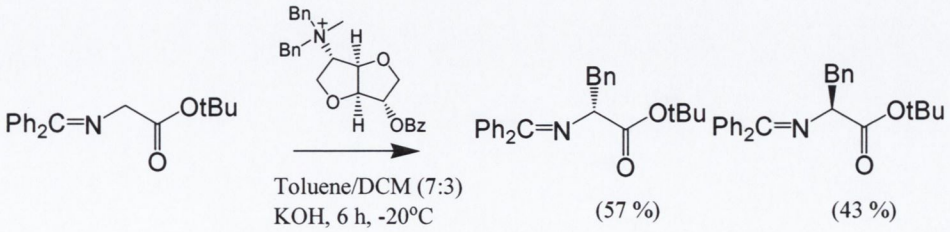
Figure 1.12

1.7.7.2. CHIRAL AUXILIARIES IN ASYMMETRIC SYNTHESIS

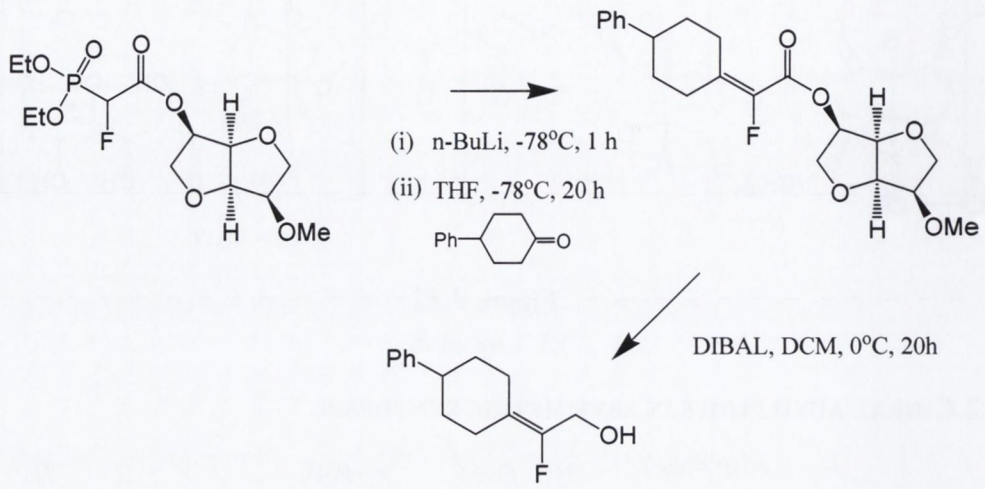
1,4:3,6 dianhydrohexitols have been employed as chiral auxiliaries in asymmetric synthesis in the preparation of α,γ-substituted γ-butyrolactones[144, 145] (Scheme 1.20) and chiral tertiary α-hydroxy acids[146] and have been used as phase transfer catalysts in the benzylation of *N*-(diphenylmethylene)glycine *tert*-butyl ester[147] (Scheme 1.21). They have been employed in Horner-Wadsworth-Emmons reactions[148] (Scheme 1.22) and as homo-chiral ligand catalysts for use in Diels-Alder reactions[135] (Scheme 1.23).



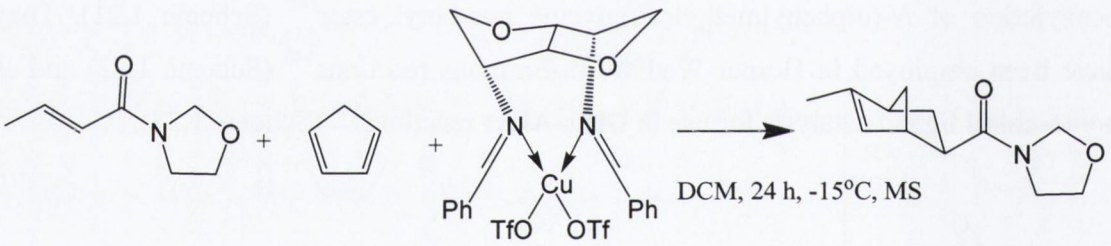
Scheme 1.20



Scheme 1.21



Scheme 1.22

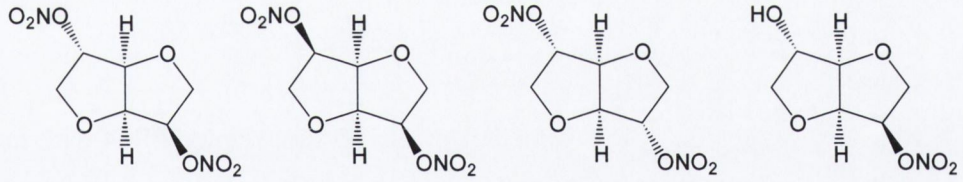


Scheme 1.23

1.7.7.3. Biomedical Applications

In a biological setting, some use has been made of isosorbide **1** and isomannide **2** as non-toxic diuretics where they have been employed in intravenous and oral dosages[132]. However, the chief application of 1,4:3,6 dianhydrohexitols is that their mono and di nitrate esters are used as coronary vasodilators which have found continuous pharmaceutical applications worldwide as antiangina agents for chronic prophylaxis of angina pectoris and in the treatment of acute heart attack[149].

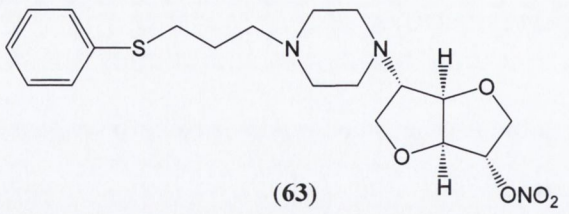
Isosorbide dinitrate (ISDN) **59**, along with glyceryl trinitrate (GTN) are the primary cardiovascular therapeutic agents used in this field, although isomannide dinitrate (IMDN) **60** and isoiodide dinitrate (IIDN) **61** may also be used in this role. As these dinitrate drugs have a number of disadvantages such as low oral availability, first pass effect and the development of tolerance over time, isosorbide 5-mono-nitrate (IS-5-MN) **62**, which is an active metabolite of ISDN, has more recently been used as a cardiovascular agent due to its greater bioavailability and weak first pass effect.



(59) **(60)** **(61)** **(62)**

Figure 1.13

In other research, different functionalities have been introduced into isosorbide nitrate to improve their efficacy with respect to vasodilation. Compound **63** demonstrates greater activity in terms of the minimum effective dose given to dogs[150].



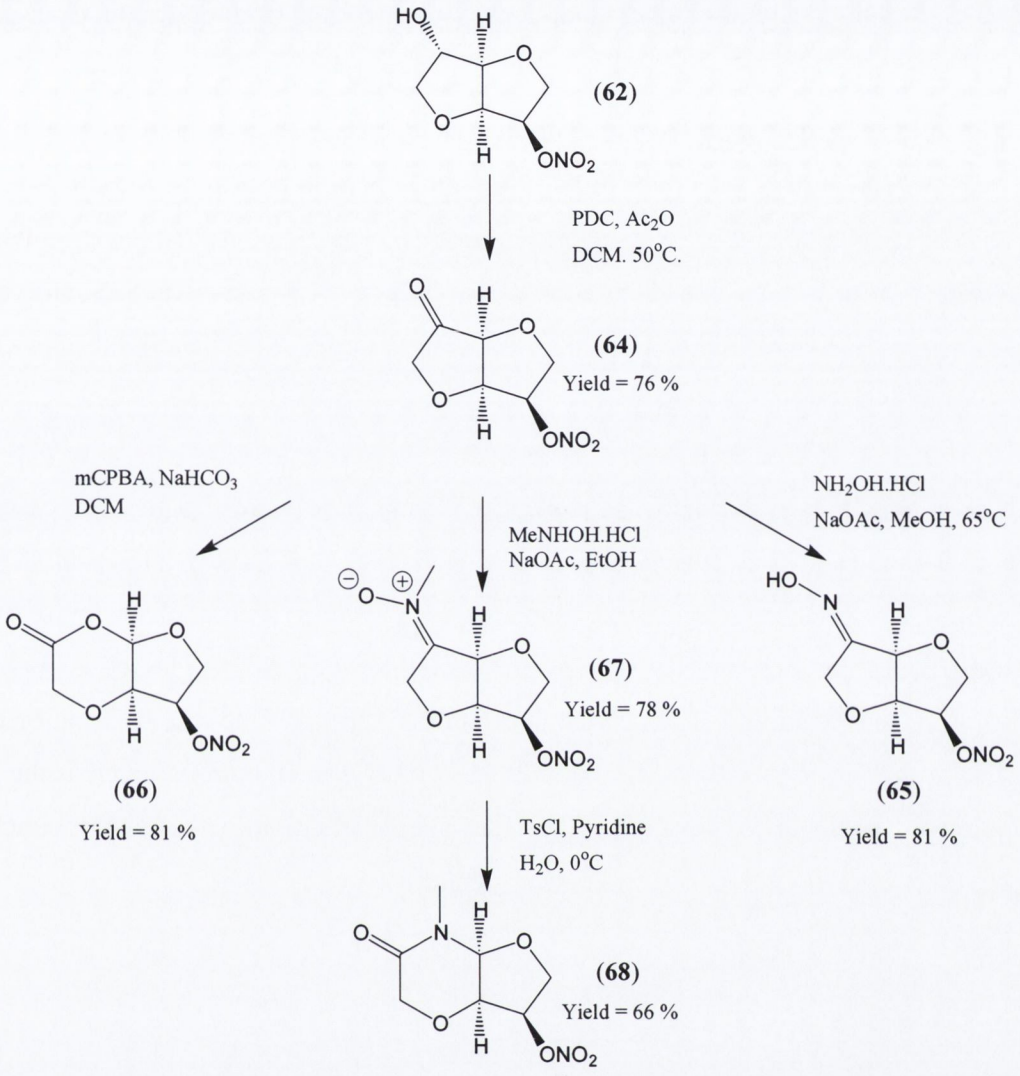
(63)

Figure 1.14

In an effort to optimise and improve the efficacy of the 1,4:3,6 dianhydrohexitol derivatives, attempts have been made to modify the molecular structure of these vasodilator drugs by introducing substituents or different functional groups or by altering the nature of the bicyclic ring system and establishing if these changes cause a change in their pharmacological properties or the biological response elicited.

IS-5-MN **62** can be converted to the ketone derivative by oxidation of the hydroxyl group in position 2 with PDC in the presence of acetic anhydride in a DCM solution. The keto compound **64** can be converted to a number of different analogues[149, 151, 152]. Firstly, the oxime of the ketone can be prepared by the reaction of **64** with hydroxylamine hydrochloride in the presence of sodium acetate in methanol to give **65**.

Baeyer Villiger rearrangement can also be carried out by the reaction of **64** with mCPBA in DCM in the presence $NaHCO_3$. This expands the ring system to include an extra oxygen atom giving a 6-membered lactone ring[152]. The ketone compound can also be subjected to a Beckmann rearrangement where a nitrogen group is introduced into the ring system containing the ketone group[151]. In this case the *N*-methyl nitrone of the ketone must be synthesised first to make the substrate from which to prepare the Beckmann rearrangement. The *N*-methyl nitrone can then be reacted with TsCl in pyridine in the presence of water to give the new compound containing an amido function in the ring system. Some of these drugs have been found to cause greater relaxation of the superior mesenteric artery in rats and therefore may be more effective as vasodilator compared to IS-5-MN[149].

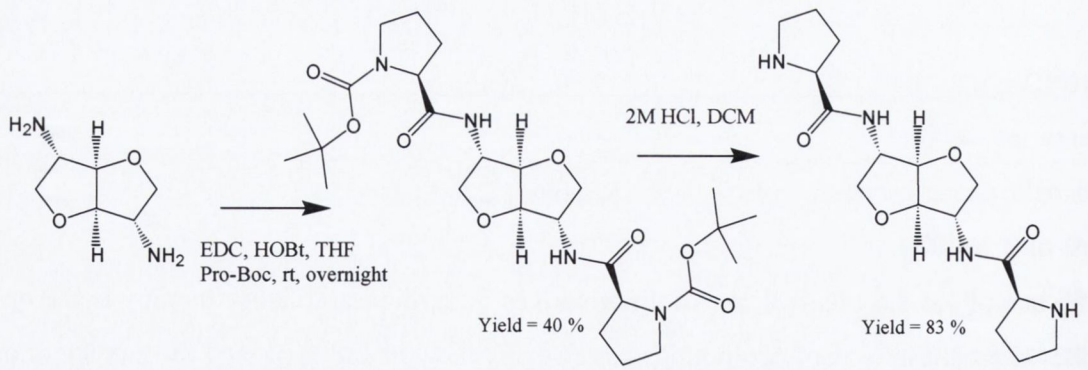


Scheme 1.24

The use of 1,4:3,6 dianhydrohexitol derivatives in cardiovasodilatory therapy is the only case where these type of compounds have been validated and approved for medicinal use and this remains the main application of these drugs today. However, the use of 1,4:3,6 dianhydrohexitols as a therapeutic agent is being widely investigated. While most of the various avenues of research are at the relatively early or speculative stages of development, several publications have shown the potential of 1,4:3,6 dianhydrohexitols in a wide range of therapeutic settings.

Among the most recent of these is where isomannide **2** has been employed to design potential inhibitors of serine proteases[153]. Flaviviruses include some 60 viruses, many of which have been found to be foremost human pathogens, such as hepatitis C, west Nile virus and dengue virus. All flaviviruses share one important serine protease, NS3pro, which is responsible for the cleavage and generation of viral proteins that are essential for viral replication and hence the maturation of the infectious viruses. The NS3pro serine protease is therefore considered a suitable therapeutic target, where the inhibition could lead to a development in the treatment of flaviviruses.

A series of *N*-t-Boc amino acid derivatives of isomannide were designed as potential inhibitors of the catalytical triad of amino acids (His 51, Asp75 and Ser135) in NS3pro. Isomannide **2** was chosen as it was found to be a useful rigid scaffold, onto which dipeptites could be constructed[154, 155]. The *N*-t-Boc amino acid amides of isomannide were synthesised by adding EDC, HOBt and N-methyl morpholine to a solution of Boc-protected amino acids and di-amine isoiodide in THF to give compounds such as the Pro-Boc protected compound given in Scheme 1.25. The evaluation of these compounds by molecular modelling and bioassay is currently underway.

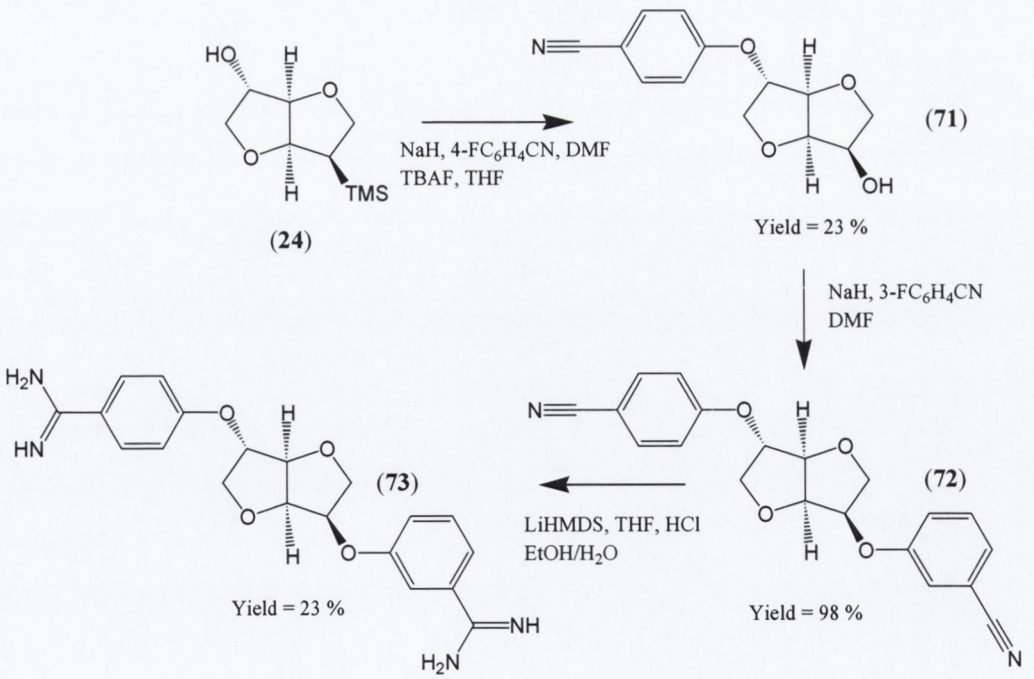


(**37**) (**69**) (**70**)

Scheme 1.25

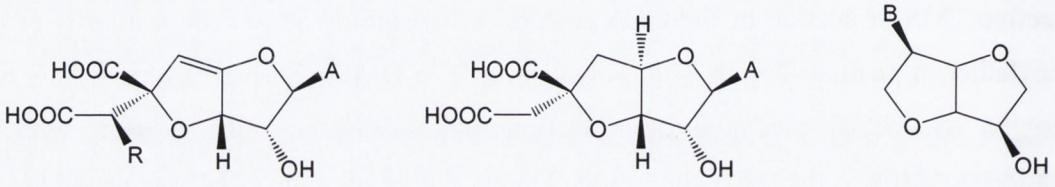
The development of inhibitors of serine protease factor Xa (fXa) has also been investigated[122]. Serine protease fXa plays a critical role in blood coagulation and is therefore responsible for venous and arterial thrombosis formation. Potent and specific inhibitors of fXa may act as anti-thrombotic agents. Again, a 1,4:3,6 dianhydrohexitol moiety, in this case, isosorbide is used as a scaffold linking two specific ligands which would interact with the active site pockets of fXa.

Selective TMS protection of the endo position of isosorbide allows the synthesis of the phenylether in position-2 with 4-fluorobenzonitrile in DMF with NaH as base. This was followed by TBAF mediated de-protection and subsequent etherification with 3-fluorobenzonitrile in the same conditions. Finally the target compound was generated by reaction with LiHMDS and subsequent hydrolysis using ethanolic HCl to give the bisamidine (**73**).



Scheme 1.26

Griseolic acids (**74**, **75** and **76**), which have been isolated from cultured broths of *streptomyces griseoaurantiacus*, are naturally occurring compounds containing a bicyclic carbohydrate system. They have been found to exhibit a wide range of interesting biological properties such as the ability to demonstrate inhibitory activity against the cyclic nucleotide-phosphodiesterase. They have also been reported to express potency as anti-hypertensive agents[134].



R = H, Griseolic acid A (**74**)
R = OH, Griseolic acid B (**75**)

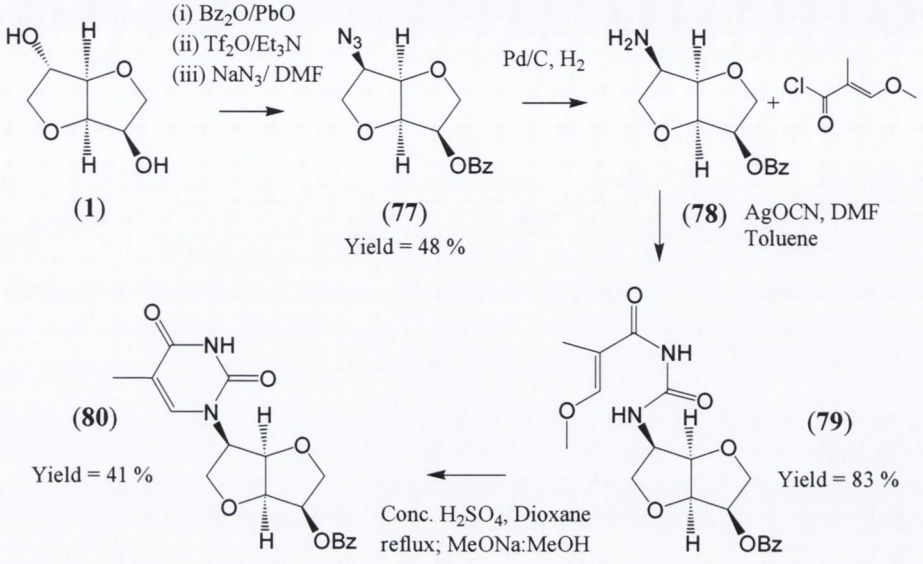
Griseolic acid C (**76**)

B = Nucleoside

Figure 1.15

Isosorbide **1** has been employed due to its similar bicyclic structural frame in an effort to make synthetic nucleoside analogues of these acids, which may also have antiviral activity. In addition to this, nucleoside derivatives of monocyclic compounds have shown inhibitory activity against HIV, highlighting the prospect that bicyclic inhibitors may have a role to play the development of a therapeutic agent in this field.

An endo-amine endo-benzyl protected derivative of isosorbide was synthesised by selective benzyl protection of position-5, followed by the introduction of a triflate group into position-2, which is then inverted by azide displacement and reduction to yield the amine in position-2. The nucleobase was then attached directly to position-2 by reaction with 3-methoxy-2-methacryloyl chloride in the presence of silver nitrate to give the urea **79**, which was converted to the nucleoside by refluxing in a mixture of 2 N sulphuric acid and dioxane. Finally, the compound was de-protected with sodium methoxide to yield the target compound **80**.



Scheme 1.27

In addition to the substitution of nucleosides to position-2 of isosorbide, analogues were also prepared where the substitution occurred at position-1 of the scaffold[156]. The reason behind this synthetic work was down to the fact that synthetic analogues of griseolic acids, with substitution at position-1, demonstrate greater potency against enzymes such as the aforementioned phosphodiesterase. In other studies, a C1 purine isosorbide derivative, which was inadvertently prepared by other researchers, was found to have significant activity against the vaccania virus with low toxicity[157]. Also, the amino sugar analogue furanodictine B **81**, which possesses a hydroxy group at position-1, demonstrates anti-tumour activity in certain models[158]

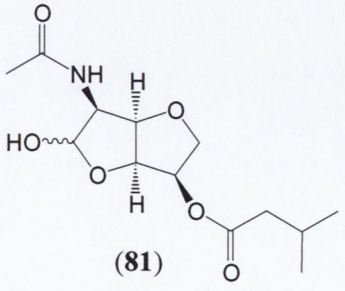
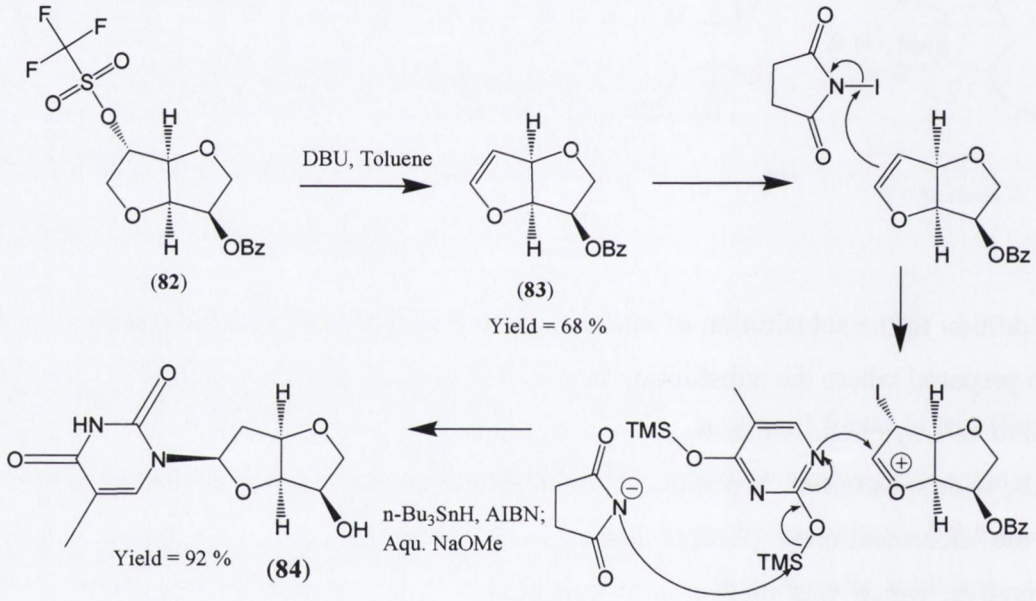


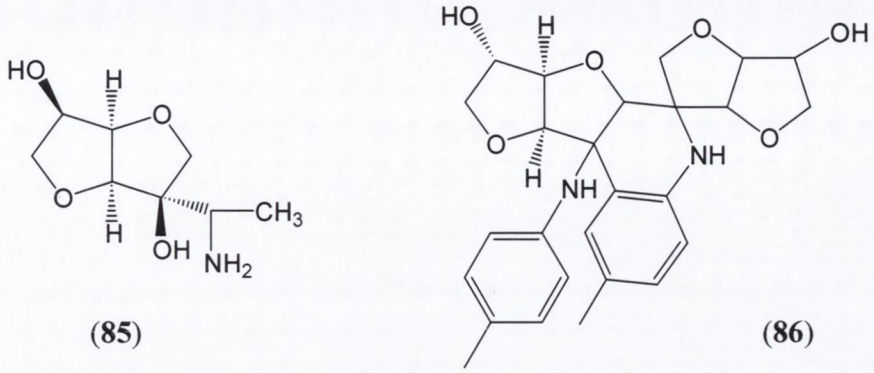
Figure 1.16

A functional group was introduced at the one position by eliminating the triflate group in position-2 with the base DBU in dry toluene to yield the alkene product **83** as the primary product with a yield of 68 %. Condensation of this compound was then initiated by nucleophilic attack from the bottom face of the molecule with N-iodosuccinimide (NIS). This gives an intermediate, which can be attacked by a silyl-protected nucleobase, in this case, thymine, to give the nucleoside compound. De-iodination with n-Bu_3SnH and de-protection of position-5 gives the target compound **84**.



Scheme 1.28

In other studies, 1,4:3,6 dianhydrohexitols were used to synthesise new amino sugar derivatives[138] **85** and were also incorporated into the structure of tetrahydroquinoline derivatives[159] **86**. Work is ongoing in these studies to see whether these types of drugs demonstrate any biological activities.



(85) (86)

Figure 1.17

1.8. SUMMARY

BuChE and AChE are two enzymes present in several species, including humans. They are capable of hydrolysing a wide range of choline and non-choline based esters. AChE has a specific physiological role in the body, as it is responsible for the hydrolysis of the endogenous substrate and neurotransmitter, ACh. However, whilst BuChE is capable of hydrolysing large exogenous esters, such as the synthetic substrate BCh, from which it takes its name, it has no known endogenous substrate and its physiological role in the body is uncertain. However, recent independent research studies into BuChE have generated a significant amount of evidence, which strongly implicates BuChE in the progression of AD.

It was recently discovered that huBuChE hydrolyses esters of isosorbide extremely rapidly and in some cases, at a rate higher than that of the prototype specific synthetic substrate, BCh. It was proposed that these highly specific substrates of huBuChE could be used to prepare novel inhibitors of huBuChE. The development of these inhibitors may prove to be very useful in determining the biological function of huBuChE and more particularly they may hold the potential to act as therapeutic agents in combating AD.

As a platform on which to conduct the synthesis of isosorbide-based huBuChE inhibitors, a comprehensive review of the literature published on 1,4:3,6 dianhydrohexitol chemistry

was undertaken in order to compile the known chemical synthetic information of this family of sugars, to which isosorbide belongs. The review also explained the nature of 1,4:3,6 dianhydrohexitols and highlighted the areas of research where 1,4:3,6 dianhydrohexitols have been applied. Moreover, the review emphasises the unique and innovative application of isosorbide derived compounds to the area of enzyme inhibitor design and furthermore the originality of the inhibitors generated in this quest.

The initial design of isosorbide based inhibitors fell into two groups. It was decided that group 1 compounds (refer to section 1.6) were to be synthesised and analysed by our sister research group based at the Athlone institute of Technology. The group 2 or 'reversed carbamates' compounds were synthesised and analysed by the author and are discussed in the following chapter of this thesis. These two groups of inhibitors represented the first generation of isosorbide-based inhibitors. The compounds were tested using biological methods to assess their inhibitory activity and SAR with respect to BuChE and AChE. Second and successive generations of inhibitors capitalised on the SAR information derived from the first and preceding generations of compounds and subsequently, a feature in the progress of this thesis going forward was the periodic biological testing of successive generations of inhibitors, preformed in order to assess the effects of the modifications and refinements of each series of inhibitor.

CHAPTER 2

SYNTHESIS AND ANALYSIS OF LIBRARY-1 COMPOUNDS: 'REVERSED CARBAMATE' DERIVATIVES OF ISOSORBIDE

2.1 INTRODUCTION

This chapter describes the synthesis and biological testing of reversed carbamate isosorbide-based inhibitors of huBuChE. The design of these inhibitors is shown in Figure 2.1.

The synthetic strategy in preparing reversed carbamate inhibitors involved replacing the hydroxyl group of isosorbide at position-2 with an amino group, with retention of configuration. Particular importance was placed on the retention of the exo orientation of position-2 so that a direct comparison could be drawn between the structures of the reversed carbamate and the group 1 compounds.

We, therefore, had to synthesise the key intermediate **87**, from which we could prepare a series of analogues.

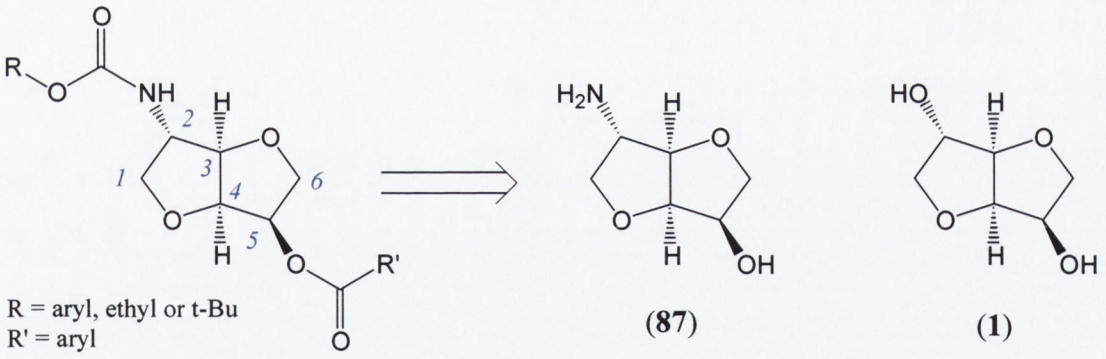


Figure 2.1

2.2 THE SYNTHESIS OF 2-DEOXY 2-AMINO ISOSORBIDE

The synthesis of the key intermediate **87** was investigated concurrently by two different approaches as shown in Figure 2.2. The first approach involved the synthesis of an oxime derivative **88** of IS-5-MN **62**, followed by the reduction of the oxime to give the desired amino intermediate **87**. The second approach involved the inversion of one of the equivalent hydroxyl groups of isomannide **2** to give the azido derivative **89**. Reduction of the azido derivative would then yield the key amino intermediate **87**.

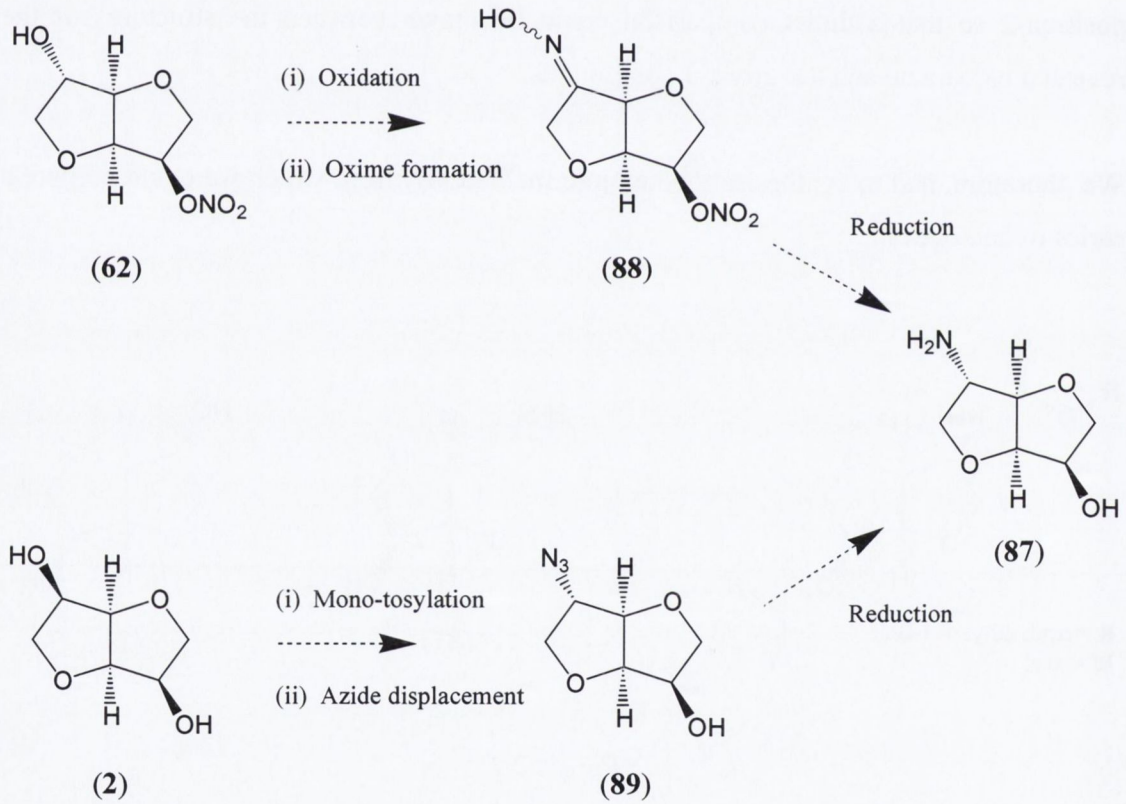
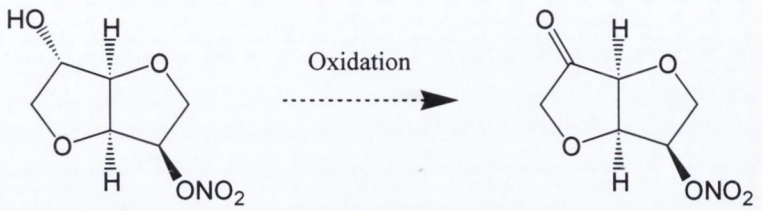


Figure 2.2

The first synthetic approach initially required the oxidation of the hydroxyl group of IS-5-MN **62** to give the ketone derivative **90** as in Figure 2.3

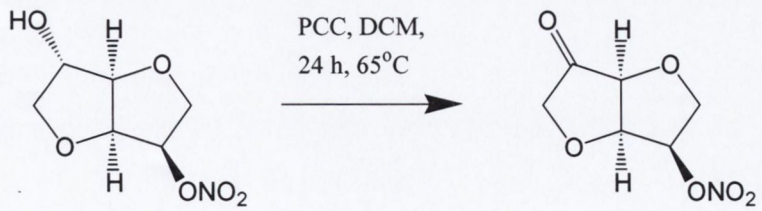


(62) **(90)**

Figure 2.3

There have only been two procedures published, which relate specifically to the oxidation of 1,4:3,6 dianhydrohexitols (refer to sections 1.7.6 and 1.7.7.3 in the introduction). These employ either PDC[149] in a solution of DCM or by using a platinum catalyst in an aqueous solution in the presence of oxygen[136]. In the present work, it was decided to investigate the PDC oxidation, being a more convenient approach.

It was found that this reaction proceeded quite slowly, with a reaction time in excess of 72 hours. The efficiency of the reaction was improved significantly when excess PCC was used instead of PDC and improved further when the reaction mixture was heated to 65°C. The NMR and IR data for **90** were consistent with those reported in the literature[149].

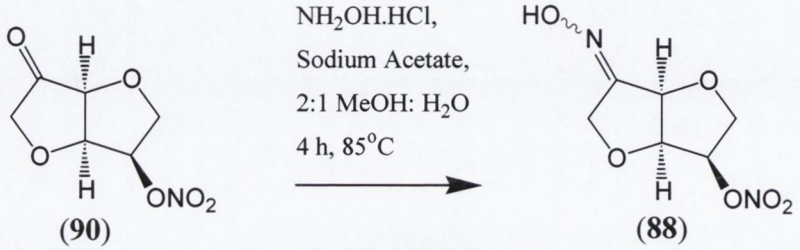


(62) **(90)**

Yield = 57 %

Scheme 2.1

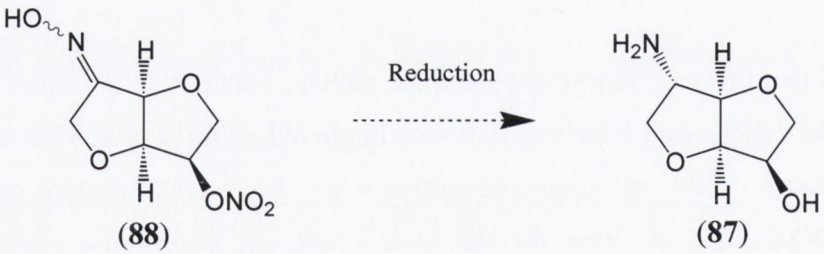
The reaction to form the oxime derivative **88** from **90,** as shown in Scheme 2.2 was a straightforward procedure, which involved the reaction of **90** with hydroxylamine hydrochloride in the presence of sodium acetate in a solution of 2:1 methanol: water at 85°C.



(**90**) (**88**)

Scheme 2.2

From **88** it was desired to synthesise the key amino intermediate with retention of the stereoselectivity shown in Figure 2.4.



(**88**) (**87**)

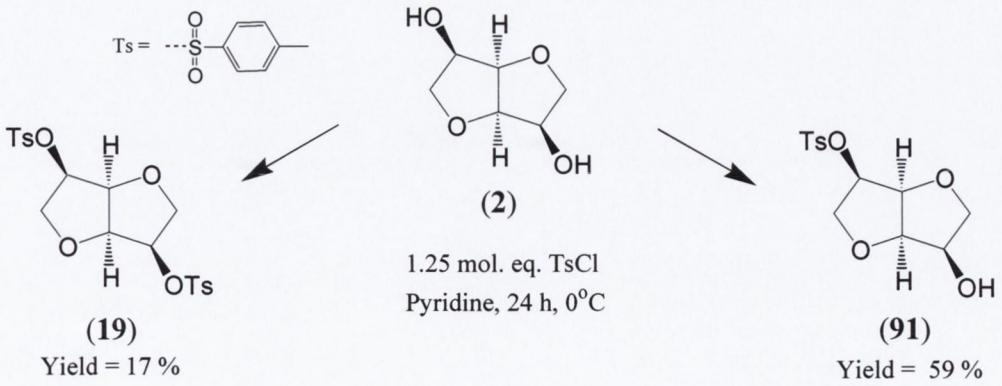
Figure 2.4

Although there have been no specific publications pertaining to the approach in Figure 2.4, it was proposed that the reduction reaction could be undertaken using one of a number of common techniques known to synthesise amines from oximes using a range of reagents. These include the use of zinc powder[160] or indium powder[161] in acetic acid, lithium aluminium hydride in acetic anhydride[162] or Pd/C under an atmosphere of H_2[163]. However, the main drawback of these types of reactions is that they produce both α- and β- isomers, which can be difficult to separate into diastereomerically pure derivatives.

In the event, the synthesis of the key intermediate **87** was successfully synthesised by the second approach (see below) and therefore the potentially problematic reduction of **88** was not pursued.

The second approach employed commercially available isomannide **2** as a building block for the synthetic procedure The first step involved the tosylation of one of the equivalent hydroxyl groups of isomannide using TsCl in a solution of pyridine. This position was then inverted by an S_n2 substitution reaction using sodium azide in DMSO to introduce the azide group, which was subsequently reduced using Pd/C in the presence of H_2 to yield the corresponding amine. The tosylation and subsequent azide displacement of isosorbide, isomannide and isoiodide derivatives have been thoroughly investigated[118, 133]. It has been widely employed in the development of chiral catalysts for asymmetric synthesis[131, 135] and the synthesis of polymers[116, 117]. However, the azide displacement of the mono-tosylate derivative of isomannide **2** has not been previously reported.

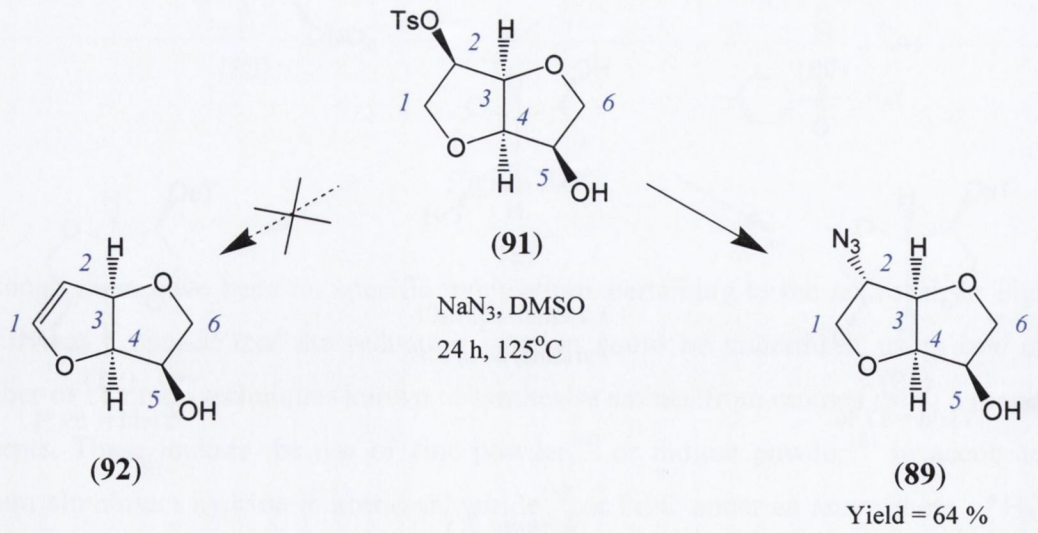
Mono-tosylation of one of the available hydroxy groups of isomannide was easily achieved by reaction with TsCl in pyridine (Scheme 2.3). Pyridine acts as both a solvent and a catalyst given its ability to neutralize the HCl generated in the reaction and shift the equilibrium of the reaction in favour of the tosylate formation[164].



Scheme 2.3

The reaction was carried out at -20°C in order to favour the formation of the mono-tosylate **91** from isomannide rather than the formation of di-tosylate **19** from the mono-tosylate. The use of 1.25 mol. eq of TsCl gave a yield of 59 % for the mono-tosylate compound **91**. This was the optimum yield achievable under these conditions, as any increase in the quantity of TsCl added, in order to push the reaction and increase the quantity of **91**, merely netted an increase in the undesired di-tosylate **19**[165].The mono-tosylate, the di-tosylate side product and any remaining starting material were separated by flash chromatography. As isomannide is a symmetrical compound, only optically pure mono-tosylate is generated from the reaction. This would not be the case for isosorbide, where two different mono-tosylate isomers can be obtained.

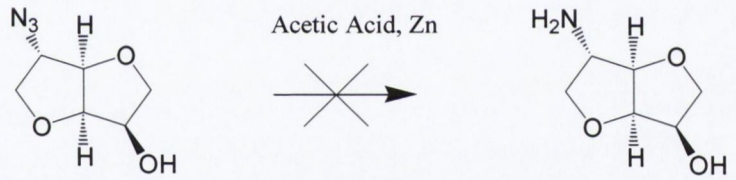
The azide displacement S_n2 reaction was performed by dissolving **91** in the polar aprotic solvent, DMSO. Excess sodium azide was added to the reaction vessel and the reaction mixture was heated to 125°C for approximately 24 hours. Other dipolar aprotic solvents such as DMF[134] and DMPU[166] can also be used in this type of reaction. These solvents were also investigated to see whether they would be appropriate solvents for the reaction. The reactions in each case appeared to progress more slowly than the reaction involving DMSO and therefore these reactions were not worked up.



Scheme 2.4

The azide displacement is an S_n2 reaction with the azide ion acting as a nucleophile. The azide ion attacks the isomannide carbon atom bonded to the tosylate group from the back of the molecule, causing the change in configuration and inversion of the stereochemistry of this position[167, 168]. As is shown in Section 1.7.5 of the introduction, reactions of this type often give an elimination product, such as **92**, however this side reaction was not observed during the synthesis of **89**.

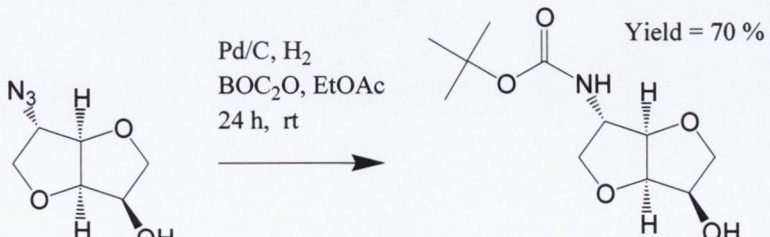
The final step of our synthetic approach to form the key intermediate **87** involved the reduction of the azide **89**. The reduction step was first attempted using zinc powder and glacial acetic acid[169], however due to the nature of the reactants, it was difficult to monitor the progress of this reaction by TLC. Therefore this procedure was abandoned in favour of another more convenient one-pot procedure[170] to give the BOC protected key intermediate **93** (Scheme 2.6).



(**89**) (**87**)

Scheme 2.5

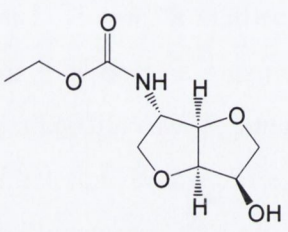
The one pot method involved adding a 200 mg of 10 % palladium on activated carbon to a solution of the azide **89** and di-*tert*-butyl dicarbonate in a solution of ethyl acetate, which was kept under as atmosphere of hydrogen gas.



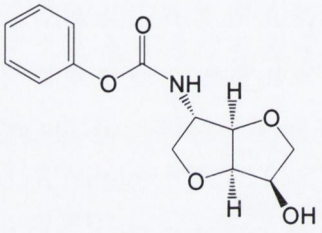
(**89**) (**93**)

Scheme 2.6

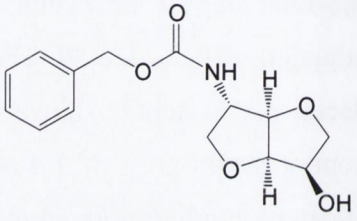
Compound **93** was synthesised in situ by the reduction of **89** followed by the reaction of the liberated amine with di-*tert*-butyl dicarbonate. The one-pot procedure was quite efficient and the target compound **93** was obtained as a white crystalline solid with a yield of 70 %. This approach was therefore applied to the synthesis of the ethyl **94**, phenyl **95** and benzyl **96** reversed carbamates, which were prepared using a relevant chloroformate reagent.



(**94**)



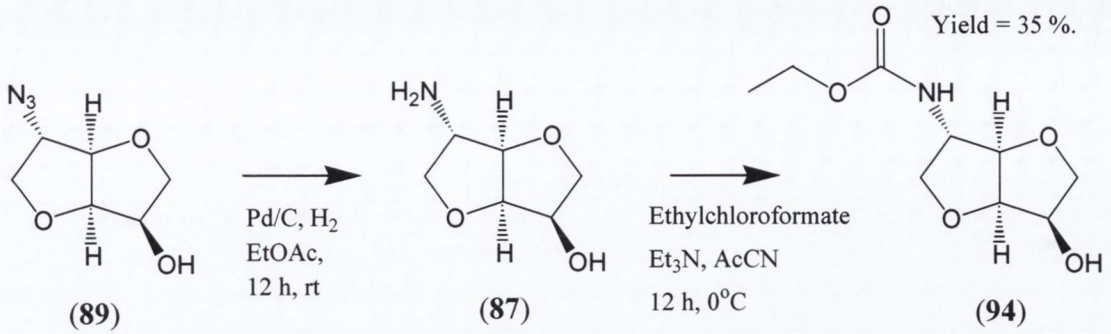
(**95**)



(**96**)

Figure 2.5

Compound **94** was first of this set of compounds synthesised. The procedure was carried out as before, however a yield of only 10 % was obtained for the ethyl carbamate **94**. There are a number of reasons why such a low yield was obtained compared to the di-*tert*-butyl dicarbonate reaction. Firstly, there was no customary base, such as triethylamine or potassium carbonate employed in the reaction mixture and therefore the HCl generated in the reaction could protonate the primary amine and thus interfere with the equilibrium and progress of the reaction. Secondly, as chloroformates are more reactive than anhydrides, it is a possible that a reaction may have occurred between the hydroxyl group in position-5 with the ethyl chloroformate. Indeed, quite a number of product spots were observed when the progress of the reaction was monitored by TLC. These side reaction products were not isolated or characterised. In order to increase the yield of the reaction the synthesis was repeated using a two-pot procedure as shown in Scheme 2.7.



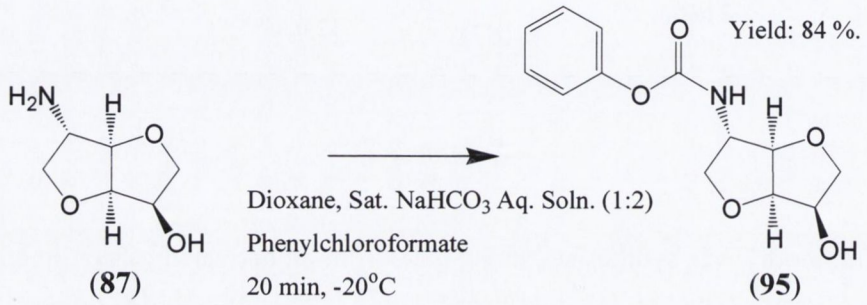
Scheme 2.7

Firstly the amino **87** was synthesised using palladium on activated carbon in ethyl acetate under an atmosphere of hydrogen gas[170]. The reduction reaction yielded the amine **87** as a clear oil after filtration of the palladium catalyst and evaporation of the organic solvent. The solubility of the amine in common laboratory solvents including acetone, DCM, chloroform, ethyl acetate, DMSO, pyridine and acetonitrile was investigated to ascertain the most appropriate solvent for the second step of the synthetic route. Of these, acetonitrile seemed to give the best solubility and therefore was chosen as the solvent for the reaction.

The second step of the procedure involved the reaction between the amine and ethyl chloroformate in the presence of triethylamine. The reaction was carried out at 0°C to discourage the reaction between the hydroxy group at position-5 and the chloroformate. These measures did improve the efficiency of the reaction, however the yield of the reaction was only increased to 35 %.

The phenyl carbamate **95** was also synthesised using the two-pot approach, giving a modest yielded of 32 %. As a sufficient quantity of this compound was required to generate a series of ester analogues in position-5, an alternative method was investigated to increase the yield of the reaction[171]. The reaction was performed in a mixture of dioxane and saturated $NaHCO_3$ aqueous solution at -20°C. The reaction mixture was stirred and phenyl chloroformate was added dropwise over ten minutes (Scheme 2.8).

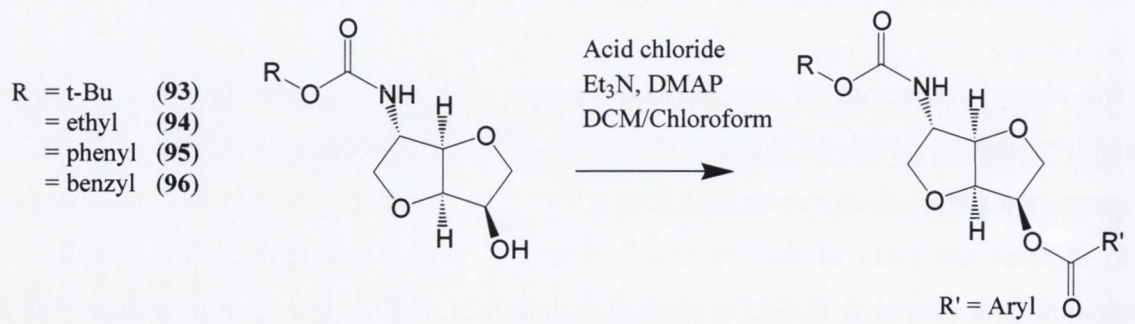
The reaction progressed quite rapidly and TLC analysis showed it was complete after a further ten minutes. Purification by flash chromatography gave **95** with remarkably improved yield of 84 %. The benzyl reverse carbamate compound **96** was also synthesised using this approach giving a yield of 63 %.



Scheme 2.8

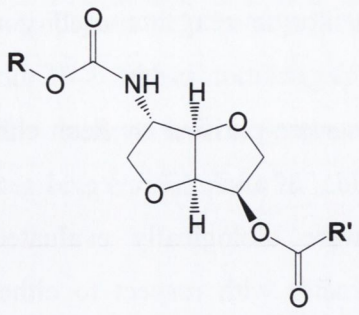
2.3 THE SYNTHESIS OF REVERSED CARBAMATE TEST COMPOUNDS

The final stage in the synthesis of reversed carbamates test compounds was to introduce the aryl ester function into position-5 of compounds **93, 94, 95** and **96.** This synthesis was achieved relatively easily by the reaction of these compounds with appropriate acid chlorides in the presence of triethylamine and the catalyst DMAP in a solution of DCM or chloroform[172, 173, 174]. Most of the reactions were carried out at room temperature, however, in some cases it was necessary to heat the reaction to reflux.



Scheme 2.9

In most reactions the acid chloride reagents used contained a certain amount of carboxylic acids, which were degradation products of the respective acid chloride, and therefore most test compounds were purified by flash chromatography to give the final target compounds in good yields. In total, 17 reversed carbamates test compounds were synthesised altogether and were biologically evaluated to ascertain whether they possessed any inhibitory character with respect to either huBuChE or AChE. These include the four compounds described in Section 2.2, which possess a reversed carbamate group in position-2 and a hydroxyl group in position-5 and a further 13 ester compounds derived from these, an aryl ester group in position-5. Figures 2.6 and 2.7 show all 13 reversed carbamate ester compounds. These 17 compounds represent the first library of compounds synthesised for this thesis.



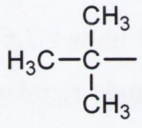
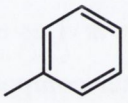
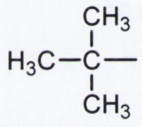
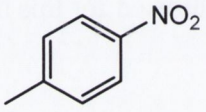
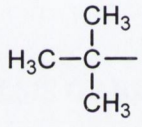
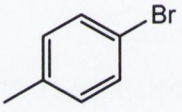
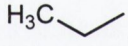
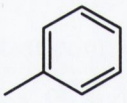
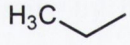
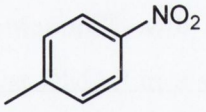
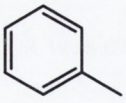
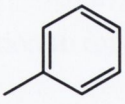
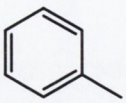
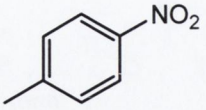
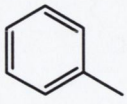
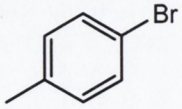
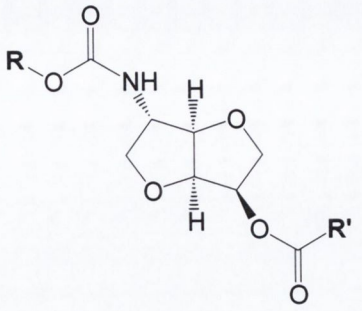
Compound No.	R	R'	Yield %
(97)			(72 %)
(98)			(59 %)
(99)			(81 %)
(100)			(54 %)
(101)			(67 %)
(102)			(93 %)
(103)			(80 %)
(104)			(89 %)

Figure 2.6



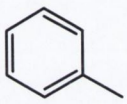
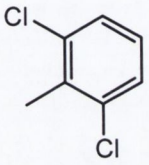
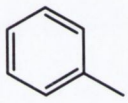
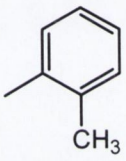
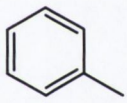
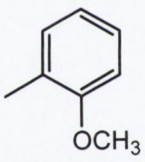
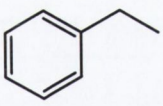
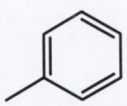
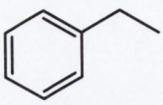
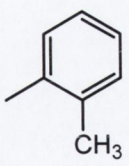
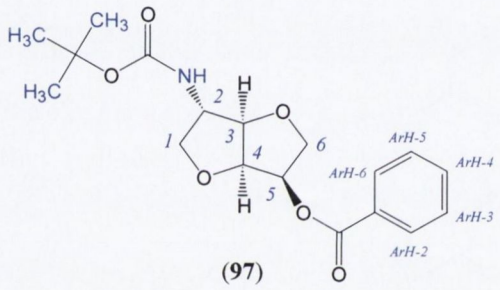
Compound No.	R	R'	Yield %
(105)			(54 %)
(106)			(67 %)
(107)			(37 %)
(108)			(36 %)
(109)			(48 %)

Figure 2.7

2.4 NMR SPECTROSCOPY OF LIBRARY-1: REVERSED CARBAMATE COMPOUNDS

The ^{1}H NMR spectrum of **97** is presented below in Figure 2.8 and is typical of nearly all the final test compounds in this library, except for compounds where there is no ester substitution at position-5. The eight hydrogens of the isosorbide moiety are all in different chemical environments due to the asymmetrical nature of the structure[175]. As each proton is coupled to other protons that are non-equivalent, the spectra tend to be quite complex, giving eight separate signals for the eight individual protons.



(97)

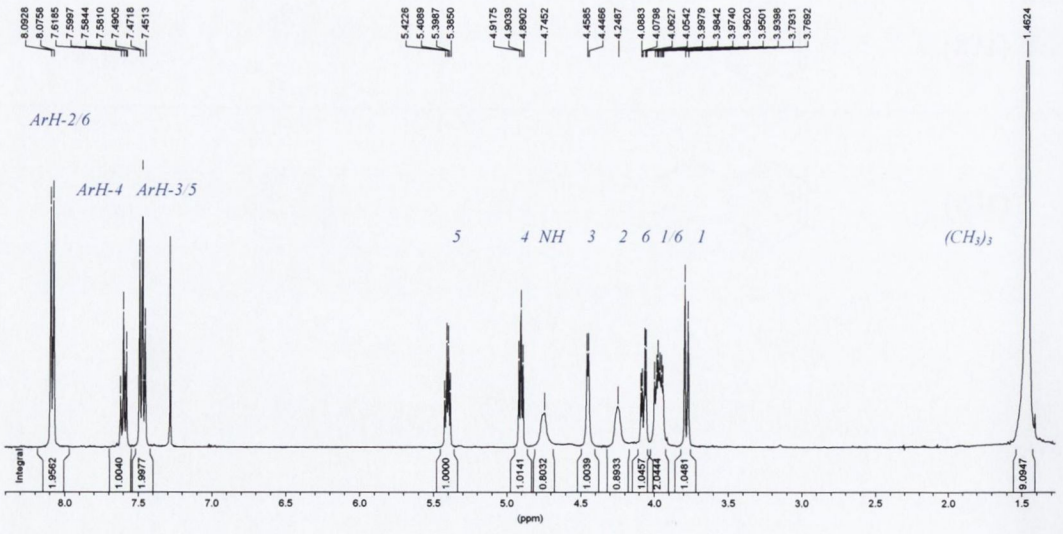


Figure 2.8

Although, the spectra of this series of compounds are complex, the same pattern of ^{1}H signals is observed from compound to compound in terms of the chemical shifts and the spin coupling. Generally speaking the order of signal from upfield to downfield are; one IsH-1 proton (doublet), a two proton signal from one IsH-6 proton and one IsH-1 proton (multiplet), one IsH-6 (double doublet), IsH-2 (broad singlet), IsH-3 (doublet), NH signal from the carbamate group (broad singlet), IsH-4 (triplet), IsH-5 (multiplet). All aromatic or aliphatic signals are detected in the characteristic regions downfield or upfield respectively.

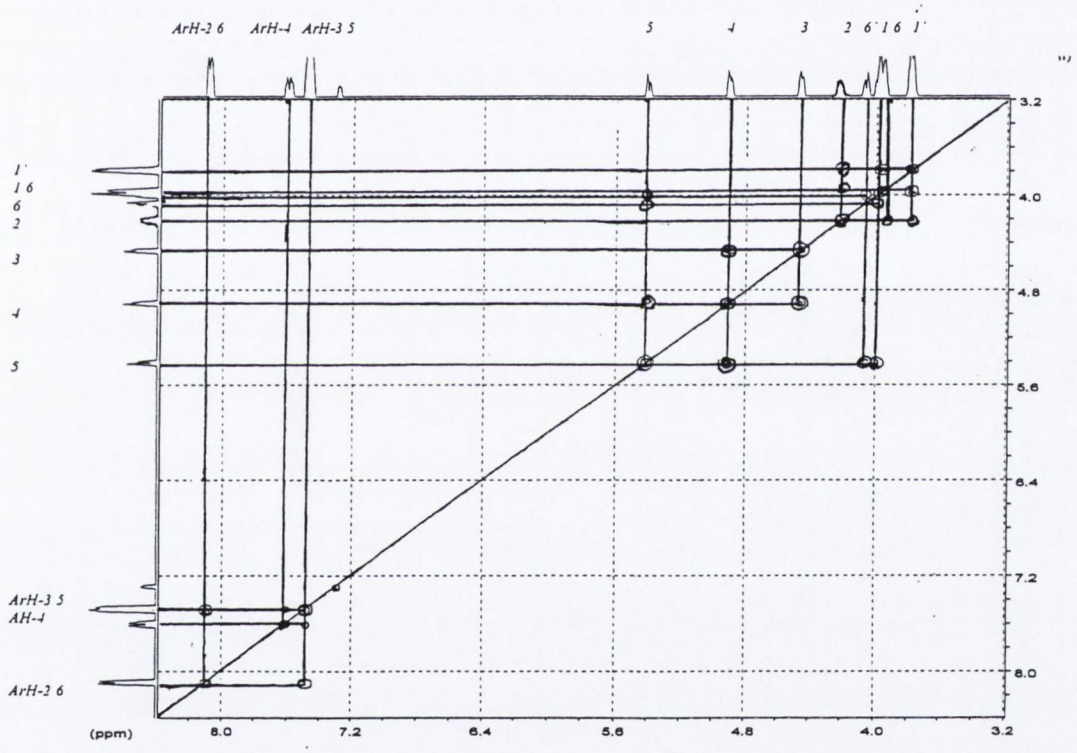


Figure 2.9

From the H-H COSY NMR spectra of these compounds (e.g. Figure 2.9) it was possible to differentiate the peaks from one other and analyse which peaks were coupled to one another. Analysis of the cross peaks of the spectrum above shows that IsH-5 couples to IsH-4 and to IsH-6 and as each of these protons are in different environments, IsH-5 is given as a multiplet. It was also possible to distinguish IsH-6 from IsH-1, given that IsH-6 couples to IsH-5 and IsH-1 couples to IsH-2, which is given as a broad singlet. IsH-1

and IsH-6 each give two separate signals for their respective pairs of hydrogen atoms, giving four signals altogether. One proton of each of these overlap in the ^{1}H spectra in most compounds of this series and as the signals are so close together, in some cases, all four proton signals overlap giving one large multiplet. IsH-4, which is in an exo position, couples to both IsH-3 and IsH-5. Both of these protons are also in exo positions and IsH-4 occurs as a triplet. IsH-3 couples only to IsH-4 and is therefore a doublet. In compounds where there is no ester substitution at position-5, the ^{1}H signals of IsH-4, IsH-5 and IsH-6 (2 hydrogens) are shielded to a greater degree than in ester substituted compounds and consequently the signals are shifted upfield to 4.56, 4.30, 3.88 and 3.62 respectively.

The ^{13}C spectra of this series of compounds also follow a characteristic pattern with the IsC-3 carbon of the isosorbide moiety being the signal shifted most downfield followed by IsC-4, IsH-5, IsC-1, IsC-6, IsC-2 respectively progressing upfield. C-H COSY NMR spectra were used to assign the isosorbide peaks observed in the ^{13}C spectra. It was also possible to assign the aromatic peaks to the respective aromatic carbon atoms.

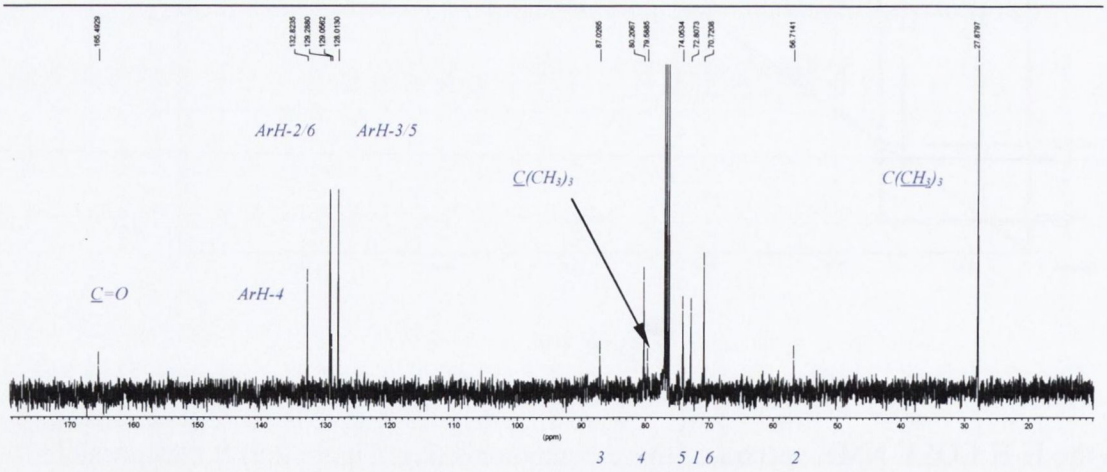


Figure 2.10

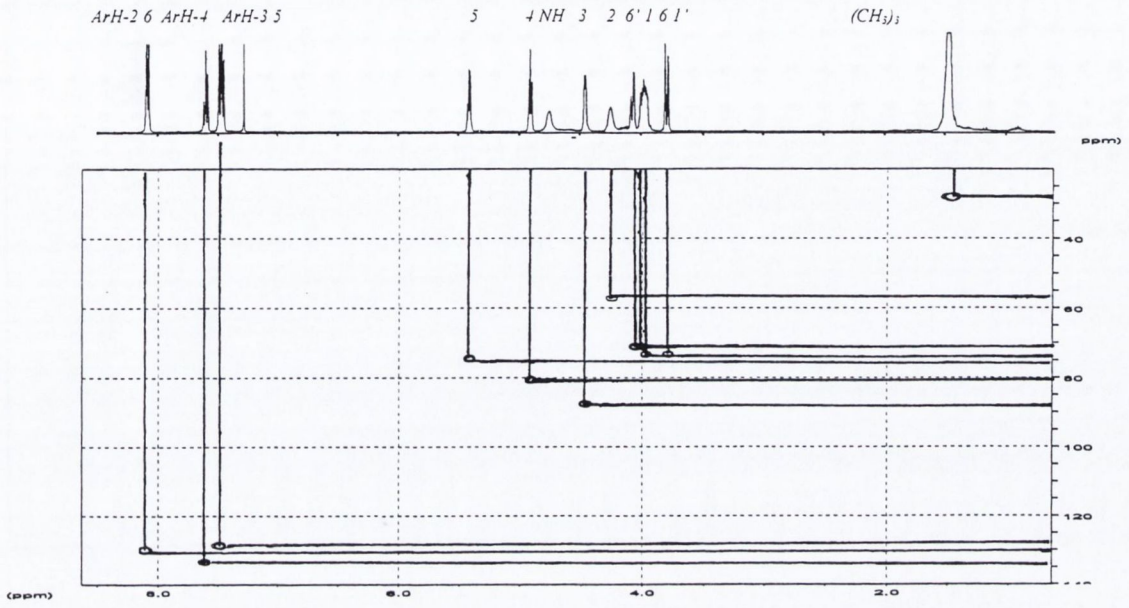


Figure 2.11

NMR analyses were also used to gain an understanding of the chemical structure of the compounds of this series. It was critical that an unequivocal understanding of the stereochemistry of these compounds was obtained in order to make the correct assessments and draw the necessary conclusions from these compounds with respect to their SARs. NMR data was collected for intermediate compounds, such as 2-*O*-*p*-tosyl-1,4:3,6-dianhydro-D-glucitol **91** and 2-deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **93** in order to confirm the successful inversion of position-2 from a endo-tosylate functional group to an exo-carbamate functional group, as shown in Figure 2.12.

In the spectra of **91**, the IsH-2 proton signal is coupled to IsH-1 and IsH-3 giving a multiplet similar to the signal of IsH-5. Likewise, IsH-3 couples to IsH-2 and IsH-4 and appear as a triplet, similar to the triplet of IsH-4, which is coupled to IsH-5 and IsH-3. Inversion of this position vis-à-vis, S_n2 substitution, gives a ^1H spectra where IsH-2 is coupled only to IsH-1 and is given as a broad singlet and IsH-3 couples only to IsH-4 and is given as a doublet.

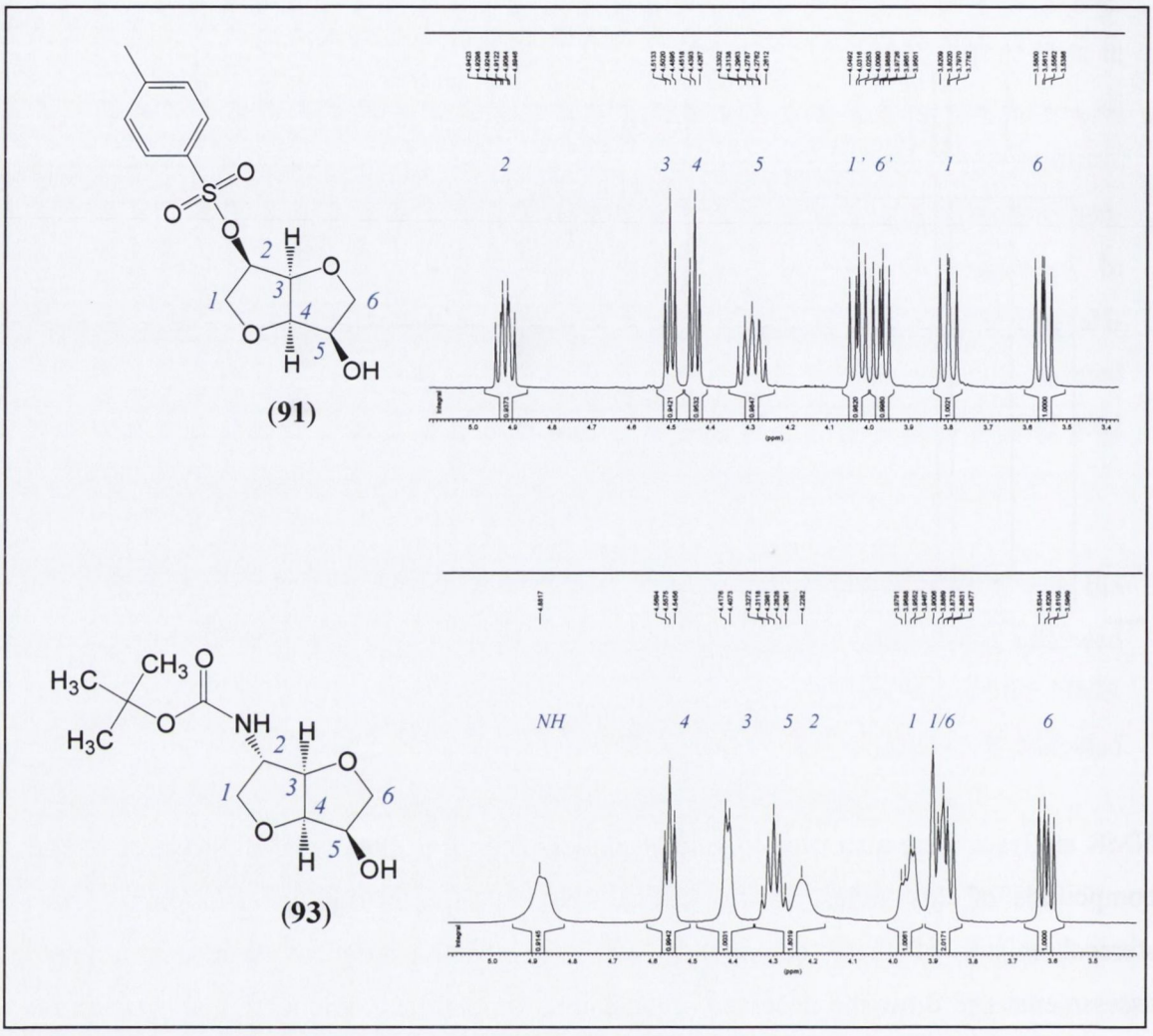


Figure 2.12

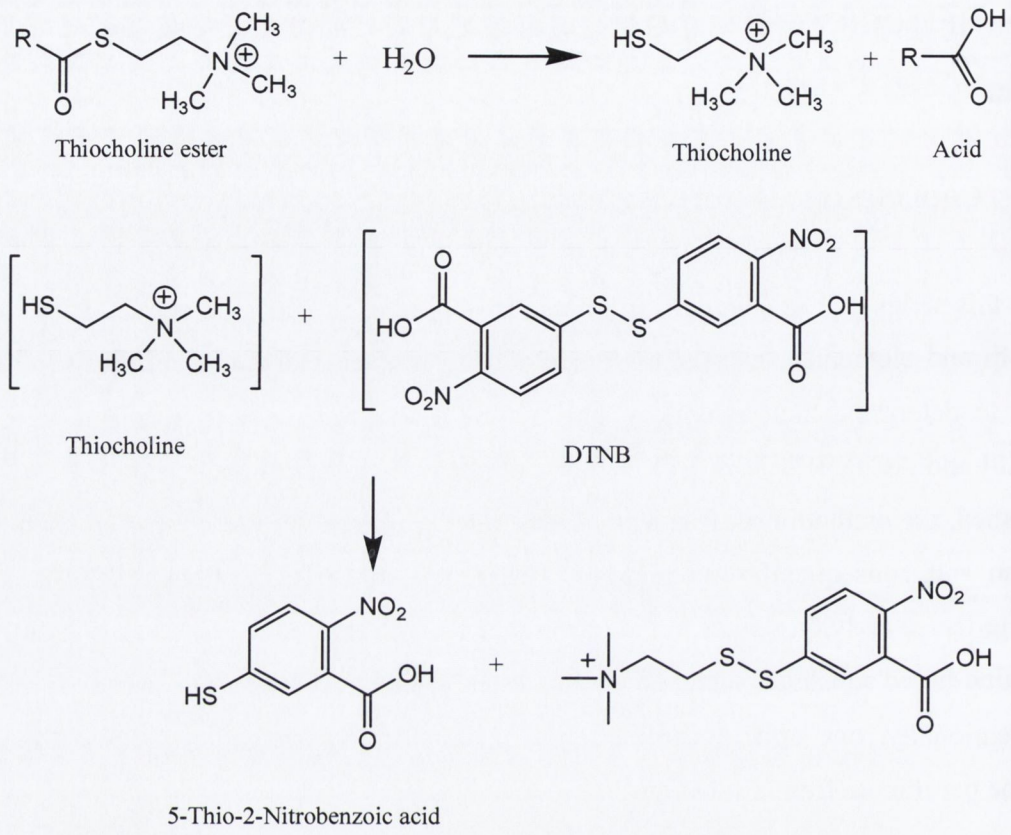
2.5 BIOLOGICAL TESTING OF LIBRARY-1: REVERSED CARBAMATE COMPOUNDS

2.5.1 CHOLINESTERASE ACTIVITY

After this series of compounds was successfully synthesised and characterised by NMR, IR, MS and elemental analysis, biological testing was carried out on the compounds in order to determine their inhibitory activity and value with respect to huBuChE [E.C. 3.1.1.8] and AChE [E.C. 3.1.1.7]. While a number of biological test methods have been published, the method most frequently used to determine cholinesterase activity is that of Ellman and consequently is commonly referred to as the Ellman assay[176]. The term cholinesterase activity, relates to the rate at which a cholinesterase enzyme can hydrolyse a choline-based substrate, such as a choline ester, to liberate choline as a primary product. Conventionally, one unit of cholinesterase activity is that which produces 1 μmol of choline per minute from a substrate[177].

2.5.2 THE ELLMAN ASSAY

The Ellman assay involves measuring the cholinesterase activity in an aqueous solution containing a choline ester substrate in the presence of a relevant cholinesterase[176]. The cholinesterase substrates generally used are ATCI, when determining the activity of AChE, and BTCI when determining the activity BuChE[178]. The substrate is catalytically hydrolysed to give thiocholine and the corresponding carboxylic acid. The liberated thiocholine then reacts with DTNB to give 5-thio-2-nitrobenzoic acid, which is yellow in colour. The rate of evolution of the yellow colour can be monitored at a wavelength of 412 nm using a UV/Vis spectrometer[176].



Scheme 2.10

This assay is based on measuring the amount of product formed as opposed to the amount of substrate remaining[176]. The substrates used are specific for the cholinesterase under analysis, i.e. BTCI is only hydrolysed by BuChE under the analytical conditions of the method[178]. As the reaction between the thiocholine liberated and DTNB is practically instantaneous, the rate of evolution of the yellow colour is directly proportional to the rate of ester hydrolysis and as the assay is linear, the slope of the rate of the reaction can be used to calculate the enzyme activity[179]. In order to determine the inhibition activity of the test compounds, a certain concentration of an inhibitor is included in the test solution. The cholinesterase activity is then determined in the inhibition solution and compared to the uninhibited activity of the enzyme to give an inhibition value for that compound at a specific concentration[180].

A 96 well plate method for the biological analysis of the reversed carbamate compounds was developed, which could carry out a number of IC_{50} assays very rapidly. The 96 well plate method was adapted from and based on the same principles as the Ellman method. In the Ellman method, 3.16 ml test samples were prepared individually and analysed one by one using a conventional UV spectrometer. This process is quite time consuming and labour intensive. On a 96 well plate, each well can hold a volume of approximately 350 µl and therefore the 96 well plate method simply scaled down the volume of each reagent used in the assay. The assay procedure was validated by determining the IC_{50} value of physostigmine in replicate. The IC_{50} values obtained were consistent with those obtained by the conventional Ellman method and those published in the literature concerning physostigmine inhibition[7, 56, 181].

By using the 96 well plate method, it was possible to simultaneously determine the activity of huBuChE (or AChE) and analyse a number of inhibitors on a single plate. It was also possible to analyse more than one inhibitor over a range of concentrations, where the concentrations of each inhibitor could be performed in replicate. This method not only decreased the time spent performing the assays but also improved the accuracy and reliability of the results by the virtue of the simultaneous replicate analysis of each test solution. It was also possible to simultaneously determine the IC_{50} value of physostigmine, which could act as a control to validate the assay result. The method also involved less sample preparation of stock solutions due to the small sample volume of reagents required for each assay. The assay procedure for determining enzyme and inhibitor activities is included in the experimental chapter of this thesis.

A huBuChE inhibition assay was performed on all reversed carbamate test compounds, where the concentration of the inhibitor in the test solution was 100 µM. This was done in order to 'screen' all the compounds to assess their inhibitory activity at a relatively high concentration. Compounds showing good inhibition at 100 µM was then analysed over a range of lower concentrations to determine their respective IC_{50} values (an IC_{50} value is the concentration of an inhibitor compound, which can inhibit an enzyme by 50 % under a certain set of test conditions[182]). IC_{50} values give a measure of how potent an

inhibitor is with respect to an inhibited enzyme and are used to compare inhibitor compounds with the published IC_{50} values of established inhibitors such as physostigmine[181], *iso*OMPA[183], tacrine[184] etc.

The assay procedure was also carried out to determine the AChE inhibition values of the test compounds using electric eel AChE. While it was the primary objective of this project to synthesise potent and selective inhibitors of huBuChE based on new templates, the possibility of synthesising potent and selective inhibitors of AChE would not necessarily represent an unfavourable outcome given that AChEI's have been employed as therapeutic agents in combating AD[185, 186, 187]. Indeed, some AD therapeutic agents such as rivastigmine are mutual inhibitors of both AChE and huBuChE[188, 189]. In any event, the determination of AChE inhibition was necessary to show the selectivity of huBuChE over AChE for compounds showing huBuChE inhibition and visa versa if appropriate.

2.5.3 BIOLOGICAL TEST RESULTS OF LIBRARY-1 COMPOUNDS

The biological screening test results of Library-1 compounds for huBuChE and AChE inhibition are given in Table 2.1. The results of the biological testing gave a detailed assessment of the SARs of the test compounds. The findings show that **93**, **97, 98** and **99**, bearing a t-butyl reversed carbamate a position-2 are very poor inhibitors of huBuChE. This may be due to the bulky nature of the BOC group, which may experience some steric hindrance either entering the active site gorge or interacting with the catalytic triad of amino acids within the gorge. This is substantiated by molecular modelling studies, which show that the structure of **97** does not fit particularly well within the active site due to the proximity of the BOC group to a number of amino residues. Compounds bearing an ethyl carbamate of position-2, **94, 100** and **101**, were marginally superior inhibitors of huBuChE compared to the t-butyl carbamates, however with inhibition of huBuChE ranging between 9.3 and 35.6 % at 100 μM, these were also considered quite poor inhibitors of huBuChE.

Table 2.1: % huBuChE and AChE inhibition of Library-1 compounds at 100 μM

No.	**Name** (Refer to page 62 and 63 for the chemical structure of each compound)	% BuChE[a] 100 μM	% AChE[b] 100 μM
93	2-deoxy-2-(t-butyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol	13.8	3.5
97	2-deoxy-2-(t-butyloxycarbonylamino-) 5-*O*-benzoyl-1,4:3,6-dianhydro-D-glucitol	2.0	5.7
98	2-deoxy-2-(t-butyloxycarbonylamino-) 5-*O*- *p*-nitrobenzoyl-1,4:3,6-dianhydro-D-glucitol	9.5	4.6
99	2-deoxy-2-(t-butyloxycarbonylamino-) 5-*O*- *p*-bromobenzoyl-1,4:3,6-dianhydro-D-glucitol	9.6	2.8
94	2-deoxy-2-(ethyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol	9.3	2.0
100	2-deoxy-2-(ethyloxycarbonylamino-) 5-*O*-benzoyl-1,4:3,6-dianhydro-D-glucitol	29.3	0.8
101	2-deoxy-2-(ethyloxycarbonylamino-) 5-*O*- *p*-nitrobenzoyl-1,4:3,6-dianhydro-D-glucitol	35.6	0.4
95	2-deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol	25.0	0.8
102	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*-benzoyl-1,4:3,6-dianhydro-D-glucitol	78.5	0.4
103	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*- *p*-nitrobenzoyl-1,4:3,6-dianhydro-D-glucitol	92.1	45.4
104	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*- *p*-bromobenzoyl-1,4:3,6-dianhydro-D-glucitol	89.4	68.1
105	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*- 2,6 dichlorobenzoyl-1,4:3,6-dianhydro-D-glucitol	5.1	45.3
106	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*-*o*-methylbenzoyl-1,4:3,6-dianhydro-D-glucitol	95.2	1.0
107	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*-*o*-methyloxybenzoyl-1,4:3,6-dianhydro-D-glucitol	65.3	0.3
96	2-deoxy-2-(benzyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol	3.5	0
108	2-deoxy-2-(benzyloxycarbonylamino-) 5-*O*-benzoyl-1,4:3,6-dianhydro-D-glucitol	8.3	65.0
109	2-deoxy-2-(benzyloxycarbonylamino-) 5-*O*-*o*-methylbenzoyl-1,4:3,6-dianhydro-D-glucitol	4.4	45.2

[a] BuChE from human plasma [b] AChE obtained from electric eel
Values shown are the mean of three readings

Compounds bearing a phenyl carbamate at position-2 showed good inhibition of huBuChE at 100 μM, with the exception of compounds **95** and **105**. This group of compounds also highlighted the importance of ester substitution at position-5, where the unsubstituted compound **95** only gave 25 % inhibition of huBuChE at 100 μM, whilst **106**, which was substituted with a toluoyl ester at position-5, showed a significant increase in inhibitory activity giving 95.2 % inhibition at 100 μM. The SARs also show that the type of substitution on the aryl group has an affect on the inhibitory activity of the compounds, with **103, 104** and **106** giving better inhibition than the unsubstituted benzoate ester **102**. Conversely, the di-chloro substituted compound **105** gave very poor inhibition of huBuChE with an inhibition value of only 5.1 % at 100 μM.

These results show that ester substitution at position-5 is important for the interaction and binding of the inhibitor within the active site. Molecular modelling of these compounds shows the interaction between the aryl groups of the esters and the amino acid residue, Tryptophan 82. These interactions stabilise the inhibitor within the active site thus allowing the compound to successfully inhibit the enzyme. This point was further investigated and is discussed in greater detail in Chapters 3 and 4.

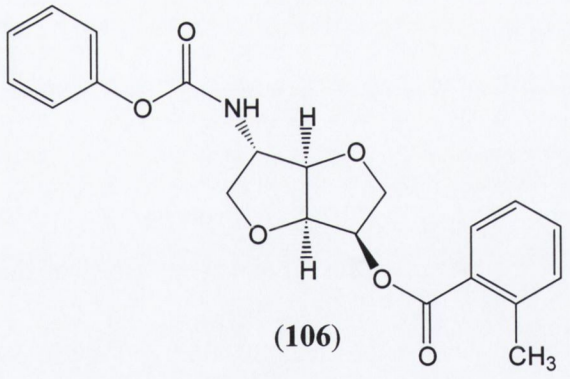
Curiously, **108** and **109**, bearing a benzyl carbamate at position-2, were very poor inhibitors of huBuChE, while they exhibited some degree of inhibition of AChE at 100 μM. Compounds **103, 104** and **105**, bearing a phenyl carbamate at position-2 also demonstrated some AChE inhibition at this concentration. However even the most potent of these compounds, **104** and **108**, showed inhibition of less than 50 % in AChE at 80 μM and therefore the IC_{50} values of these compounds were not determined.

Compounds showing good inhibition of huBuChE at 100 μM were analysed at concentrations of inhibitor ranging between 100 and 10 nM to determine the IC_{50} values of these compounds. The IC_{50} results of these compounds are given in Table 2.2.

Table 2.2: IC_{50} values determined of Library 1 compounds

No.	Name	IC_{50} (μM)
102	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*-benzoyl-1,4:3,6-dianhydro-D-glucitol	32.8
103	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*- *p*-nitrobenzoyl-1,4:3,6-dianhydro-D-glucitol	16.1
104	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*- *p*-bromobenzoyl-1,4:3,6-dianhydro-D-glucitol	21.9
106	2-deoxy-2-(phenyloxycarbonylamino-) 5-*O*-*o*-methylbenzoyl-1,4:3,6-dianhydro-D-glucitol	1.5

The most potent inhibitor of the reverse phenyl carbamate compounds was found to be **106**, which gave 95.2 % inhibition of huBuChE at 100 μM and an IC_{50} of 1.5 μM. This result not only represented the most potent inhibitor in this library of compounds but also demonstrated an interesting SAR, with the presence of the toluoyl group substituted in the ortho position of the 5-benzoate ester. The effect of the toluoyl substitution can be shown by the increase in inhibition of huBuChE at 100 μM from 78.5 % to 95.2 % in **102** and **106** respectively and also by the significant decrease in IC_{50} from 32.8 to 1.5 μM.



(106)

Figure 2.13

2.6 DISCUSSION

The overall evaluation of reversed carbamate test compounds as huBuChE inhibitors is that they show moderate and selective inhibition of huBuChE in some cases, with the lead compound **106** giving an IC_{50} value of 1.5 μM and is at least 66-fold more selective for huBuChE over AChE*. However, the results for Library-1 compounds seem quite poor when compared to established huBuChE inhibitors as shown in Table 2.3.

Table 2.3: IC_{50} values of huBuChE inhibitors

Name	huBuChE (nM)
Compound **106**	1500
Physostigmine[6]	16
Rivastigmine[6]	37
*Iso*OMPA[6]	980
MF-8622[6]	9
Compound **110**	4.3
Compound **111**	50

More important however, is how the compounds compared with the group 1 compounds, which were synthesised by the sister research group based at the Athlone Institute of Technology, Ireland.

This group reported highly potent and selective inhibitors of huBuChE, with their lead compounds having IC_{50} values of 4.3 and 50 nM and selectivity of 10,000 and 2,000 for **110** and **111** respectively. These results were reported in a joint paper, which included the synthesis and SAR of both group 1 and reversed carbamate compounds[190].

* The inhibition of AChE by compound **106** is negligible at 100μM. Calculating the selectivity based on this concentration and IC_{50} for BChE of compound gives a selectivity of at least 66.6 for huBuChE over AChE.

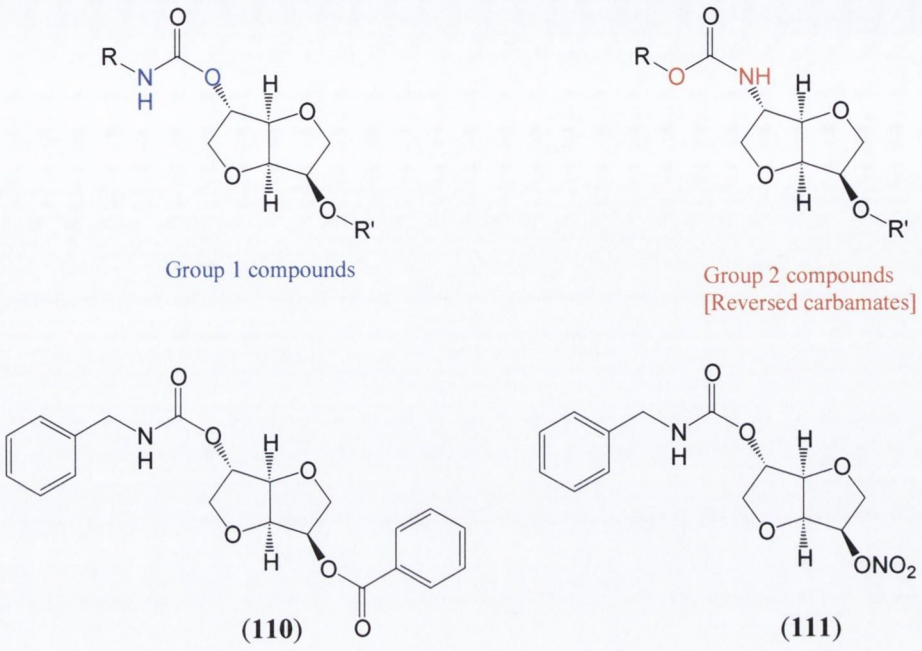


Figure 2.14

By comparing the SARs of both group 1 and reversed carbamate compounds, it was abundantly clear that compounds bearing a group 1 structured carbamates, particularly compounds bearing a benzyl carbamate as in compound **110**, acted as far superior inhibitors of huBuChE compared to reverse carbamate compounds. This SAR information was used to further refine the isosorbide-based inhibitor model. Therefore, it was decided that the next library of compounds would involve the synthesis of compounds bearing a group 1 benzyl carbamate group in position-2 while varying substitution in position-5. The positive SAR effects evidenced by the substitution of the aryl group in Library-1 compounds, particularly in the case of ortho substitution in compound **106**, was also borne in mind when considering the design and synthesis of future compounds. This type of synthesis had not been investigated by the sister project group in the Athlone Institute of Technology, who went on to focus on enzyme and inhibitor interactions including the competitive/uncompetitive nature, kinetics and dialysis of isosorbide based huBuChE inhibitors. At the time of writing, the results of this work are in the process of being published in scientific literature.

2.7 SUMMARY

To summarise Chapter 2, a library of reversed carbamate compounds was prepared with a view to the synthesis of potent and selective inhibitors of huBuChE using isosorbide as a building block and to compare the SAR of these compounds with group 1 compounds, which were synthesised by the sister research group based at the Athlone Institute of Technology, Ireland. There were 17 reversed carbamate test compounds prepared altogether and characterised by a range of analytical methods including NMR, MS, IR and elemental analysis and they were also subjected to biological analysis to determine their inhibitory activity with respect to huBuChE and AChE.

While the results of the biological testing showed that some reversed carbamate compounds were moderately good inhibitors of huBuChE, the compounds were not as efficient in inhibiting huBuChE or AChE as group 1 compounds or established cholinesterase inhibitors, such as physostigmine and rivastigmine. However, the synthesis and design of reversed compounds was completely novel and unlike any other compounds published in scientific literature. The synthesis and results of this chapter and the synthesis and results of group 1 compounds synthesised and evaluated by the sister research group were published in a joint paper[190].

As a result of this work, it was decided that the next body of work would involve the synthesise of a library of compounds bearing an group 1 benzyl carbamate group at position-2 while varying the substitution at position-5. It was hoped that this would yield compounds with increased potency and selectivity of huBuChE.

CHAPTER 3

THE SYNTHESIS AND BIOLOGICAL TESTING OF LIBRARY-2 COMPOUNDS: DERIVATIVES OF 2-BENZYL CARBAMATE ISOSORBIDE

3.1 INTRODUCTION

This chapter describes the development of a second library of huBuChE inhibitors using isosorbide as a building block. The design of the inhibitors in this library was based on the SAR information obtained from Chapter 2 and from the group 1 compounds that were prepared by our broader research group[190].

Research showed that in group 1, a benzyl carbamate at position-2 was the most effective group in giving inhibition of huBuChE. The design for the second library of inhibitors was therefore based on the structure of **110** shown in Figure 3.1. In this chapter we wished to retain the group 1 benzyl carbamate at position-2, whilst probing the SAR at position-5. Molecular modelling studies showed that the ester group of **110** pointed back up through the active site gorge. These studies suggested that the dimensions of the gorge might be able to accommodate larger esters at position-5, than the benzoate ester of **110,** and subsequently this prompted us to construct inhibitors bearing large aromatic and aliphatic ester groups in this position. It was hoped that these large esters might increase the binding of the inhibitors to the active site and therefore to give inhibitors with increased potency and selectivity of huBuChE. It was also possible that the SAR of the group might inform on the binding mode of isosorbide-based inhibitors in general.

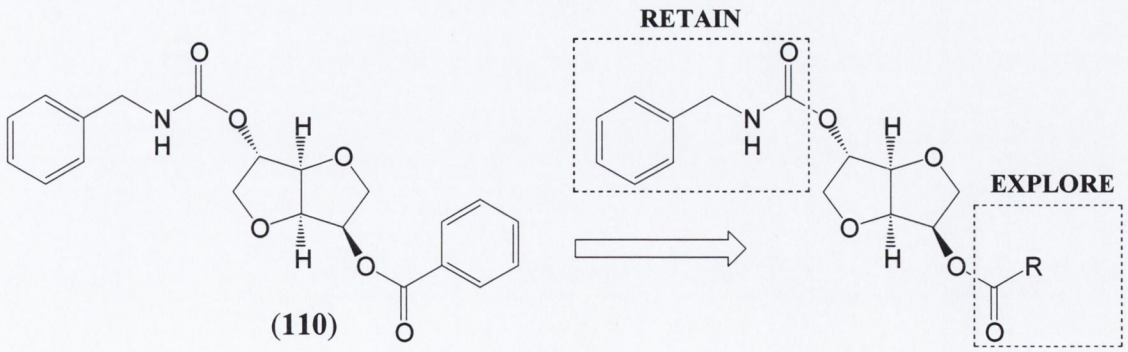


Figure 3.1

It was also intended to further probe the SAR of position-5 and increase the scope of this chapter by including the synthesis of inhibitors with other groups at position-5, such as ethers, amides and ketones, as shown in Figure 3.2. In addition, from the ketone **113**, it was possible to attempt the synthesis of several other compounds, including oximes, alkenes, lactones and lactams.

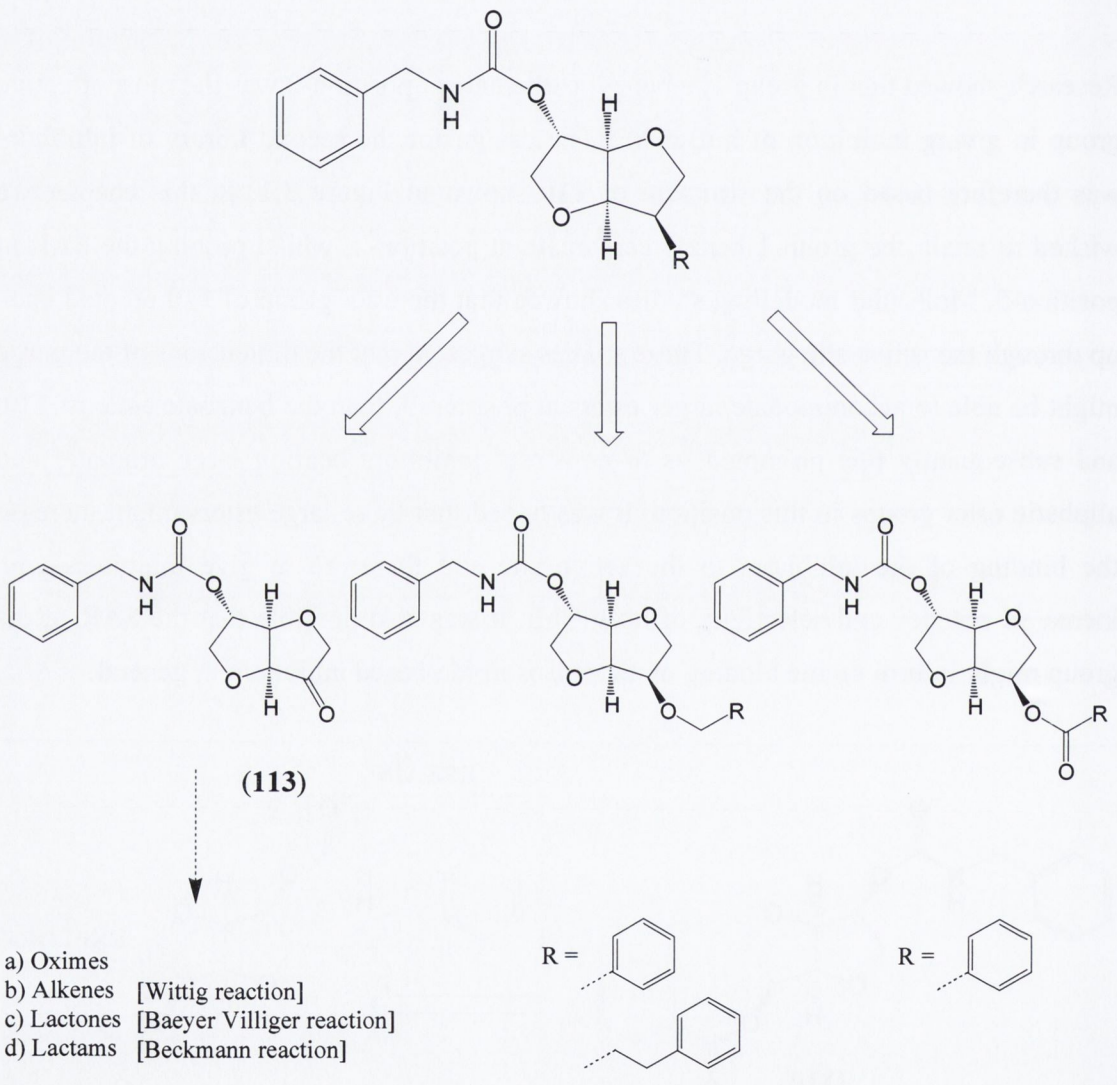


Figure 3.2

The synthesis of the ester, ketone, ether and amide compounds required several different approaches. The approach to the synthesis of ester and the ketone analogues is shown in Figure 3.3 and discussed first, whilst the synthetic approaches taken for the synthesis of the ether and amide compounds are discussed later in the chapter.

The approach taken to the ester and ketone analogues involved the synthesis of the key intermediate **112** from IS-5-MN **62**. This was achieved by the carbamoylation of position-2 with benzyl isocyanate to give the benzyl carbamate group, followed by reduction of the nitrate group at position-5 to give the hydroxyl group. From **112** it was possible to synthesise a range of aliphatic and aromatic esters using acid chlorides, acid anhydrides or carboxylic acids. It was also possible to synthesise the ketone **113** by the oxidation of the hydroxyl group at position-5 of **112**. From this compound it was possible to prepare a number of derivatives including oximes and alkenes as well as lactones and lactams.

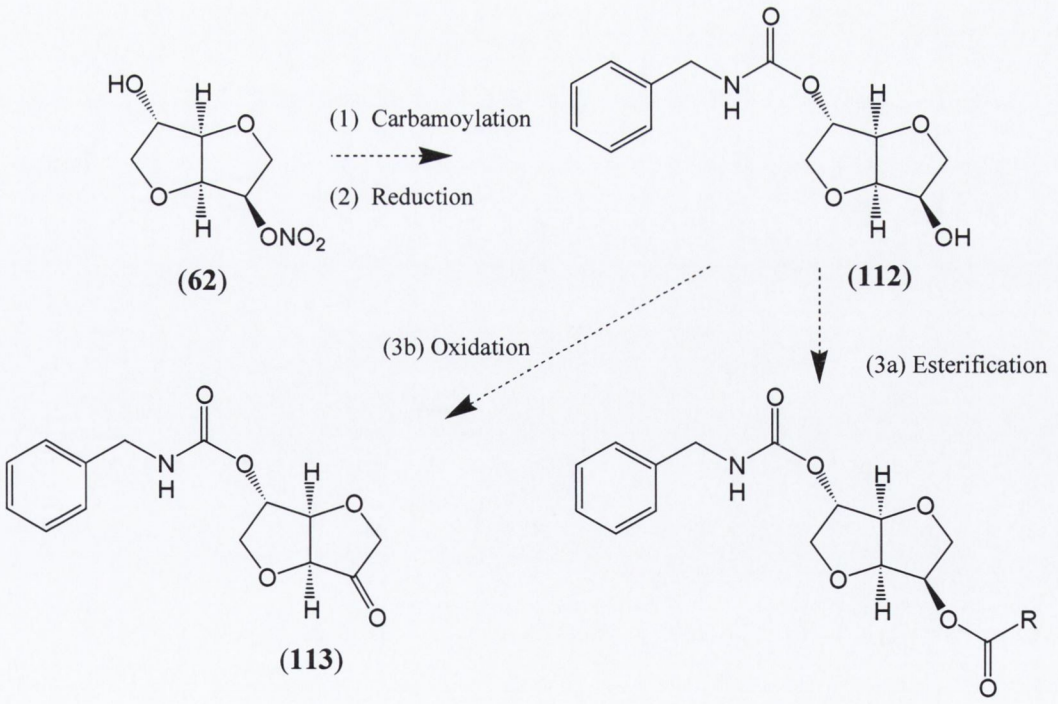
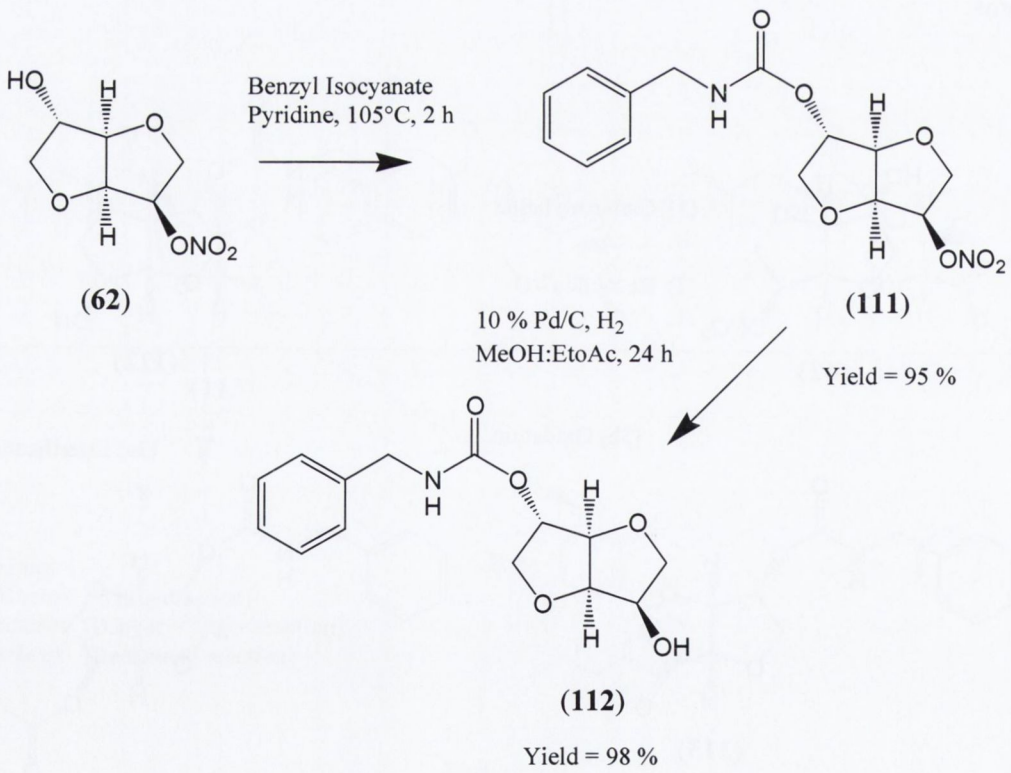


Figure 3.3

3.2 THE SYNTHESIS OF ESTER AND KETONE DERIVATIVES OF 2-BENZYL CARBAMATE ISOSORBIDE

3.2.1 THE SYNTHESIS OF 2- BENZYL CARBAMATE ISOSORBIDE

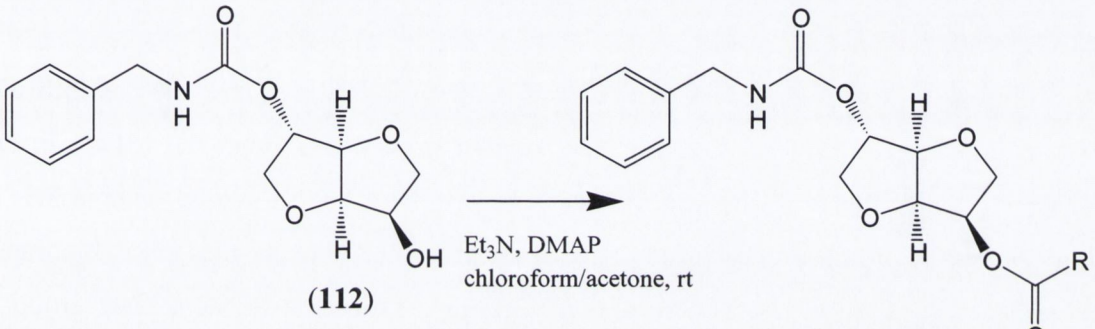
The synthesis of the key intermediate **112** is shown in Scheme 3.1. This approach first involved the reaction of IS-5-MN **62** with benzyl isocyanate in pyridine to give **111**[191]. This type of reaction has also been reported using an isocyanate in the presence of triethylamine and DMAP in DCM[192] or in the presence of dimethylaminoethanol in a solution of benzene[193]. The second step involved the reduction of the nitrate group at position-5 with 10 % Pd/C and H_2[194] to give the desired intermediate **112** as a white crystalline product in an excellent yield.



Scheme 3.1

3.2.2 SYNTHESIS OF ALIPHATIC ESTERS AT POSITION-5

A series of aliphatic esters were prepared by reaction of **112** with acid anhydrides in the presence of triethylamine and the catalyst DMAP[195]. A solution of chloroform: acetone (50:50) was used as solvent to ensure the solubility of the polar starting material, **112**. The acid anhydrides used were acetic anhydride, propionic anhydride and valeric anhydride respectively and therefore, the esters produced varied in terms of the numbers of carbons in the aliphatic chain.



(112)

	R	Yield
(114)	methyl	(90 %)
(115)	ethyl	(84 %)
(116)	butyl	(77 %)

Scheme 3.2

The aliphatic esters were obtained easily in good yields and purification by flash chromatography was not necessary as the compounds were recrystallised efficiently from hot ethyl acetate and hexane.

The mesylate and triflate sulfonyl esters were also synthesised using a similar approach as for the synthesis of the aliphatic esters. Methanesulfonyl chloride reagent was used for the synthesis of the mesylate compound **117**[196], while trifluoromethanesulfonic anhydride was used for the preparation of the triflate compound **118**[197]. Due to the highly reactive nature of trifluorosulfonic anhydride, this reaction was carried out with the careful exclusion of moisture by using dry glassware and anhydrous solvent.

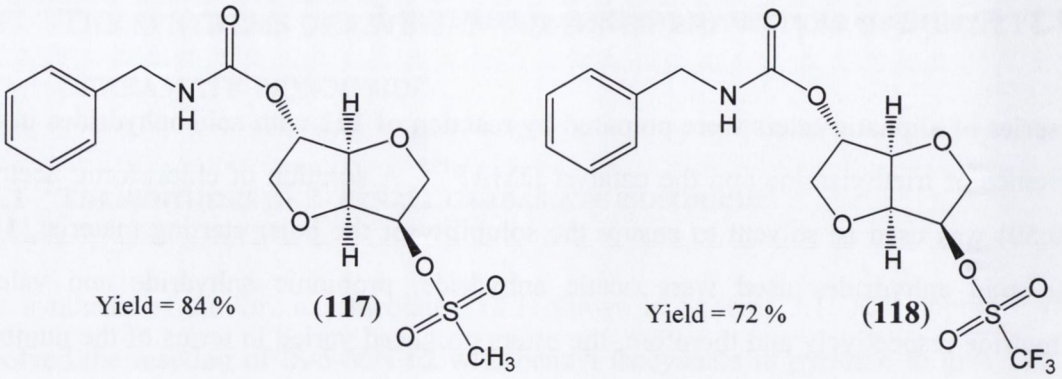
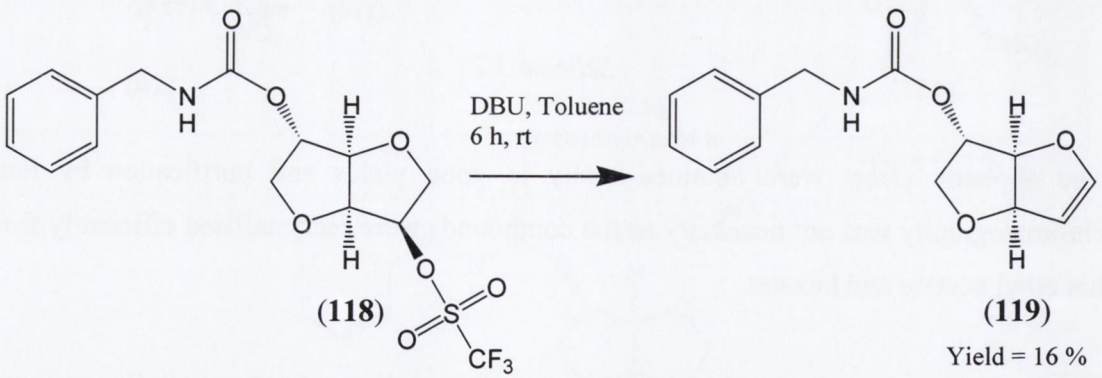


Figure 3.4

As an extension to this work, the synthesis of the triflate **118** presented the possibility to pursue the alkene **119.** The synthesis was carried out by dissolving the triflate in dry toluene in the presence of the tertiary amino base, DBU[156] as shown in Scheme 3.3. In this reaction S_n2 substitution at position-5 is prevented due to the steric hindrance experienced by the bulky structure of DBU giving elimination of the triflate group at position-5 and formation of the double bond between carbon-5 and 6[156].

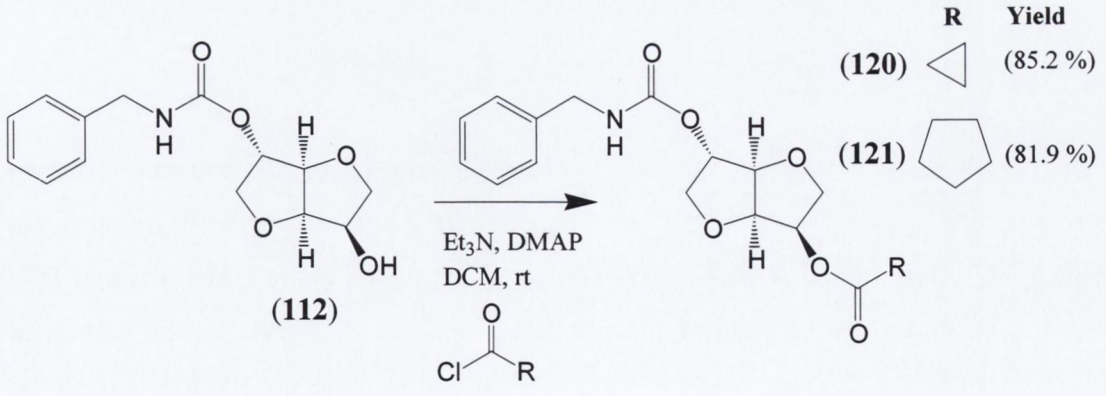


Scheme 3.3

The progress of the reaction from triflate to alkene was monitored by a number of TLC analyses using mobile phases of varying polarity. The Rf values obtained for the reactant and reaction mixture remained the same in each individual analysis, indicating that the reaction had not been successful. However, it was noticed that when the TLC plates were stained with vanillin developing solution, the test spot for the reaction mixture turned dark brown whilst the triflate compound did not give any colour change. The IR spectrum of the reaction product showed a peak at 1611 cm^{-1} indicating the presence of an alkene group and therefore successful formation of the alkene **119**. This peak was absent in the IR spectrum of the triflate **118**. The identity of the alkene **119** was corroborated by NMR data.

3.2.3 SYNTHESIS OF CYCLOALKYL ALIPHATIC ESTERS AT POSITION-5

Two cycloalkyl esters were also synthesised in order to compare their activity, not only with the aliphatic compounds, but also with the cyclic aromatic compounds covered later in this chapter. Compounds **120** and **121** were synthesised from acid chlorides[198, 199, 200], in the presence of triethylamine and the catalyst DMAP in a solution of DCM. These compounds transpired to be the most potent alkyl ester inhibitors with IC_{50} values of 5.8 nM and 334.6 nM for **120** and **121** respectively.



Scheme 3.4

3.2.4 NMR SPECTROSCOPY OF ALIPHATIC ESTERS AND DERIVATIVES

Analogues bearing the benzyl carbamate group in position-2 with an ester in position-5 follow a pattern to a large degree in terms of their ^{1}H and ^{13}C spectra. Figure 3.5 shows the ^{1}H spectrum of **114**. The four hydrogen atoms on the carbons at positions one and six (IsH-1 and IsH-6) are in the region between 3.7 and 4.2 ppm. The two IsH-1 signals and one IsH-6 are observed as one large overlapping multiplet between 4.0 and 4.2 ppm, while one IsH-6 signal is shifted slightly more upfield at between 3.7 and 3.9.

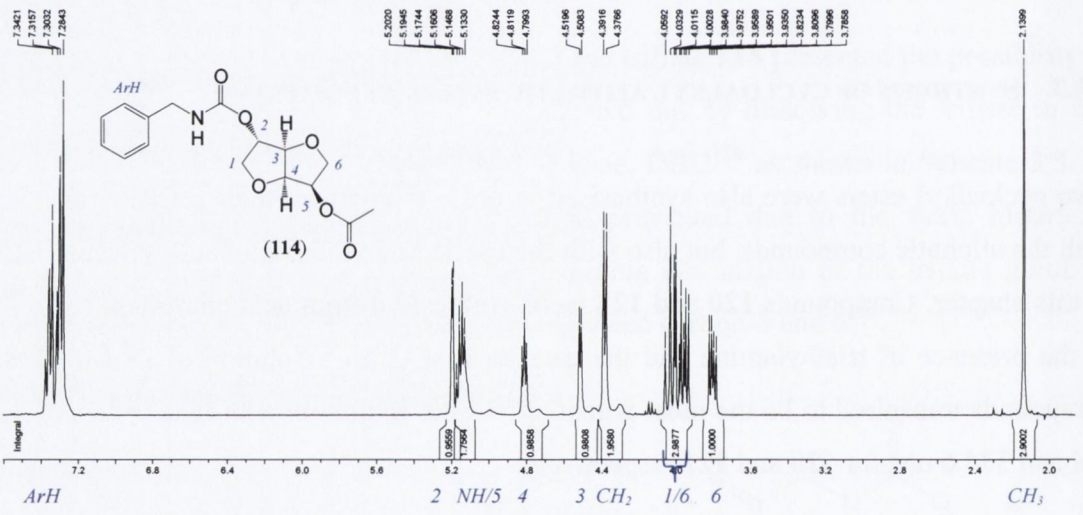


Figure 3.5

The hydrogen on the carbon at position-3 (IsH-3) is in an exo-position and can be shown in the HH COSY spectrum in Figure 3.6 to couple only to the hydrogen on the carbon at position-4 (IsH-4), which is also in an exo-position, and therefore, gives a signal as a doublet at 4.5 ppm. IsH-4 couples to both IsH-3 and the hydrogen on the carbon at position-5 (IsH-5), both of which are in an exo-position. IsH-4 is observed as a triplet at 4.8 ppm approx. The hydrogen atoms on the carbon atoms at position-5 and 2 (IsH-5 and IsH-2) and the hydrogen on nitrogen atom of the carbamate (NH) are observed in the region between 5.0 and 5.2 ppm. IsH-5 couples to IsH-4 and IsH-6 and is shown as a

multiplet, while IsH-2 couples to IsH-1 and is given as a doublet. The NH signal of the carbamate group is not as strong as the other ^1H signals and is given as a broad singlet. In more concentrated NMR solutions, this signal shows up as a triplet due to the splitting caused by the neighbouring CH_2 group. Each of these compounds exhibits slight chemical shifts in their signals in the 5.0 to 5.2 ppm region causing these signals to overlap in certain cases from compound to compound.

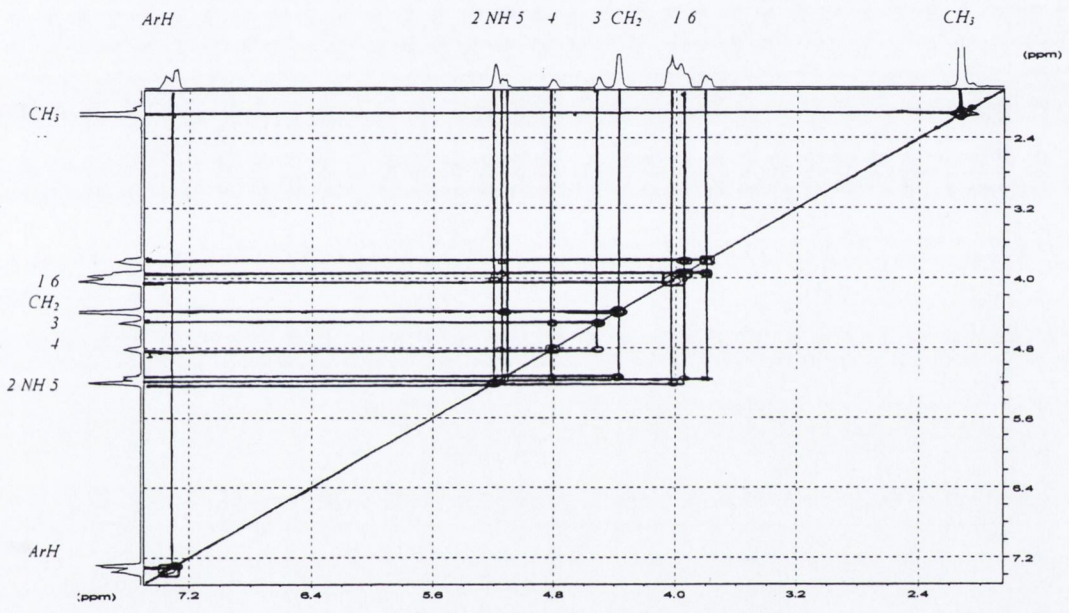


Figure 3.6

The NMR spectra of **111** and **112** show a similar pattern in their ^1H NMR signals to those discussed above. However in the case of **112**, the signals for IsH-6 are shifted upfield to 3.57 and 3.90, giving four separate signals for IsH-1 and IsH-6 between 3.5 and 4.1 ppm. This is due to the greater degree of shielding offered by the hydroxyl group at position-5, instead of an ester or nitrate group. Similarly, this shielding effect causes a large shift upfield of IsH-5 to 4.31 ppm, while IsH-4 is shifted to 4.61 ppm. All other signals remain unaffected.

The successful synthesis of the alkene **119** was proven by NMR. Only seven isosorbide proton signals were detected as opposed to the eight proton signals usually expressed by the isosorbide moiety. There are only two proton signals, due to IsH-1, in the 3.50 to 4.10 ppm region, where IsH-1 and IsH-6 signals are usually found. The one remaining IsH-6 signal is shifted downfield to 6.55 ppm due to the deshielding effect experienced due to its proximity to the alkene and the adjacent oxygen atom. The remaining four proton signals were also shifted downfield. Figure 3.7 shows the two NMR spectra of the triflate **118** and the alkene **119** derivatives.

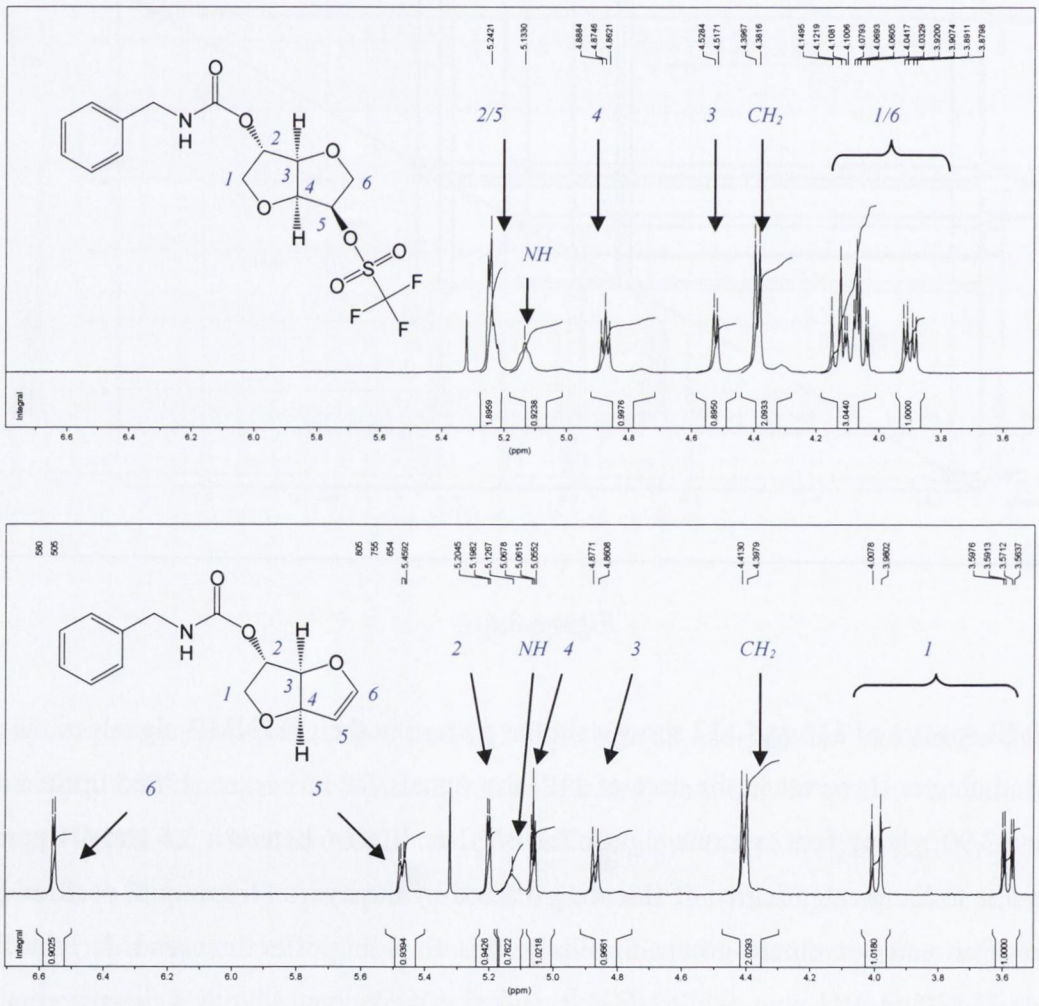


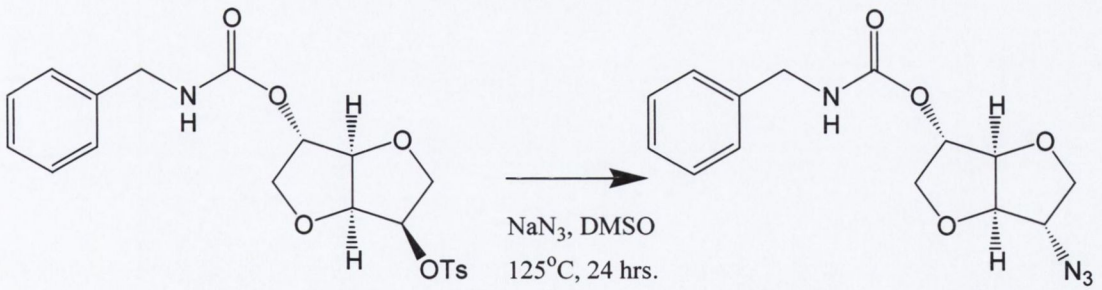
Figure 3.7

3.2.5 SYNTHESIS OF AROMATIC ESTERS AT POSITION-5

A series of compounds were synthesised where an aromatic ester group was incorporated into position-5. The esters produced are shown in Figure 3.8.

Compound **122** to **126** were prepared by reaction of **112** with acid chlorides following the same approach outlined in Scheme 3.4. These reactions gave the final compounds in excellent yields and in most cases could be recrystallised from hot methanol or ethyl acetate to give pure compounds without the need for additional purification by flash chromatography. Compounds **127** to **129** were synthesised using an alternative approach. The strategy to the synthesis of these compounds was more similar to that used in the synthesis of ether compounds and therefore is described later in the chapter.

In addition to the final test compounds shown in Figure 3.8, a tosylate compound **130** was also produced to compare the activity of this sulfonyl aromatic ester with the carbonyl ester compounds and also with the aliphatic mesylate **117** and triflate **118** esters. Compound **130** was prepared by reaction of **112** with TsCl following the approach shown in Scheme 2.3. It was then also possible to prepare the exo azide **131** from the tosylate as shown in Scheme 3.5.



(130) **(131)**

Yield = 74 %

Scheme 3.5

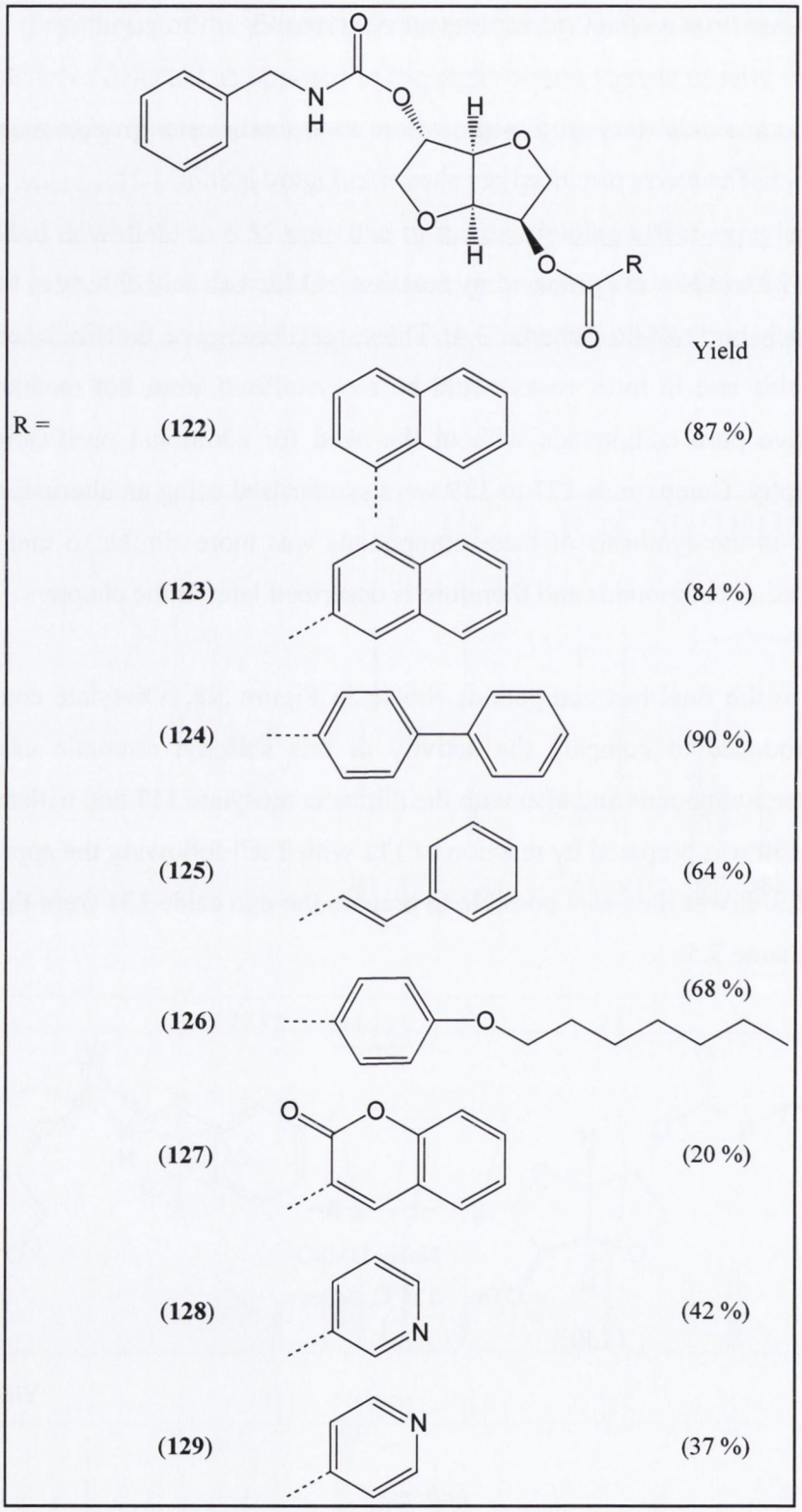


Figure 3.8

3.2.6 NMR SPECTROSCOPY OF 5-ARYL ESTERS COMPOUNDS

Figure 3.9 shows a ^1H spectrum of 2-benzyl carbamate 5-(1-naphthoyl) isosorbide **122**. The spectrum is typical of the aromatic esters in this series in terms of the chemical shifts and splitting of ^1H signals. The order of ^1H signals that are common to all compounds, in moving from 3.9 ppm, downfield to 5.5 ppm is; IsH-6 and IsH-1 (observed as one large multiplet), benzyl carbamate CH_2 (doublet), IsH-3 (doublet), IsH-4 (triplet), benzyl carbamate NH (singlet), IsH-2 (doublet) and IsH-5 (quartet). These signals are consistent in all ^1H spectra except for the spectrum of **130**, where the IsH-5 signal was shifted from approximately 5.5 to 4.9 ppm.

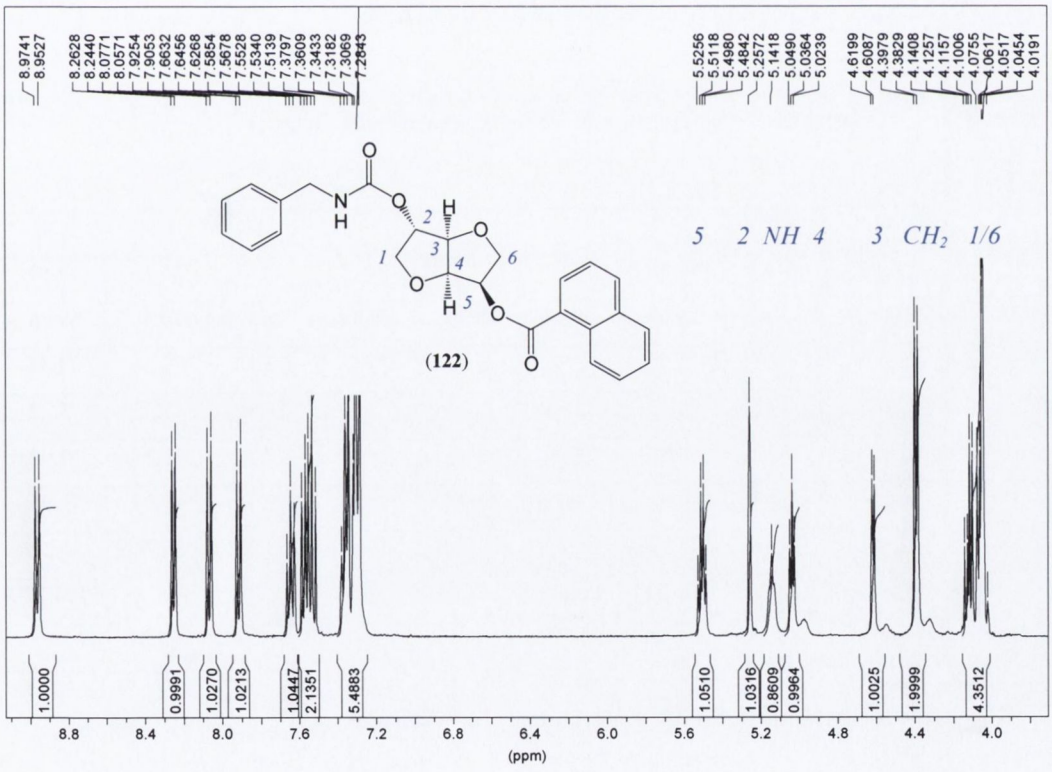


Figure 3.9

All aromatic ^{1}H signals are accounted for and assigned in the experimental chapter. In **126** all aliphatic proton signals are present in the region between 0.9 and 1.9 ppm except for the $-OCH_2-$ signal, which is shifted upfield to 4.0 ppm and appears as a multiplet with IsH-1 and IsH-6. With compound **125**, its ^{1}H spectrum shows two characteristic CH doublets at 6.50 and 7.75 corresponding to the two CH signals on the carbon-carbon double bond of the cinnamoyl group, while in compound **130**, the ^{1}H shows the CH_3 peak as expected at 2.5 ppm.

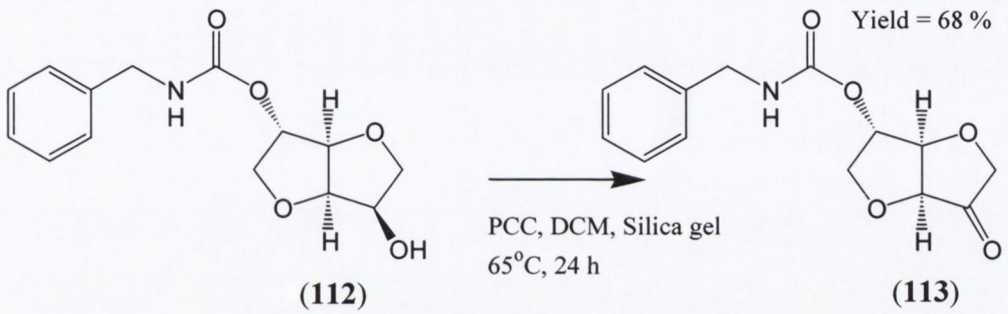
The consistency in NMR spectra is also mirrored in the ^{13}C spectra of the compounds where ^{13}C shifts common to all compounds differ little from compound to compound (Table 3.1). Again, the presence of a tosylate ester instead of a carbonyl ester gives a change in the signal at position-5 in **130**, where the ^{13}C signal for IsC-5 is shifted slightly downfield compared to the other compounds in this series.

Table 3.1: ^{13}C signals of the aromatic ester compounds (ppm)

	122	**123**	**124**	**125**	**126**	**127**	**128**	**129**	**130**
CH_2	44.69	44.67	44.67	44.67	44.65	44.81	44.64	45.10	44.57
IsC-6	70.23	70.30	70.31	69.84	70.28	71.18	70.17	70.58	69.06
IsC-1	73.24	73.27	73.25	73.33	73.22	73.51	73.18	73.64	73.45
IsC-5	74.11	74.18	74.25	73.68	73.69	75.79	74.50	75.22	78.22
IsC-2	78.14	78.18	78.06	78.04	78.09	78.62	77.85	78.27	77.92
IsC-4	80.56	80.64	80.61	80.45	80.64	81.51	80.44	80.83	79.95
IsC-3	85.80	85.80	85.79	85.62	85.72	86.97	85.82	86.24	85.32
-C(O)N-	154.77	154.87	154.81	154.80	154.83	156.17	154.81	158.05	154.77
-C(O)Ar_2	166.33	165.64	165.36	165.85	165.25	165.31	165.36	164.48	-

3.2.7 THE SYNTHESIS OF THE KETONE 113 AND ASSOCIATED DERIVATIVES

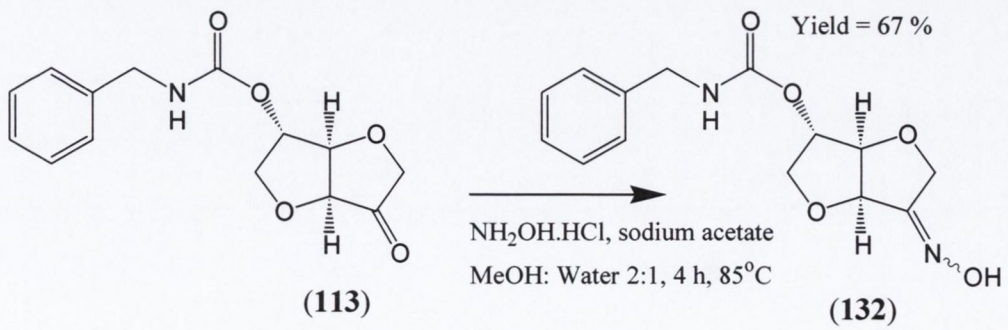
The ketone **113** was synthesised from the intermediate **112** using the same oxidation procedure[149] investigated in Section 2.2. Compound **113** was a final test compound in itself but it was also used as a key intermediate to synthesise a number of other compounds. The ^{1}H NMR spectra of **113** differed from that of **112** by the absence as expected of the signal for IsH-5 and also that IsH-4, which was a triplet in the ^{1}H spectrum of **112**, was observed as doublet, as it can only couple to IsH-3. In the ^{13}C spectra, the signal for the IsC-5, was observed at 209.28 ppm.



(112) **(113)**

Scheme 3.6

The oxime derivative **132** was easily prepared by reaction of **113** with hydroxylamine hydrochloride in the presence of sodium acetate in a solution of 2:1 methanol: water at 85°C[149].

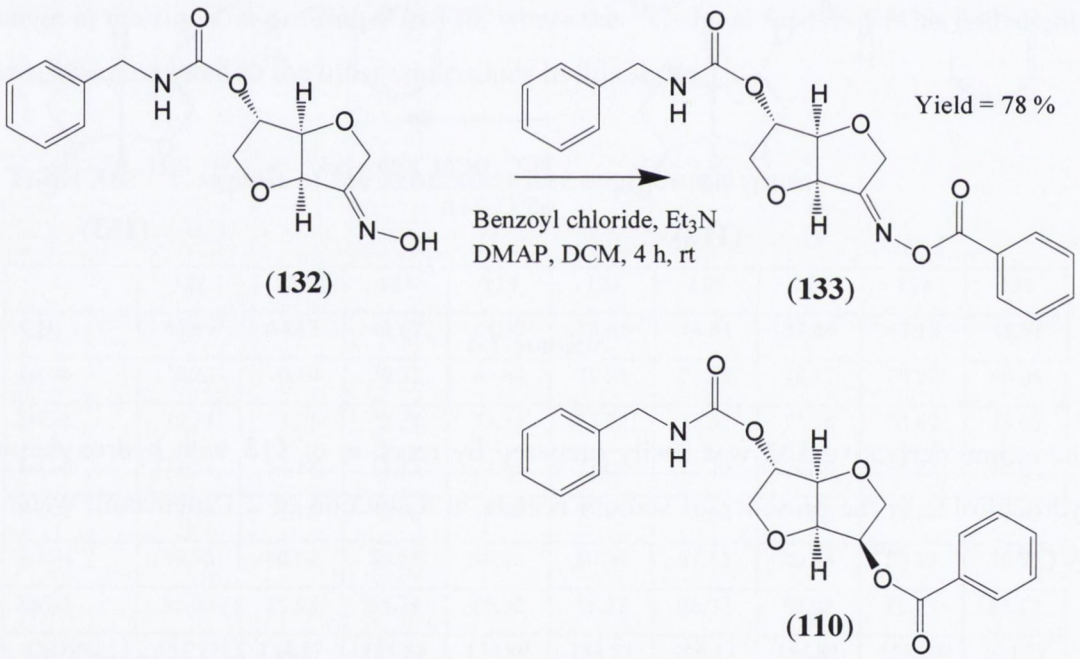


(113) **(132)**

Scheme 3.7

In the ^{13}C NMR spectra of **132**, the position of IsC-5 is shifted from 209.28, as observed in **113,** to 159.82 ppm. In the ^{1}H spectrum, similar signals are observed as in the spectrum for **113**, only the proton signal for IsH-6 is shifted downfield to between 4.4 and 4.7, where it appears as a multiplet and overlaps with the signal for IsH-3.

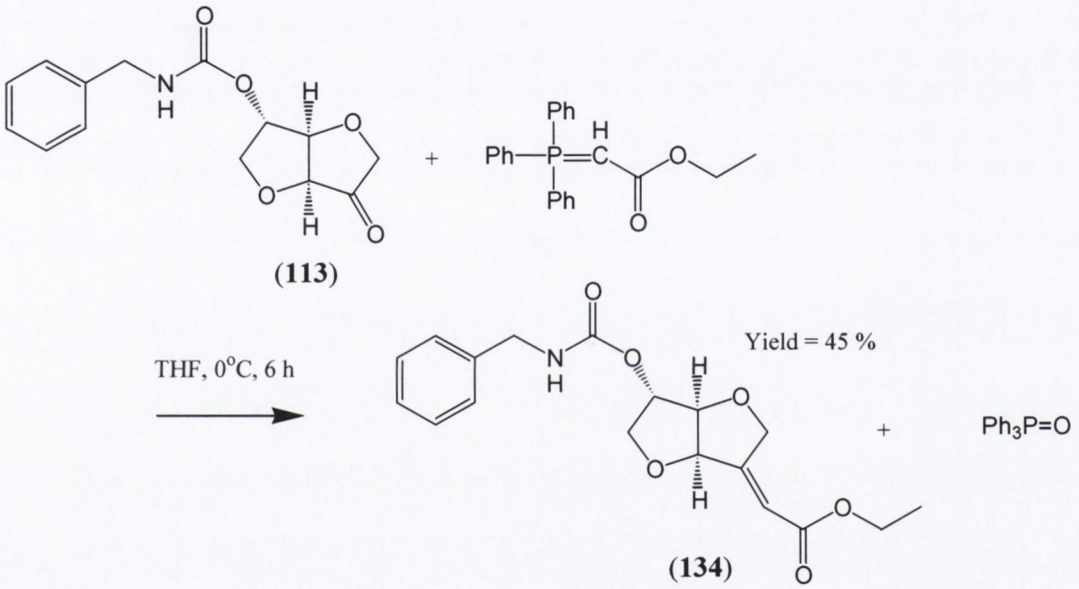
From **132** it was subsequently possible to synthesise an ester derivative **133** using the conventional esterification method involving benzoyl chloride, triethylamine and DMAP in a solution of DCM (Scheme 3.8). Through biological testing it was possible to assess the significance of the oxime function in **133** by comparing its activity with that of the potent and selective inhibitor **110**.



Scheme 3.8

The NMR spectra of **133** were similar to that of **132** but in addition displayed the expected extra aromatic signals in both ^{1}H and ^{13}C spectra.

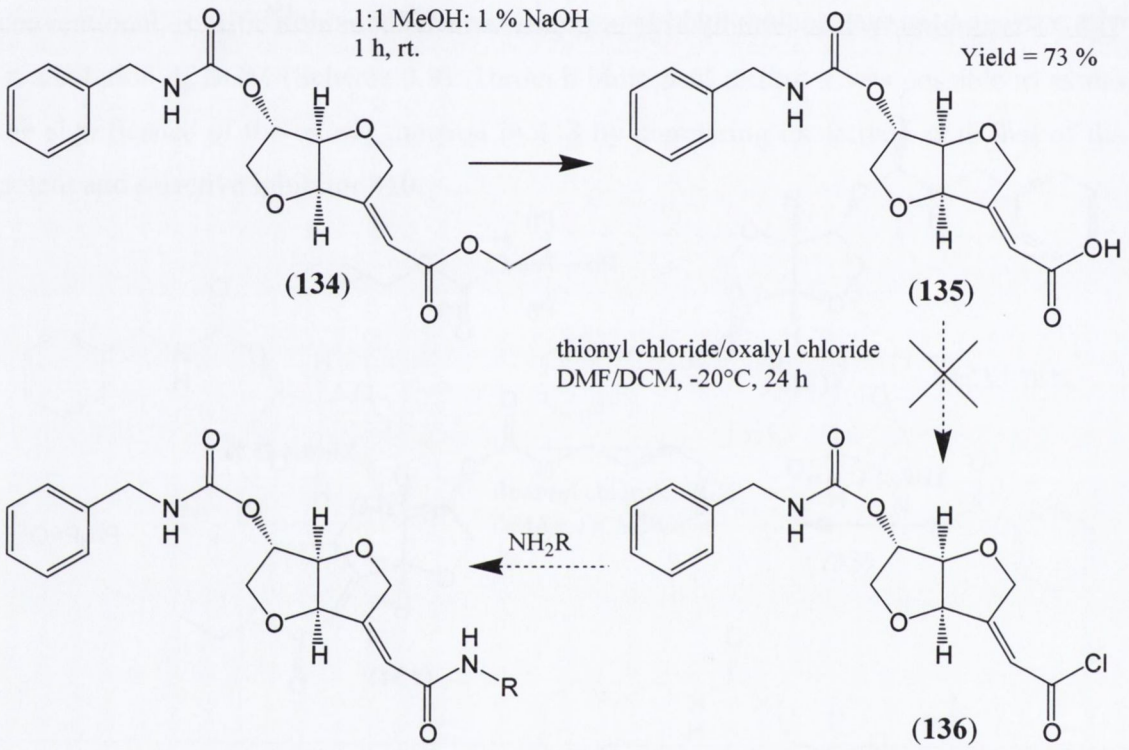
The Wittig reaction was carried out on **113** using a commercially available ylide, carbethoxymethylene triphenyl phosphorane, as shown in Scheme 3.9. This ylide possessed an ethyl ester and therefore gave **134** after reaction with **113**. Ylides used in a Wittig reaction can either be purchased commercially or prepared synthetically. Common ylides such as methyltriphenylphosphonium bromide[201] or iodide[156] are prepared by reacting triphenyl phosphine with an alkyl halide which is then followed by treatment with a strong base such sodium hydride or potassium tert-butoxide[156].



Scheme 3.9

The ^{1}H spectrum of **134** displayed the characteristic CH_3 and CH_2 signals of the ester at 1.30 and 4.12 ppm respectively. The spectrum also shows the additional hydrogen signal of the alkene group at 6.1 ppm and as in the spectra of compounds **132** and **133**, the proton signal corresponding to IsH-6 are shifted downfield, to between 4.7 and 5.0 ppm. In the ^{13}C spectrum the characteristic signals of the CH_3, CH_2 and carbonyl groups of the ester portion of the molecule in position-5 are displayed at 13.79, 60.16 and 165.30 ppm respectively. With respect to the alkene group of the molecule, the signal for IsC-5 is observed at 154.71 ppm, whilst the corresponding carbon atom of the alkene is at 116.12 ppm.

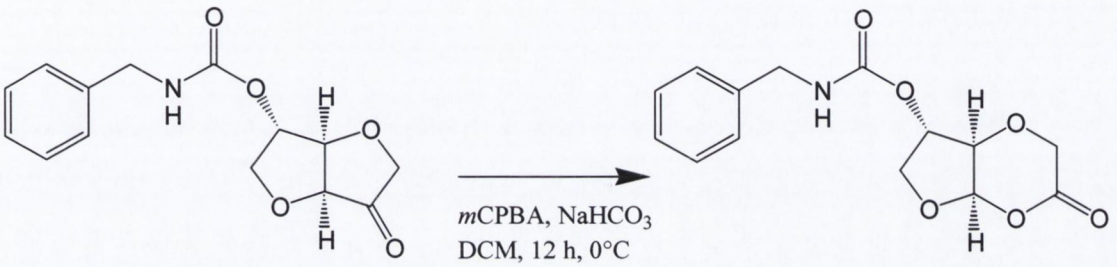
From **134** it was possible to cleave the ester in position by hydrolysis in an aqueous basic solution of 1:1 methanol: 0.25 M NaOH aqueous solution, to give the carboxylic acid **135**. From this compound it was hoped to synthesise the acid chloride **136** and from this to prepare a number of amides, as shown in Scheme 3.10, by reaction of **136** with primary amines.



Scheme 3.10

The reaction to give the acid chloride **136** was attempted several times by reacting **135** with either thionyl chloride or oxalyl chloride in the presence of a small volume of DMF in a solution of DCM at -20°C, however, the reaction proved unsuccessfully in each case.

Baeyer-Villiger oxidation is a reaction in which a ketone or aldehyde can be converted to an ester by treatment with peroxy acids or hydrogen peroxide[202]. The oxidation mechanism is commonly used in the formation of lactones[203, 204] from cyclic ketones and therefore it was an obvious choice of reaction to apply to **113** in order to probe the SAR of ketone derivatives.

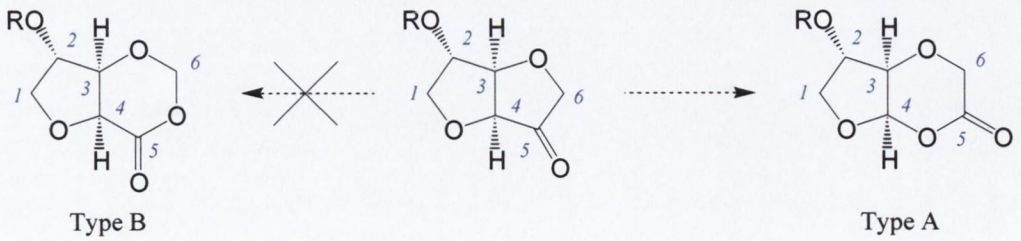


(113) **(137)**

Yield = 39 %

Scheme 3.11

Compound **137** was synthesised by dissolving **113** in DCM and cooling the solution to 0°C. Sodium hydrogen carbonate and *m*CPBA (1.5 mol. eq. of each) were added to the mixture, which was stirred for 12 hours[152]. The reaction product was obtained as a clear oil which was characterised by NMR and mass spectrometry. Studies into the Baeyer-Villiger reaction with isosorbide-based compounds have shown that rearrangement occurs with migration of the more substituted α-carbon (Figure 3.10)[152].



Type B Type A

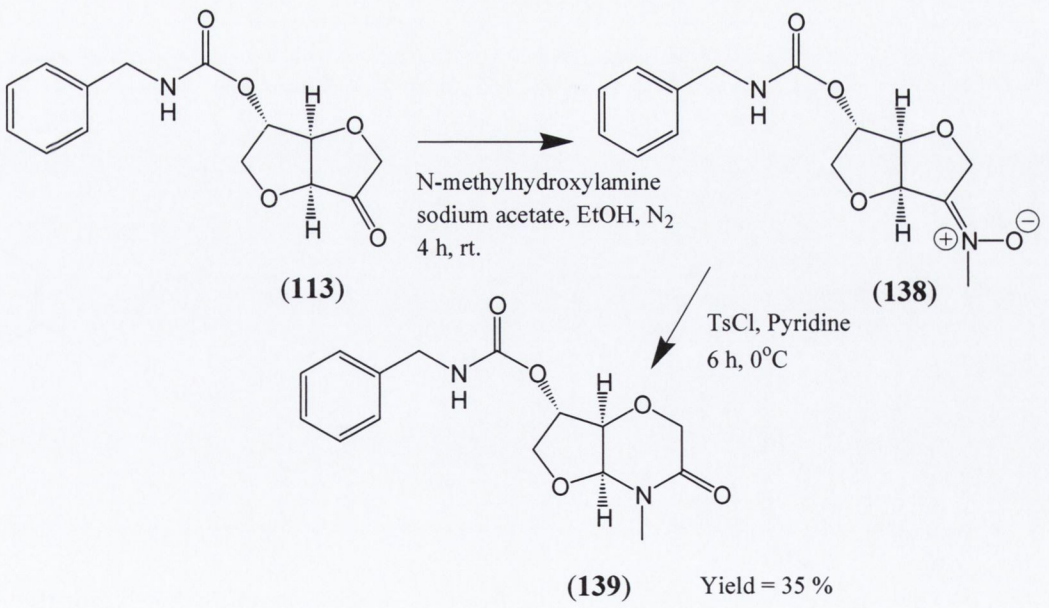
Figure 3.10

Therefore the Baeyer-Villiger rearrangement of isosorbide-based compounds tends to give products with the structure of type A compounds, as shown in Figure 3.10, and not that of type B compounds. The identity type A structured compounds can be shown by ^{1}H NMR, where the signal for IsH-4 is shifted downfield to 5.9 ppm with the two proton signal for IsH-6 lying between 3.9 and 4.5 ppm. Conversely, the NMR of type B structured compounds would show a two proton signal downfield for IsH-6 and a single signal for IsH-4 at approximately 4.0 ppm

The ^{1}H NMR spectrum of **137** is highly similar to the NMR data for compounds with type A structure, published in the literature, with the signal for IsH-4 being shifted downfield to 5.85 ppm while in the ^{13}C spectrum the signal for IsC-4 is shifted to 100.42 ppm and the signal corresponding to the carbon atom of the carbonyl group observed at 165.29 ppm.

The rearrangement of ketoximes to a corresponding amide is known as a Beckmann reaction and can be used to insert an amide into a cyclic system called δ-lactam by a mechanism not unlike the formation of lactones by the Baeyer-Villiger reaction. It accomplishes in one stroke both the cleavage of a carbon-carbon bond and the formation of a carbon-nitrogen bond[205]. Generally, Beckmann rearrangement is carried out by treating a compound, possessing an oxime function, with a strong Lewis acid such as *p*-toluenesulfonic acid, tin chloride or phosphorus pentachloride[206]. However, the reaction can also be achieved using mild basic[207] or acidic[208] conditions employing non-toxic and inexpensive reagent, which are more environmental friendly.

The Beckmann reaction was employed to rearrange the structure of **113** to introduce a nitrogen atom into its ring system giving a δ-lactam **139**. The synthesis of **139** was achieved by initially preparing the methyl ketoxime **138** with N-methylhydroxylamine in the presence of sodium acetate in a solution of anhydrous ethanol under an atmosphere of nitrogen gas. This was followed by Beckmann rearrangement of **138** with *p*-toluenesulfonyl chloride in a solution of pyridine[151].



(113) **(138)** **(139)** Yield = 35 %

Scheme 3.12

As with the lactone product **137** of the Baeyer-Villiger reaction, the ^{1}H NMR spectrum of δ-lactam **139** is similar to that reported in the literature[151] with the signals for IsH-4 and IsH-6 being shifted downfield to 4.98 and 4.22 ppm respectively. The ^{1}H spectrum also showed the characteristic signal pertaining to the methyl group, attached to the nitrogen atom, at 3.05 ppm. The ^{13}C spectrum the signal for IsC-4 is shifted to 87.01 ppm and the signal corresponding to the carbon atom of the carbonyl group of the δ-lactam is observed at 166.12 ppm, whilst the carbon atom of the methyl group is observed at 31.55.

3.3 SYNTHESIS OF AROMATIC ETHERS AT POSITION-5

It was also desirable to prepare non-ester aromatic derivatives in position-5, which could be compared to the ester analogues to gauge the significance of the carbonyl function at position-5. It is possible that such compounds might also be more stable metabolically *in vivo.* For this purpose an ether group was to be introduced into position-5. The first ether to be synthesised was the benzyl ether **140** whose activity could be directly compared to that of the highly potent and selective huBuChE inhibitor **110** to assess the importance of the ester group in conferring potency.

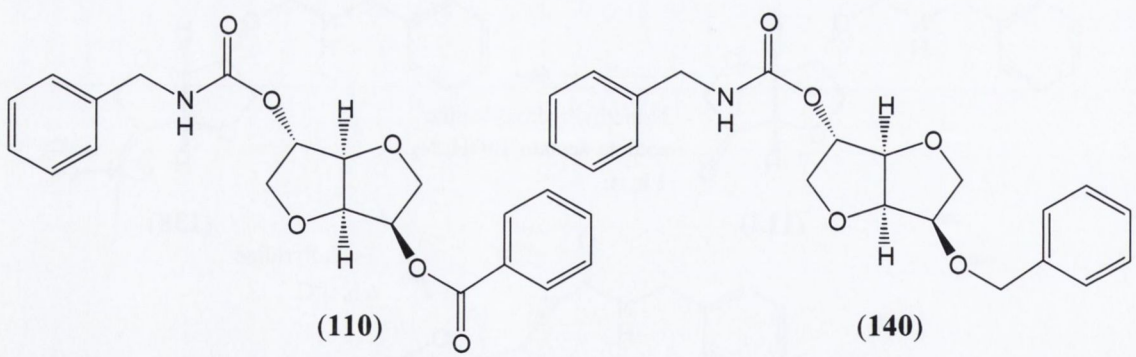
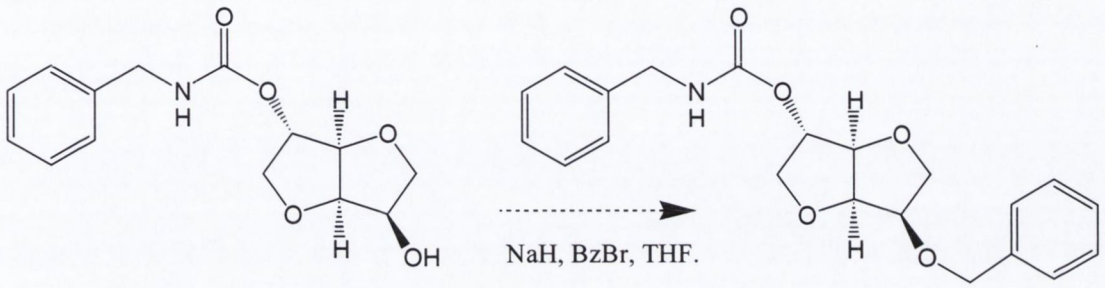


Figure 3.11

Ethers can be prepared in a number of ways from secondary alcohols[209, 210], but they are generally synthesised using a strong base such as sodium hydride in the presence of a relevant halide[211]. The first approach taken to synthesise **140** involved a direct reaction

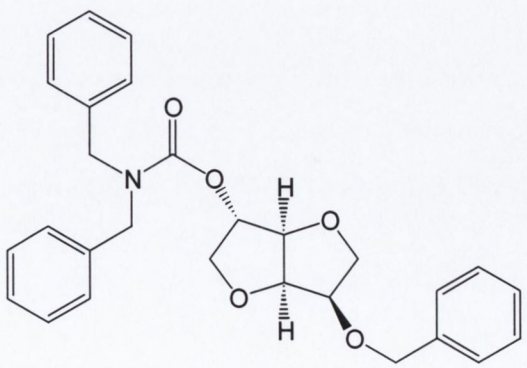
with the intermediate **112** as shown in Figure 3.12. This approach involved reacting **112** with benzyl bromide in the presence of sodium hydride in a solution of anhydrous THF, which was kept under an atmosphere of nitrogen gas[212]. The reaction was carried out with the careful exclusion of moisture.



(112) **(140)**

Figure 3.12

Only one reaction product was formed, which was analysed by NMR spectroscopy. The ^{1}H spectrum of the reaction product showed 15 proton signals in the aromatic region between and 7.0 and 8.0 ppm, indicating that the reaction had succeeded in benzylating the carbamate as well as the hydroxyl group at position-5, giving **141**. This was confirmed from the ^{13}C and DEPT 135 spectra giving five CH_2 signals, which can be attributed to IsC-1 and IsC-6 and three CH_2 groups from the three benzyl groups present.



(141)

Figure 3.13

It was therefore decided that an alternative approach was required in order to synthesise ether analogues. This approach, shown in Figure 3.14, would involve the protection of position-2 of IS-5-MN **62**, followed by the reduction of the nitrate group at position-5. Etherification of the protected intermediate would give the relevant ether group in position-5 and this could be followed by the deprotection and carbamoylation of position-2, to give the required ether compound.

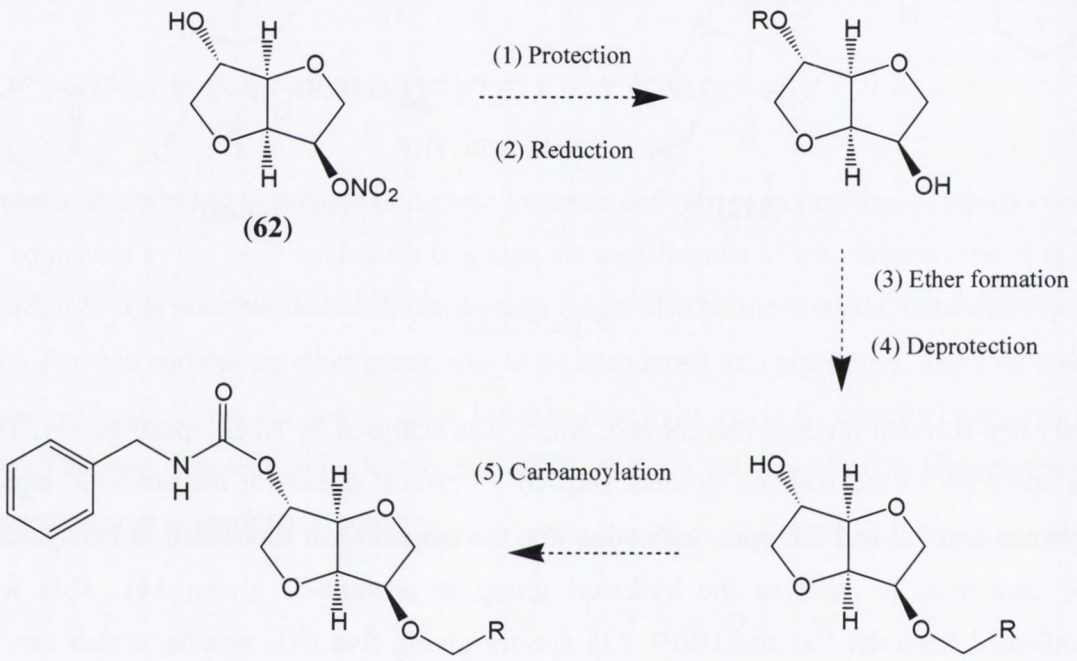
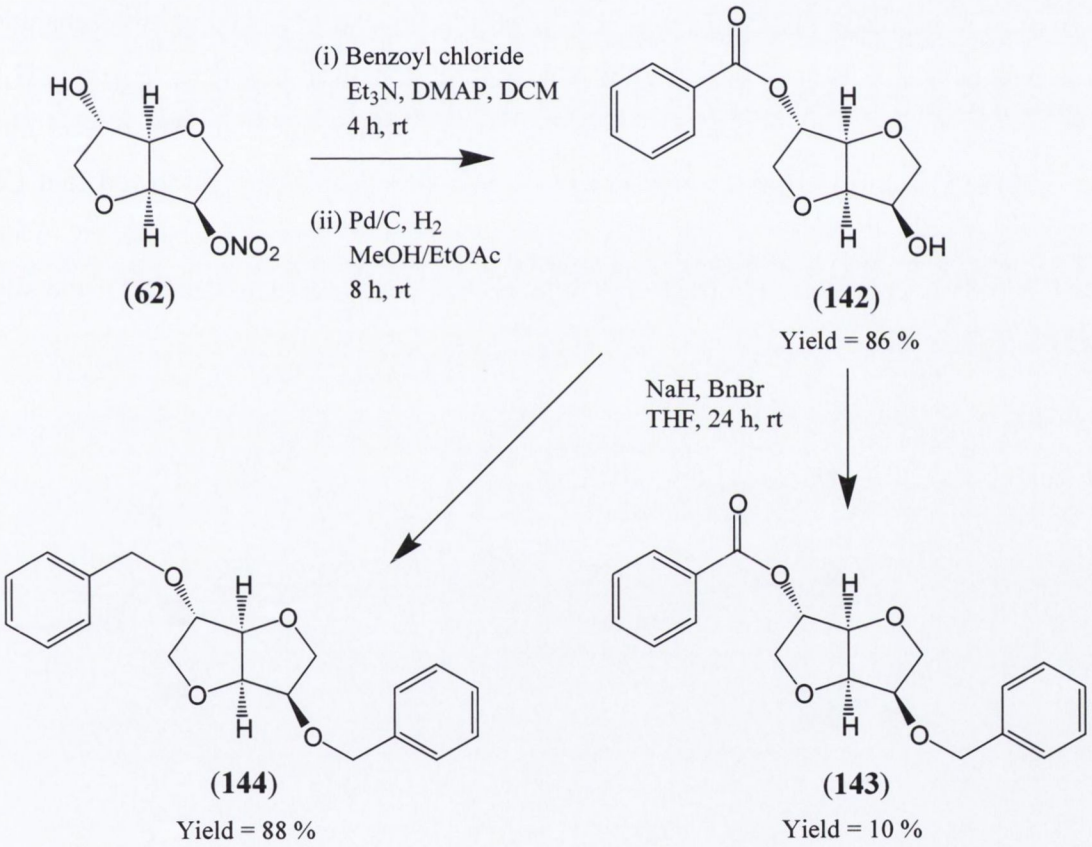


Figure 3.14

The first attempt to protect position-2 used benzoyl chloride[213] to prepare a benzoate ester of IS-5-MN. This was followed by the reduction of the nitrate group in position-5 using 10 % palladium on activated carbon and hydrogen gas, giving **142** (Scheme 3.13). The synthesis of the benzyl ether followed the same approach used in the synthesis of compound **141**.

The procedure gave two products, which were worked up as before and separated using column chromatography giving two white crystalline products, which were subjected to NMR analysis. Although a small quantity of the desired compound **143** was formed, the

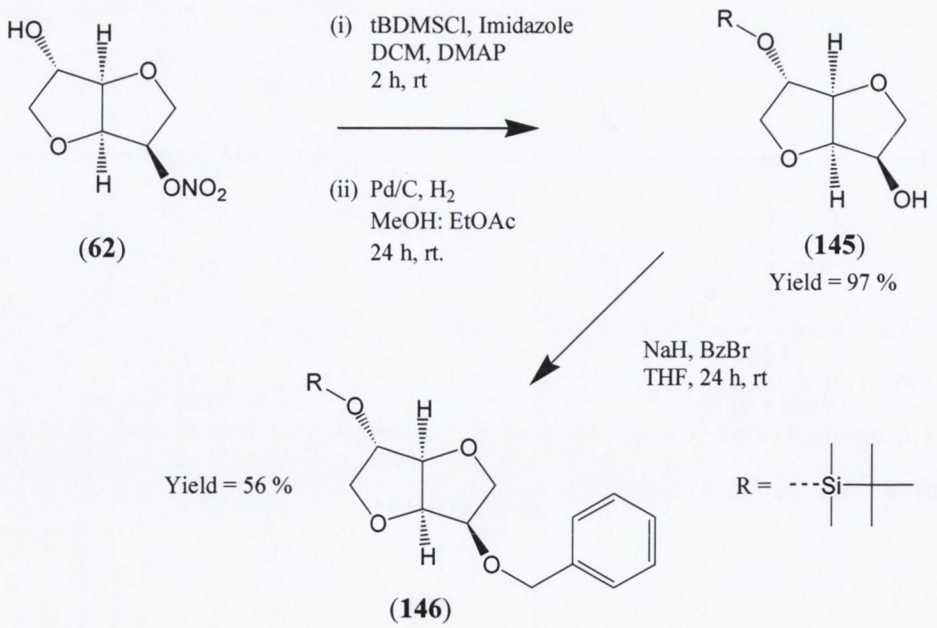
primary product, with a yield of 88 %, was the di-benzyl ether derivative of isosorbide **144**, where the benzoyl group in position-2 had been cleaved under basic conditions and replaced with the benzyl ether group. Compounds **143** and **144** could be distinguished from each other from their respective proton and carbon NMR data. Compound **143** showed ten proton signals in the 3.5 to 5.0 ppm region compared to 12 in **144**. The formation of **144** as the primary product was confirmed by the ^{13}C and DEPT 135 spectra, which showed four separate CH_2 signals accounting for IsC-1, IsC-6 and two CH_2 signals from the benzyl ether groups.



Scheme 3.13

Protection of position-2 was successfully achieved by employing tBDMSCl, in the presence of imidazole and DMAP[214], to introduce the tBDMS-protecting group. The reduction of the nitrate group at position-5 was again carried out using 10 % palladium on activated carbon and hydrogen gas, giving **145**, as shown in Scheme 3.14. The synthesis of the benzyl ether was carried out as before giving one reaction product, which was obtained as a clear oil.

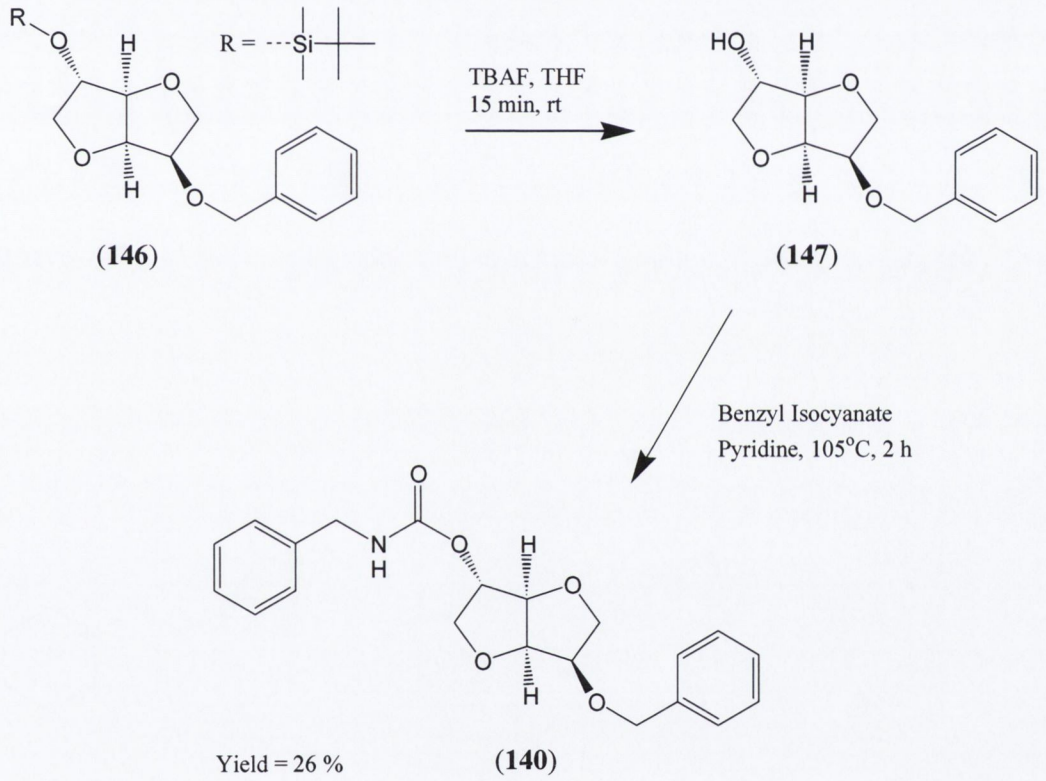
Successful synthesis of the tBDMS protected ether **146** was verified by the NMR spectra with the ^{1}H spectrum showing the relevant five proton signals for the aromatic group between 7.20 and 7.40 ppm and the CH_2 signal, which was observed as two separated proton signals at 4.58 and 4.80 ppm. The spectrum also showed the characteristic $(CH_3)_2$ and $(CH_3)_3$ singlets of the tBDMS protecting group, which integrate for six and nine protons at 0.11 and 0.91 ppm respectively. ^{13}C and DEPT 135 spectra showed four CH signals for IsC-3, IsC-4, IsC-5 and IsC-2 as well as three CH_2 signals for IsC-1, IsC-6 and the CH_2 of the benzyl group. They also showed the relevant carbon signals for the silyl and aromatic groups.



Scheme 3.14

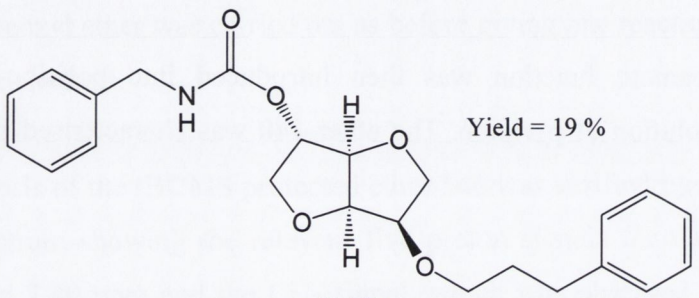
The deprotection to give the free hydroxyl group in position-2 is easily achieved by diluting **146** with THF and adding excess TBAF[215, 216]. The reaction to remove the protecting group was complete in 15 minutes. NMR analysis of **147** showed that there were no peaks present relating to the protecting group in both ^{1}H and ^{13}C spectra.

The benzyl carbamate function was then introduced into position-2 using benzyl isocyanate in a solution of pyridine. The ether **140** was characterised by NMR, IR and elemental analysis.



Scheme 3.15

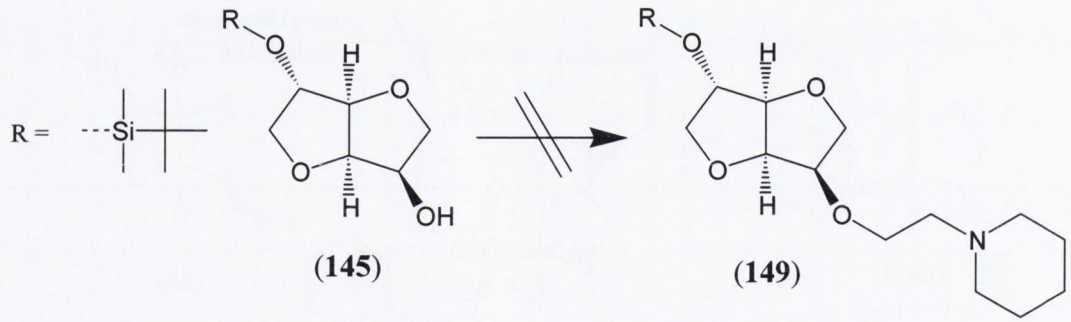
A propyl phenyl ether was also synthesised using the same approach as for the benzyl ether **140**. It was necessary however to add excess sodium hydride and heat the reaction under reflux for it to proceed to completion.



(148)

Figure 3.15

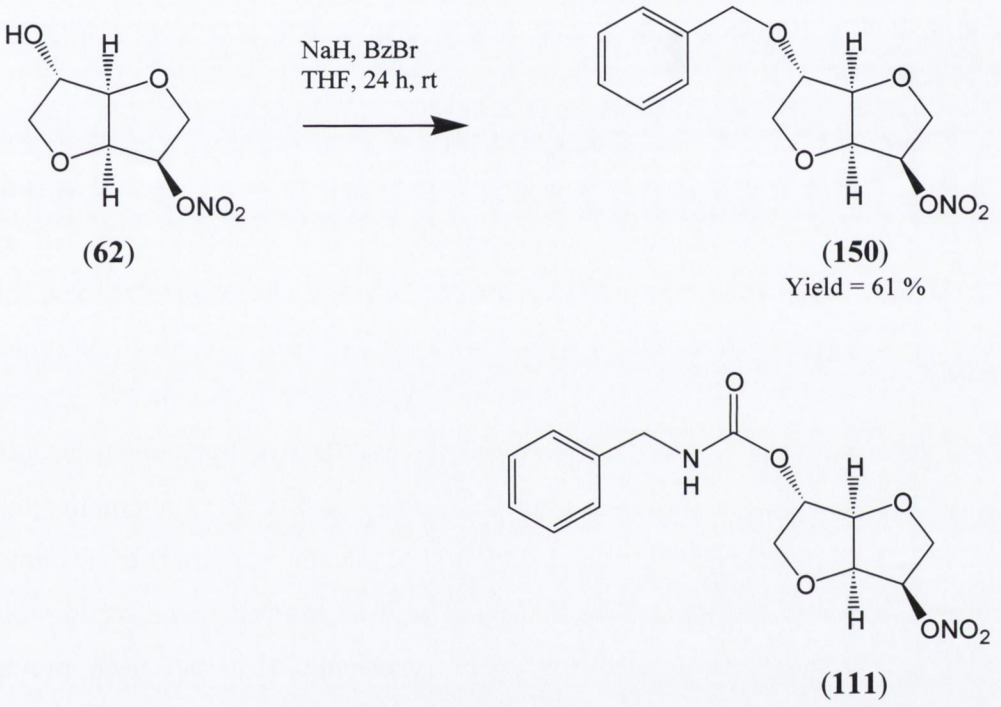
An attempt was also made to synthesise a morpholine substituted ether **149**, however this reaction proved unsuccessful.



(145) **(149)**

Scheme 3.16

With the synthesis of the ethers **140** and **148** successfully achieved, it was considered worthwhile to synthesise a compound bearing a benzyl ether in position-2 of IS-5-MN. As mentioned in Chapter 1, **111** gave an IC_{50} of 50 nM, therefore **150** was synthesised to see if it was possible to synthesise a non-carbamate inhibitor of huBuChE and to determine the importance of the carbamate group for inhibition. It was speculated that the ether, somewhat isosteric with the carbamate group, might exhibit reversible inhibition.



(62)

(150)
Yield = 61 %

(111)

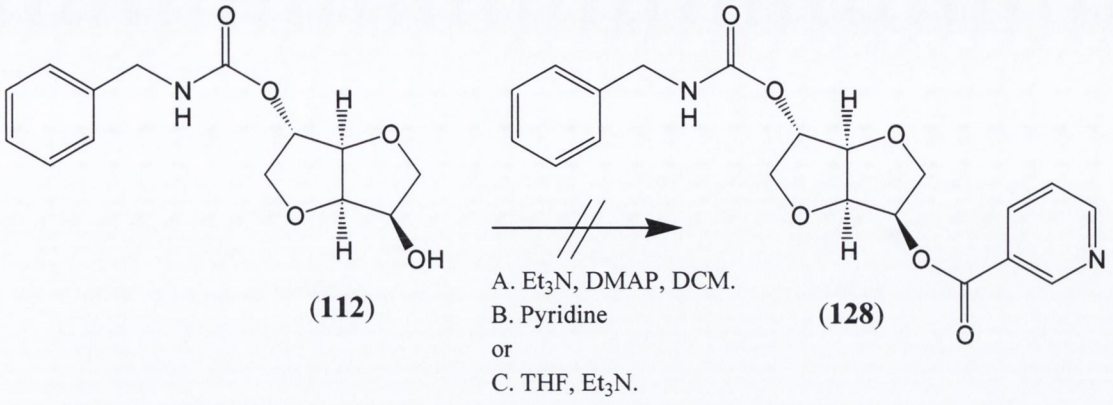
Scheme 3.17

3.4 SYNTHESIS OF NICOTINIC ESTERS AT POSITION-5

It was desired to synthesise two nicotinic esters (refer to **128** and **129** in Figure 3.8 on page 91) in order to investigate whether the inclusion of a nitrogen atom in the benzene ring of the ester could improve upon the inhibition demonstrated by **110**. It was speculated that these compounds might also be more water soluble than **110**. The synthesis of the 2-tBDMS protected isosorbide **145** proved very useful as a starting material from which the synthesis of other compounds, such as the nicotinic esters, could be achieved.

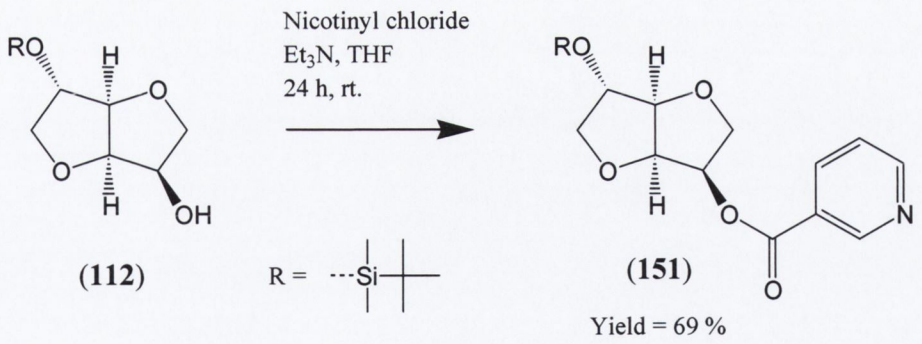
Several attempts had been made in order to synthesise the nicotinic esters **128** and **129**. Efforts to synthesise the compounds using conventional methods of esterification, as had previously been used to synthesise **122** to **126**, proved unsuccessful. These efforts included the esterification of the starting material **112** with nicotinyl chloride and isonicotinyl chloride in a solution of either triethylamine in DCM in the presence of DMAP[217], or in pyridine[218]. While it was considered that the reaction may be successfully realised used nicotinic acid and the catalyst DCC, the pursuit with an alternative method was preferred using nicotinyl chloride in anhydrous THF and anhydrous triethylamine to avoid the problematic removal of the undesired urea by-product prevalent in reactions involving DCC. The method used followed a procedure that had been previously published, outlining the synthesis of di-nicotinic esters of isosorbide and isomannide to make molecular tectonics[219 220].

The procedure was carried out by preparing a solution of **112** in anhydrous THF. To this was added excess nicotinyl chloride hydrochloride and the reaction was stirred vigorously and kept under an atmosphere of nitrogen. After 15 minutes, triethylamine was added to the reaction vessel by a syringe and the reaction was stirred overnight. This reaction again proved unsuccessful as only starting material appeared after TLC analysis of the reaction the next day.



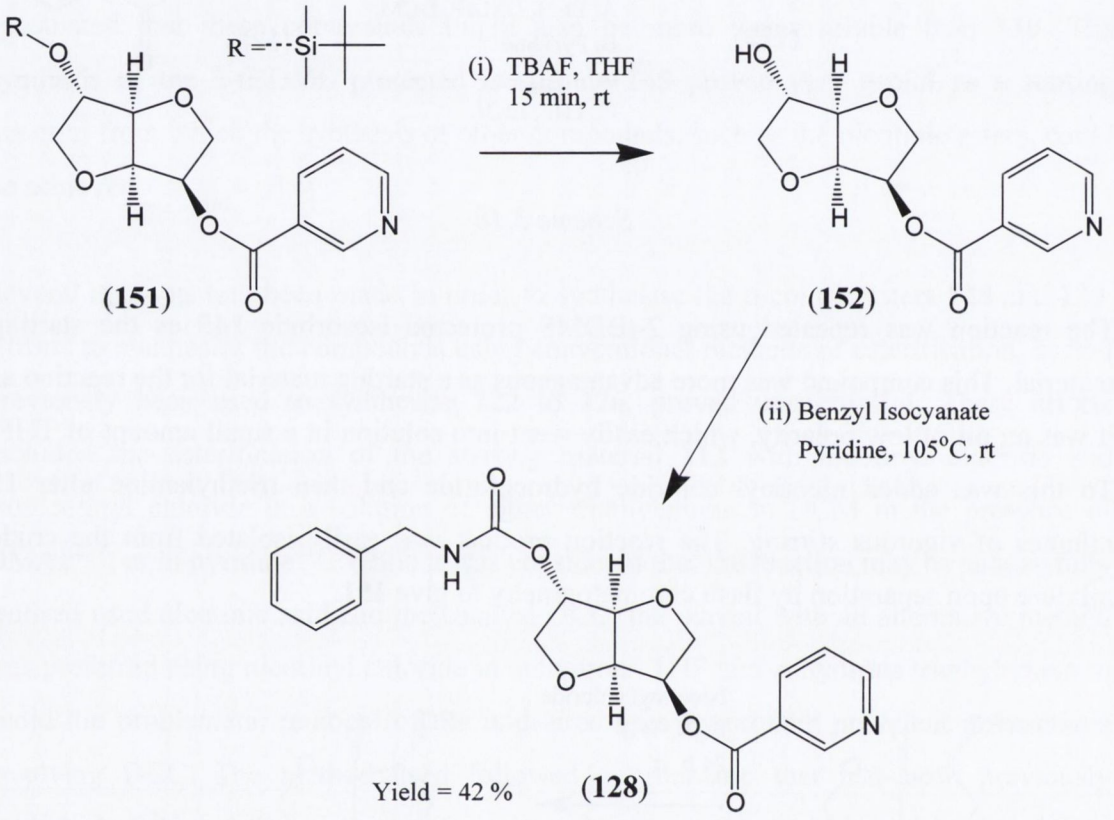
Scheme 3.18

The reaction was repeated using 2-tBDMS protected isosorbide **145** as the starting material. This compound was more advantageous as a starting material for the reaction as it was an oil of low polarity, which easily went into solution in a small amount of THF. To this was added nicotinyl chloride hydrochloride and then triethylamine after 15 minutes of vigorous stirring. The reaction product was easily isolated from the crude mixture upon separation by flash chromatography to give **151**.



Scheme 3.19

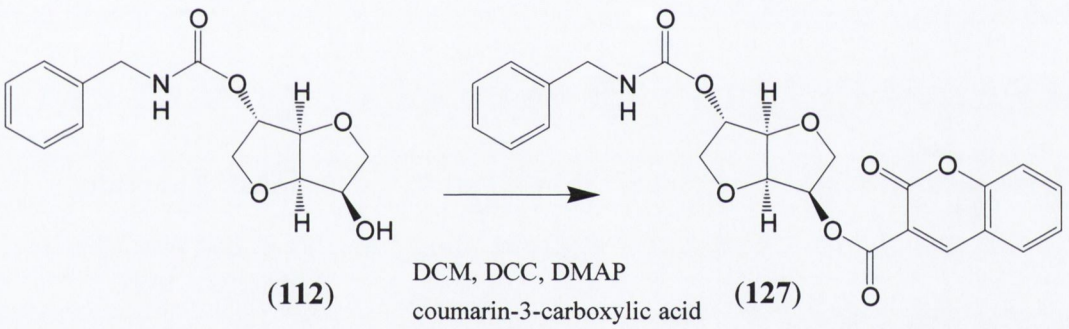
The synthesis of **128** was accomplished in two steps, where **151** was firstly de-protected with TBAF in THF to give **152**. This was followed by the reaction with benzyl isocyanate in pyridine to give **128**. The synthesis of **129** using iso-nicotinyl chloride was carried out using the same approach.



Scheme 3.20

3.5 SYNTHESIS OF COUMARIN ESTER AT POSITION-5

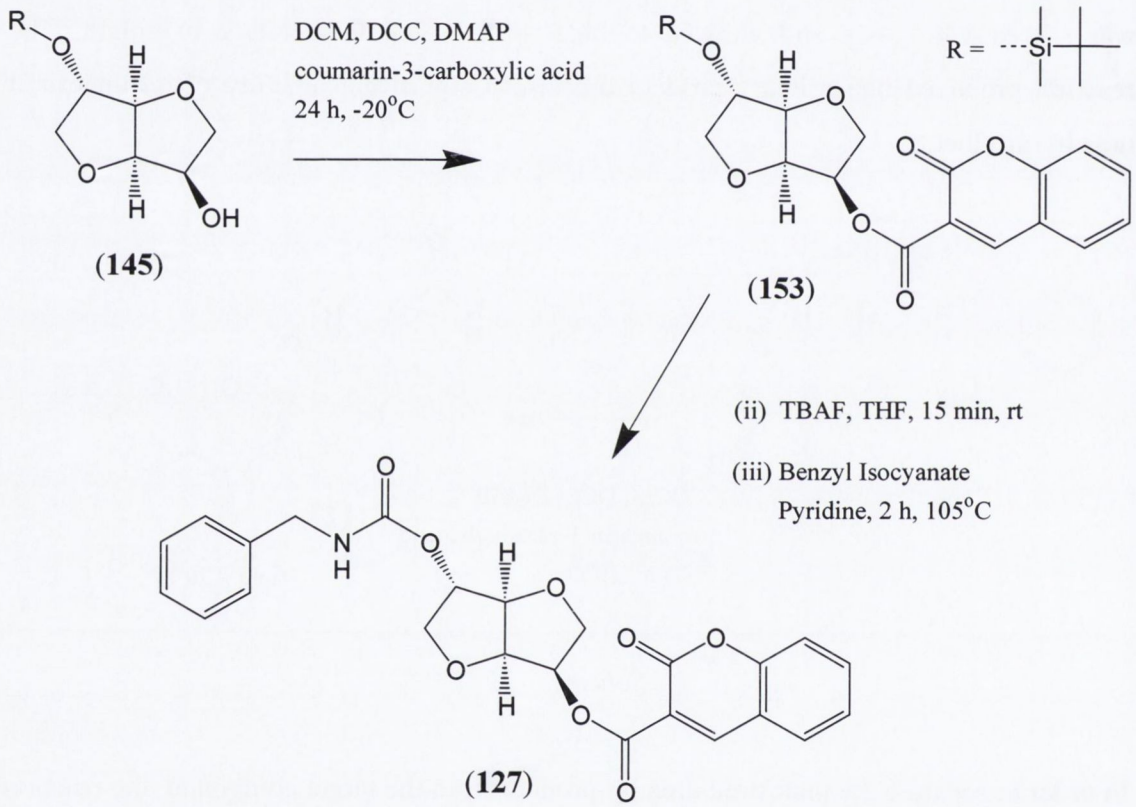
The synthesis of the **127** involved the coupling of the starting material **112** to coumarin-3-carboxylic acid[221], a commercially available carboxylic acid. One of the reasons for our interest in the coumarin analogue **127** was the possibility that it might fluoresce and therefore be potentially useful in tracking huBuChE activity *in vitro* and *in vivo*. The reaction procedure first involved dissolving **112** in DCM and cooling the solution to -20°C. Coumarin-3-carboxylic acid, DCC and DMAP were added to the reaction, which was stirred vigorously and allowed to heat up to room temperature overnight. The reaction produced one primary product but also a significant quantity of an undesired urea by-product.



Scheme 3.21

In order to separate the undesired urea by-product from the target compound, the reaction mixture was extracted with DCM and washed with 1 M HCl, $NaHCO_3$ and brine, filtered and purified by flash chromatography. This only succeeded in partially removing the urea product from the target product. The use of 0.45 mm acro discs was finally employed to filter the mixture, which completely eliminate the urea by-product. Unfortunately, the purification procedure affected the yield of the reaction (20 %).

Compound **145** was also used to make the coumarin analogue **127**. In this case, DCC coupling gave compound **153**, which was much less polar than **112** and was therefore very soluble in only a small volume of DCM. As the unwanted urea impurity is insoluble in DCM, more urea by-product could be successfully filtered out without affecting the yield of the protected coumarin intermediate **153**. This compound was easily isolated in a good yield and was de-protected with TBAF and carbamoylated to give **127** in an improved yield of 52.8 %



Yield = 53 %

Scheme 3.22

3.6 THE SYNTHESIS OF 2-BENZYL CARBAMATE 5-DEOXY-5-BENZYLAMIDE

From Chapter 2 we saw that the subtle difference in structure between group 1 and reversed carbamate inhibitors had a significant impact of the biological efficacy of the inhibitors of each case. With this in mind, it seemed pertinent to synthesise the benzylamide **154** and to compare its activity with that of its ester analogue, the potent inhibitor **110**.

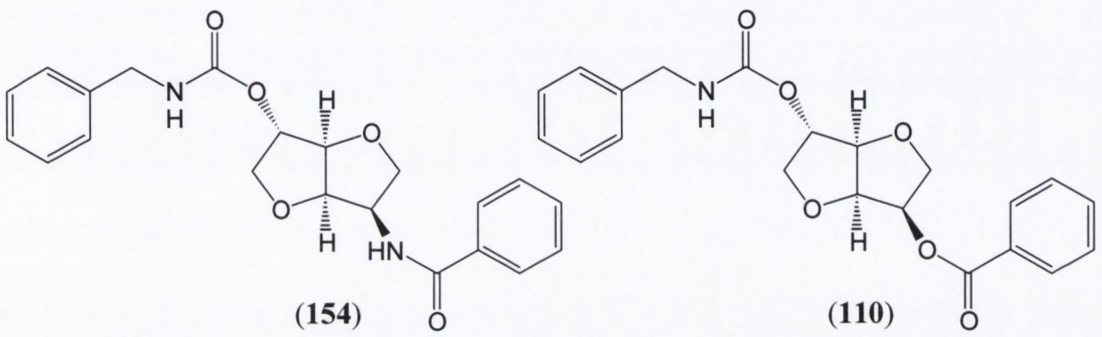


Figure 3.16

The approach taken to synthesise the benzylamide **154** involved the replacement of the hydroxyl group of isosorbide at position-5 with an azide group, with retention of configuration, as in Figure 3.17.

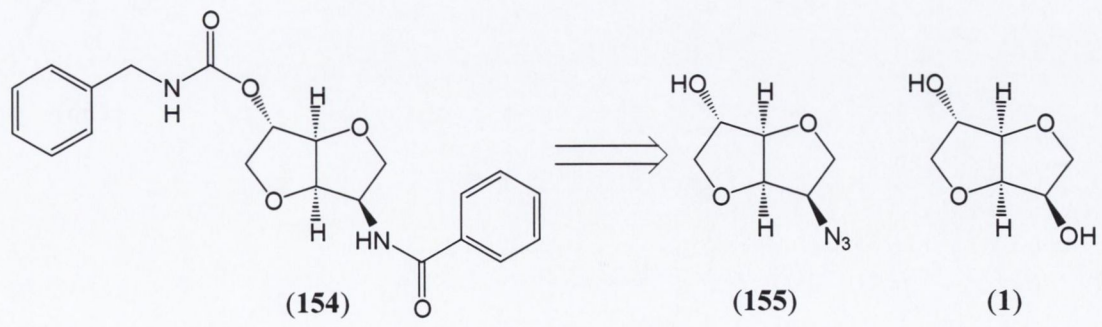


Figure 3.17

This approach was similar to that used to synthesise the reverse carbamates in Chapter 2, however, as the amino group was required at position-5, isoiodide **3** was used as the starting material as opposed to isomannide **2**, as was used in the synthesis of the reverse carbamates. Figure 3.18 shows the approach taken to the synthesis of **154**. This approach involved the mono-tosylation and azide displacement to introduce the azido group at position-5 in **155**. From this compound it would be possible to carbamoylate position-5 with benzyl isocyanate. Reduction of the azide group at position-5 and subsequent reaction with benzoyl chloride would give the target benzylamide **154**

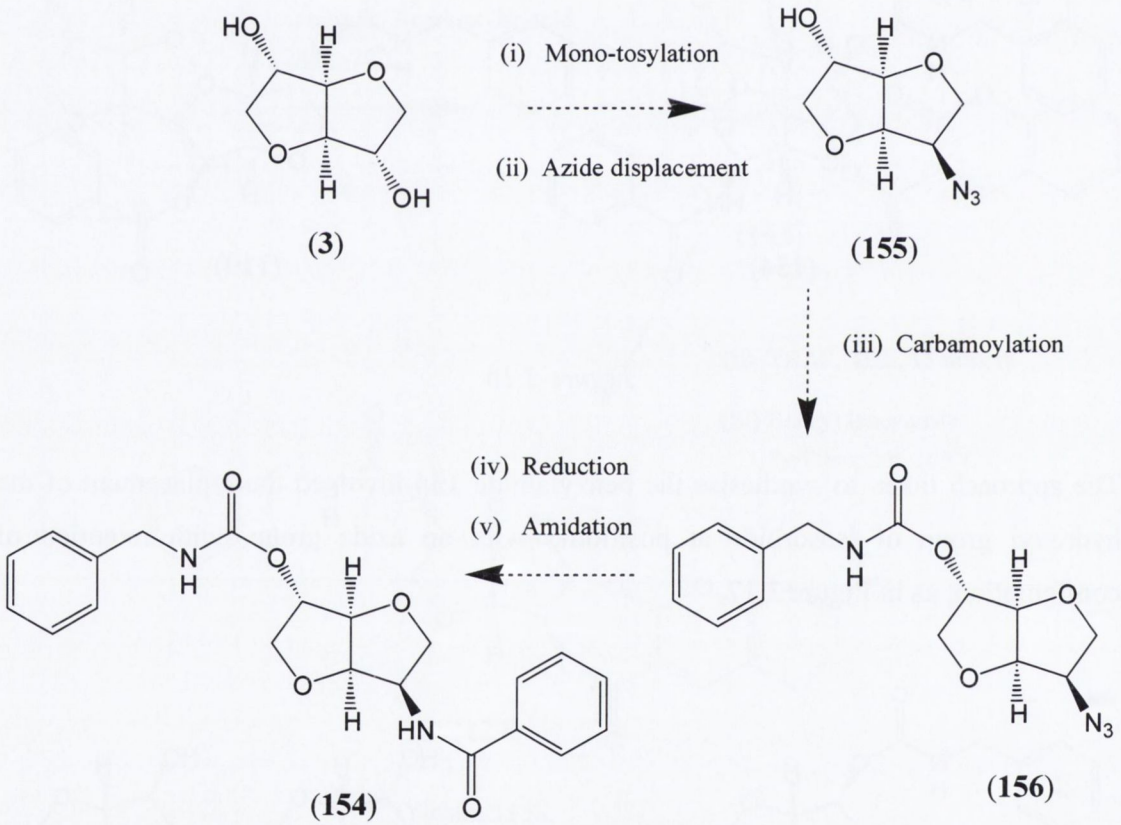
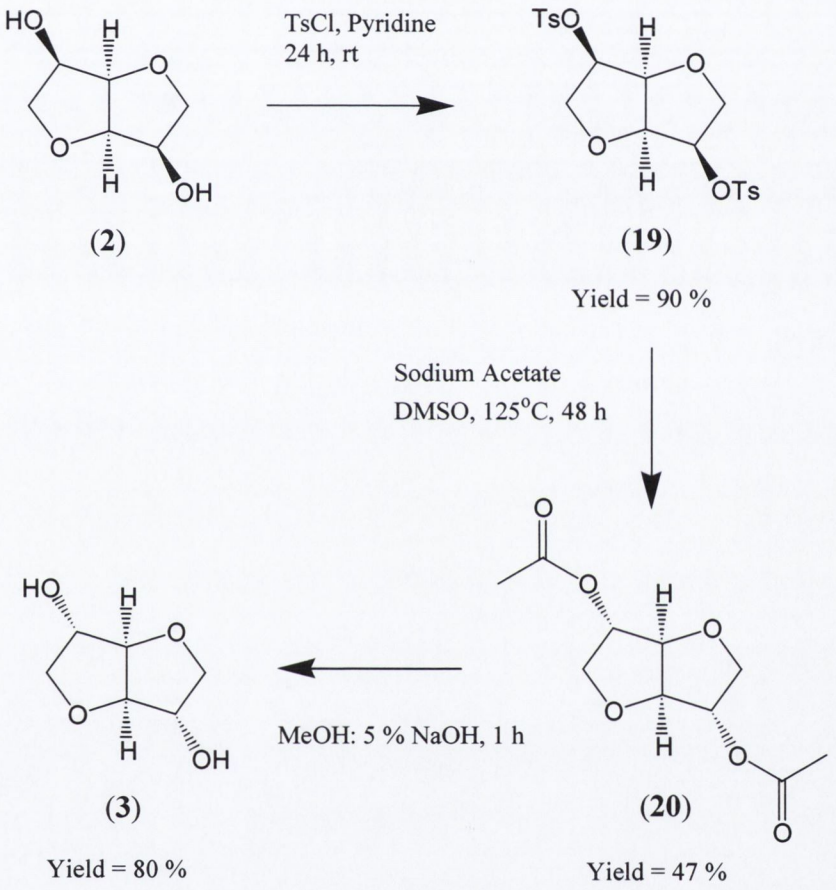


Figure 3.18

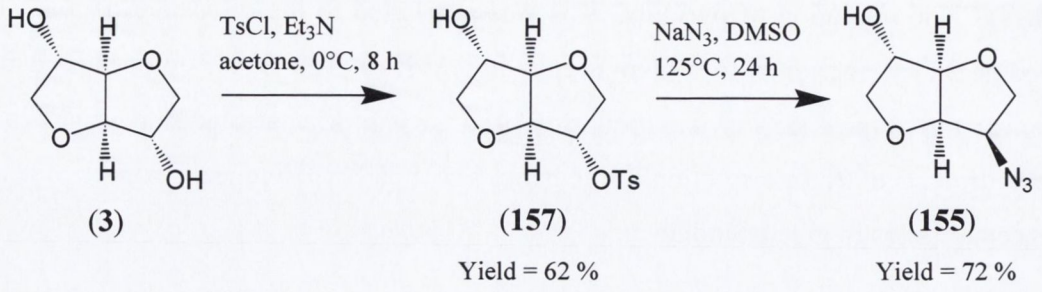
In order to take this approach it was first necessary to synthesise isoiodide **3**, which is not commercially available. Isoiodide **3** was synthesised from isomannide **2** using the approach outlined in Scheme 3.23. The di-tosylate of isomannide **19** was first prepared

from TsCl in a solution of pyridine. This was converted to the di-acetate derivative of isoiodide **20** by reaction with sodium acetate in DMSO at 120°C. This procedure caused the inversion of the endo-tosylate groups of **19** to the exo orientated acetate groups of **20**. The hydrolysis of **20** in a solution of methanol and 5 % NaOH aqueous solution cleaved the acetate esters to give isoiodide **3**.



Scheme 3.23

The mono-tosylate derivative of isoiodide **157** was synthesised by reaction with TsCl in the presence of triethylamine in a solution of acetone at 0°C. The azide displacement S_n2 reaction was carried out with excess sodium azide in DMSO at 125°C to give the azido intermediate **155** (Scheme 3.24).

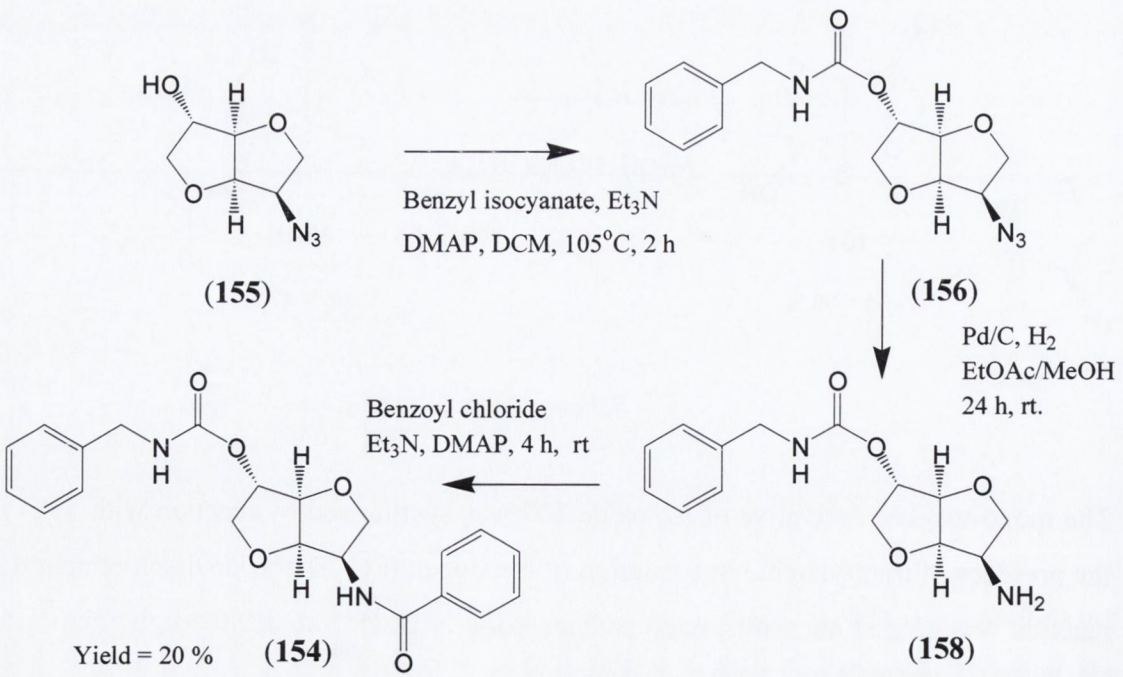


(3) **(157)** **(155)**

Yield = 62 % Yield = 72 %

Scheme 3.24

The synthesis of **154** was accomplished in three steps by from **155** Firstly, **156** was synthesised from **155** by reaction with benzyl isocyanate in the presence of triethylamine and DMAP. Secondly, the reduction of compound **156** to give **158** was achieved using 10 % palladium on activated carbon under an atmosphere of hydrogen gas. Finally, the target compound was prepared using benzoyl chloride, triethylamine, and DMAP. This procedure gave **154** in a yield of 20 % from isoiodide. Compound **154** was characterised by NMR and IR and mass spectrometry.



Scheme 3.25

H-H and C-H COSY spectra allowed for the correlation and assignment of all proton and carbon signals detected in the ^{1}H and ^{13}C spectra of **154**. The spectra were similar in terms of the signal coupling and splitting as the 5-ester substituted compounds. In the ^{1}H spectrum, the main differences were that **154** exhibited an extra NH signal at 6.73 ppm correlating to the amide in position-5. The IsH-5 signal was shifted slightly upfield compared to the ester inhibitors and gave a broad multiplet, due to overlapping with IsH-3 and IsH-4. The two protons of IsH-6 gave two different signals, which were observed at 4.31 and 3.46 reflecting their different chemical environments and non-equivalent orientation.

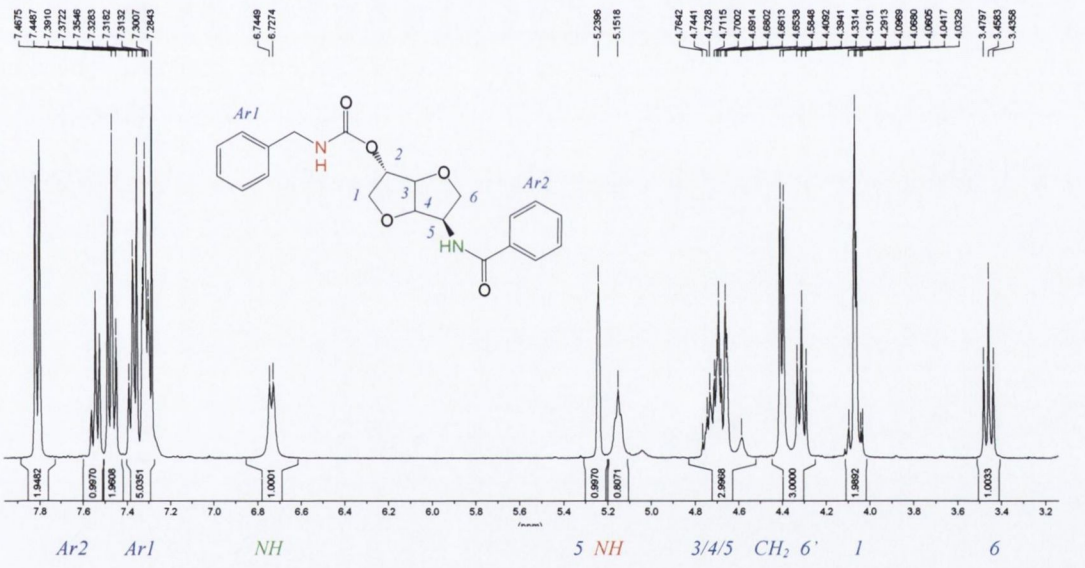
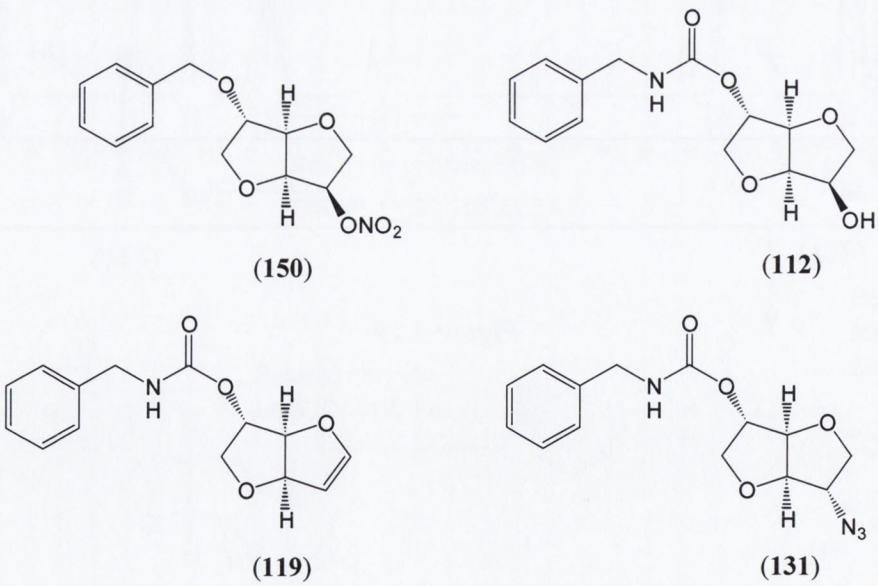


Figure 3.19

3.7 BIOLOGICAL TEST RESULTS AND DISCUSSION OF LIBRARY-2 COMPOUNDS

The results of the biological testing of Library-2 compounds are given in Tables 3.2 and 3.4, whilst the information in Table 3.3 is an example of a set of data generated for each compound. Table 3.5 shows the selectivity of huBuChE over AChE for the most potent compounds. As with the Library-1 compounds, all test compounds in this series were 'screened' at a concentration of 100 μM to determine whether they demonstrated sufficient inhibitory activity to merit further investigation. The compounds are identified in each table by their specific number and also by the group substituted at position-5.

Figure 3.20 shows the first set of compounds of Library-2 that were analysed. The non-carbamate **150**, which had a benzyl group, instead of a carbamate group, substituted at position-2 gave virtually no inhibition of either huBuChE or AChE. This compound emphasised the importance of including a carbamate group at position-2 in the design of isosorbide-based inhibitors.



(150) **(112)**

(119) **(131)**

Figure 3.20

Molecular modelling studies suggests that carbamate inhibitors cause the inhibition of huBuChE when serine 198 of the catalytic triad, attacks the carbonyl bond of the carbamate group, giving a stable tetrahedral structural, which inactivates the enzyme. The tetrahedral structure is stabilised by hydrogen bonding between the oxygen atom of the carbonyl group and the amino acid residues alanine 199 and glycine 116 and 117. In the case of Library-2 compounds, the inhibitor is further stabilised within the active site by hydrogen bonding between the aryl ring of the benzyl carbamate and the two aliphatic amino acid residues in the acyl pocket, leucine 286 and valine 288.

Compounds **112, 119,** and **131** which were unsubstituted at position-5, demonstrated good or moderate inhibition of huBuChE at 100 μM. All of these compounds possessed a benzyl carbamate at position-2, again emphasising the role of the carbamate group in the inhibition of huBuChE. However, these compounds showed poor inhibition of huBuChE at lower concentrations emphasising the need for substitution at position-5 to improve the overall inhibitory activity of these compounds.

Table 3.2: % huBuChE inhibition by Library-2 compounds *

No.	Name	% huBuChE 100μM	IC_{50} (nM) huBuChE	
	Non-carbamate compound			
150	2-*O*-benzyl-5-*O*-nitro	4.0		-
	5 -non-substituted compounds			
112	5-hydroxyl	94.5	3720	(3374 to 4102)
131	5-deoxy-5-azido -1,4:3,6-dianhydro-L-iditol	50.4		-
119	5-deoxy-L-xylohex-6-enitol	91.8	10250	(9190 to 11433)
	5-aliphatic ester substituted compounds			
114	5-*O*-acetyl	96.7	4018	(3273 to 4933)
115	5-*O*-propionyl	96.7	986.14	(801.16 to 1213.8)
116	5-*O*-valeryl	95.2	701.79	(521.27 to 944.82)
	5-cycloalkyl ester substituted compounds			
120	5-(cyclopropylcarbonyloxy-)	96.8	334.62	(253.23 to 442.18)
121	5-(cyclopentylcarbonyloxy-)	99.3	5.77	(1.47 to 11.83)
	5-sulfonyl ester substituted compounds			
118	5-*O*-trifluoromethanesulfonyl	85.1	358.93	(146.01 to 882.33)
119	5-*O*-methanesulfonyl	9.8		-
130	5-*O*-toluenesulfonyl	25.2		-
	5-aromatic ester substituted compounds			
122	5-*O*-1-naphthoyl	98.6	28.21	(25.45 to 31.27)
123	5-*O*-2-naphthoyl	99.6	31.75	(21.57 to 46.73)
124	5-(*p*-phenyl-phenylcarbonyloxy-)	98.6	12.33	(10.42 to 14.59)
128	5-*O*-nicotinoyl	99.4	57.33	(48.53 to 67.73)
129	5-*O*-isonicotinoyl	96.9	88.30	(73.46 to 106.1)
125	5-*O*- cinnamoyl	95.5	137.02	(102.80 to 182.64)
126	5-(*p*-heptyloxyphenylcarbonyloxy-)	79.2	2787	(2372 to 3273)
127	5-(coumarincarbonyloxy-)	99.8	72.64	(60.44 to 87.30)
	5- aromatic ether substituted compounds			
140	5-*O*-benzyl	92.8	52.29	(45.98 to 59.47)
148	5-*O*-(phenylpropyloxy-)	97.8	201.45	(137.0 to 296.2)
	5-amide substituted compound			
154	5-benzylamide	88.1		-

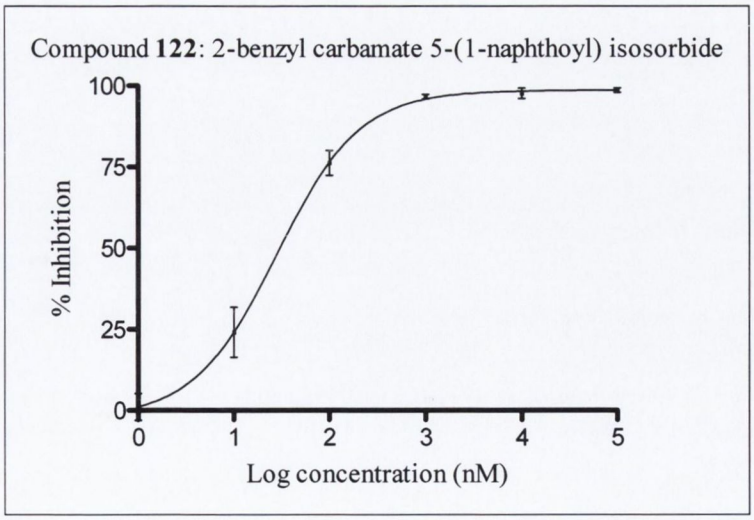
* Values shown are the mean of three readings. The IC_{50} results and 95 % confidence intervals, which are displayed in brackets, were calculated using GraphPad Prism software version 4.02. [S] 0.5 mM, [DTNB] 0.5mM.

Table 3.2 (contd.): % huBuChE inhibition by Library-2 compounds*

No.	Name	% BuChE 100μM	IC_{50} (nM) huBuChE	
	Ketone derivatives			
113	5-ketone	46.1		-
132	5-oxime	40.1		-
133	5-oxime benzoate ester	64.8		-
134	5-alkene ester [Wittig reaction]	91.3	4838	(3875 to 6040)
137	Lactone [Baeyer-Villiger reaction]	56.5		-
139	Lactam [Beckmann reaction]	63.4		-

Table 3.3: huBuChE inhibition data of compound 122.

Conc. (nM)	Log 10 Conc. (nM)	Rep1 % Inhibition	Rep2 % Inhibition	Rep3 % Inhibition	Mean % Inhibition	SD	95% Confidence
100000	5	98.4219	98.4510	98.9262	98.5997	0.2831	0.3204
10000	4	97.1730	97.3017	98.3254	97.6000	0.6315	0.7146
1000	3	96.8221	96.2766	96.6396	96.5794	0.2777	0.3142
100	2	76.5873	74.4767	77.4732	76.1790	1.5394	1.7420
10	1	23.3823	27.5134	21.4227	24.1061	3.1092	3.5183
1	0	0.1258	0.0013	2.9875	1.0382	1.6893	1.9118



* Values shown are the mean of three readings. The IC_{50} results and 95 % confidence intervals, which are displayed in brackets, were calculated using GraphPad Prism software version 4.02. [S] 0.5 mM, [DTNB] 0.5mM.

Table 3.4: % AChE inhibition by Library-2 compounds*

No.	Name	% AChE 100μM	IC_{50} (nM) AChE	
	Non-carbamate compounds			
150	2-*O*-benzyl-5-*O*-nitro	9.4		-
	5 -non-substituted compounds			
112	5-hydroxyl	14.8		-
131	5-deoxy-5-azido -1,4:3,6-dianhydro-L-iditol	4.6		-
119	5-deoxy-L-xylohex-6-enitol	9.8		-
	5-aliphatic ester substituted compounds			
114	5-*O*-acetyl	11.9		-
115	5-*O*-propionyl	4.8		-
116	5-*O*-valeryl	4.3		-
	5-cycloalkyl ester substituted compounds			
120	5-(cyclopropylcarbonyloxy-)	54.8		-
121	5-(cyclopentylcarbonyloxy-)	87.7	56.23	(29.31 to 79.14)
	5-sulfonyl ester substituted compounds			
118	5-*O*-trifluoromethanesulfonyl	5.8		-
117	5-*O*-methanesulfonyl	2.7		-
130	5-*O*-toluenesulfonyl	65.9		-
	5-aromatic ester substituted compounds			
122	5-*O*-1-naphthoyl	86.0	37.60	(20.56 to 68.79)
123	5-*O*-2-naphthoyl	78.1	43.42	(26.02 to 70.55)
124	5-(*p*-phenyl-phenylcarbonyloxy-)	89.3	51.64	(27.72 to 75.22)
128	5-*O*-nicotinoyl	32.9		-
129	5-*O*-isonicotinoyl	23.9		-
125	5-*O*- cinnamoyl	82.8		-
126	5-(*p*-heptyloxyphenylcarbonyloxy-)	-		-
127	5-(coumarincarbonyloxy-)	80.7	60.41	(40.99 to 81.48)
	5- aromatic ether substituted compounds			
140	5-*O*-benzyl	54.9		-
148	5-*O*-(phenylpropyloxy-)	50.9		-
	5-amide substituted compound			
154	5-benzylamide	46.1		-

* Values shown are the mean of three readings. The IC_{50} results and 95 % confidence intervals, which are displayed in brackets, were calculated using GraphPad Prism software version 4.02. [S] 0.5 mM, [DTNB] 0.5mM.

Table 3.4: % AChE inhibition by Library-2 compounds*

No.	Name	% AChE 100μM	IC_{50} (nM) AChE
	Ketone derivatives		
113	5-ketone	10.8	-
132	5-oxime	83.2	-
133	5-oxime benzoate ester	9.0	-
134	5-alkene ester [Wittig reaction]	4.9	-
137	Lactone [Baeyer-Villiger reaction]	9.7	-
139	Lactam [Beckmann reaction]	6.5	-

Table 3.5: Selectivity of Library-2 compounds

No.	Name	IC_{50} (nM) huBuChE	IC_{50} (μM) AChE	Selectivity
114	5-*O*-acetyl	4018	-	24.9
115	5-*O*-propionyl	986.14	-	101.4
116	5-*O*-valeryl	701.79	-	142.5
118	5-*O*-trifluoromethanesulfonyl	358.93	-	278.6
122	5-*O*-1-naphthoyl	28.21	37.60	1332.9
123	5-*O*-2-naphthoyl	31.75	43.42	1367.6
124	5-(*p*-phenyl-phenylcarbonyloxy-)	12.33	51.64	4188.2
128	5-*O*-nicotinoyl	57.33	-	1744.3
129	5-*O*-isonicotinoyl	88.30	-	1132.5
125	5-*O*-cinnamoyl	137.02	-	729.8
126	5-(*p*-heptyloxyphenylcarbonyloxy-)	2787	-	35.9
127	5-(coumarincarbonyloxy-)	72.64	60.41	831.6
120	5-(cyclopropylcarbonyloxy-)	334.62	-	298.8
121	5-(cyclopentylcarbonyloxy-)	5.77	56.23	9745.2
140	5-*O*-benzyl	52.29	-	1912.4
148	5-(phenylpropyloxy-)	201.45	-	496.4
134	5-alkene ester [Wittig product]	4838	-	20.7

* Values shown are the mean of three readings. The IC_{50} results and 95 % confidence intervals, which are displayed in brackets, were calculated using GraphPad Prism software version 4.02. [S] 0.5 mM, [DTNB] 0.5mM.

The aliphatic ester compounds **114, 115** and **116** were considered moderate inhibitors of huBuChE. They showed good inhibition of huBuChE at 100 μM and showed modest inhibition at lower concentrations. The SARs of this group of compounds showed a pattern where an increase in the length of the carbon chain of the aliphatic ester increased the inhibitory activity of the compounds, i.e. the IC_{50} results of compounds **114, 115** and **116** were 4018, 986.14 and 701.79 nM respectively.

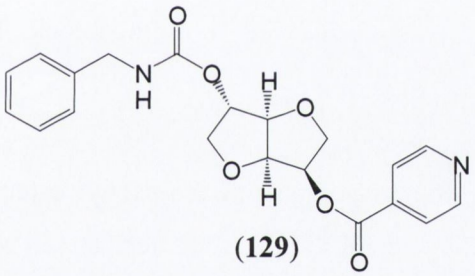
The sulfonyl ester compounds were largely poor inhibitors of huBuChE. However, **118**, whose chemical structure was the same as that of the mesylate **117**, bar the presence of a CF_3 group instead of a CH_3 group, gave inhibition of 85.1 % at 100 μM for huBuChE as opposed to 9.8 % for **117**. Compound **118** demonstrated a good IC_{50} huBuChE with an IC_{50} result of 358.93 nM.

All compounds which were substituted with an aromatic ester group at position-5 gave good inhibition of huBuChE at 100 μM. The IC_{50} values of each of these compounds were determined and, with the exception of **130**, all of these compounds were good inhibitors of huBuChE with IC_{50} results between 12.33 and 137.02 nM. Compound **126** was likely to be sterically hindered in terms of its ability to either enter the active site gorge of or interact with the catalytic triad of the active site due to the long aliphatic chain substituted at the para position of the aryl ring of the ester. Nonetheless, this compound did exhibit some huBuChE inhibitory activity and it may be possible that this inhibitor interacts with the peripheral site of the enzyme thus blocking the entrance of the substrate to the active site gorge.

The most potent aromatic substituted inhibitor was compound **124**, which bore a bi-phenyl ester at position-5 and had an IC_{50} of 12.33 nM for huBuChE. The two naphthoyl esters, **122** and **123**, also showed good inhibition of huBuChE, with similar IC_{50} values of 28.21 and 31.75 nM respectively, showing that the orientation of the naphthoyl group in the structure of the inhibitor has no significant effect on inhibitory activity (refer to page 91 for the chemical structures of compounds **122** and **123**). The nicotinate ester compounds also gave good inhibition of huBuChE with the isonicotinate **129** providing

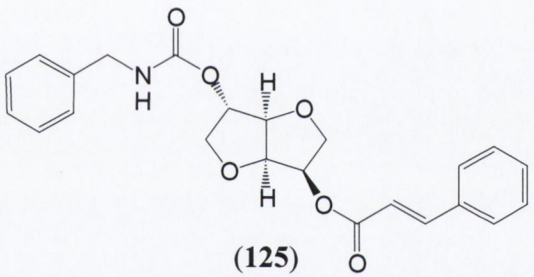
slightly superior inhibition of huBuChE, with an IC_{50} of 57.33 nM, compared to 88.30 nM for compound **128**. These inhibitors compare quite favourably with other known huBuChE inhibitors such as physostigmine and rivastigmine.

Compounds **125** and **127** also gave good inhibition of huBuChE with compound **125** showing that longer aromatic esters in position-5 give less inhibition of than shorter aromatic esters. This observation was mirrored by the results obtained for the ethers compound **140** and **148**, with the shorter ether **140** giving an IC_{50} of 52.29 nM for huBuChE, similar to that of the isonicotinate ester, while the longer ether **149** gave an IC_{50} comparable with the cinnamoyl ester **125** (see Figure 3.21). Cycloalkyl compounds **120** and **121** were also very good inhibitors of huBuChE with compound **121** proving to be highly potent with an IC_{50} of 5.77 nM for huBuChE.



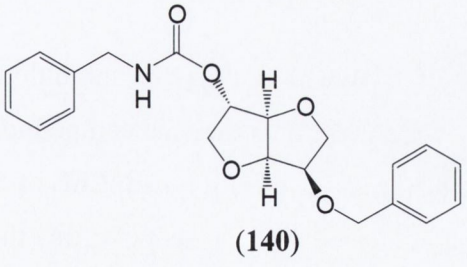
(129)

IC_{50} = 57.33 nM



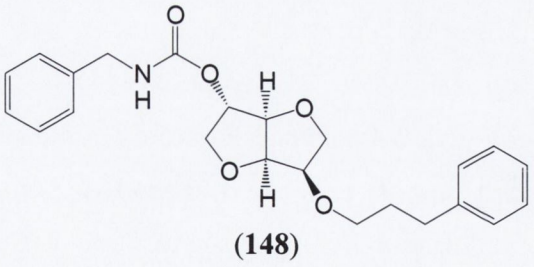
(125)

IC_{50} = 137.02 nM



(140)

IC_{50} = 52.29 nM



(148)

IC_{50} = 201.45 nM

Figure 3.21

The ketone **113** and its associated derivatives were all very poor inhibitors of huBuChE and AChE except for the alkene **134**, which showed moderate inhibition of huBuChE. These results reinforce the finding that substitution of isosorbide-based compounds at position-5 is essential to facilitate the inhibition huBuChE.

All Library-2 compounds were shown to be very poor inhibitors of AChE with even the most potent compound giving an IC_{50} of 37.60 μM for AChE. Therefore the inhibitors proved to be quite selective for huBuChE with respect to AChE.

3.8 SUMMARY

A total of 29 test compounds were synthesised in the work described in this chapter representing the second library of isosorbide-based inhibitors. All compounds except for **150** possessed a benzyl carbamate in position-2 of the isosorbide building block. The compounds varied by virtue of the group substituted at position-5.

Whilst most of the compounds produced were aromatic, aliphatic or sulfonyl esters, a range of other compounds including ethers, amides, alkenes and ketone derivatives were also prepared. The chemical structures of the compounds produced were completely novel and were synthesised using a variety of chemical pathways.

Most compounds demonstrated good inhibition of huBuChE with poor inhibition of AChE giving them good selectivity of huBuChE over AChE, with the lead compounds of this chapter, namely **121**, **124** and **122**, giving excellent IC_{50} values for huBuChE of 5.77 nM, 12.33 nM and 28.21 nM respectively. Future studies outside the scope of this thesis will determine the efficacy of these compounds *in vivo*, where they may act more efficiently than compounds demonstrating greater potency *in vitro*, in terms of water solubility and stability.

However, while the compounds synthesised compare favourably with known inhibitors and represent a marked improvement in terms of potency and selectivity compared to reversed carbamate compounds, they failed to improve upon the potency and selectivity of compound **112**, which exhibits a potency of 4.3 nM with respect to huBuChE and a selectivity of over 20,000 of huBuChE over AChE. In the work detailed in the following chapter it was decided to investigate the effects of substitution on the aryl group in position-5 of **112**. Therefore the design of compounds synthesised in Chapter 4 was based on the model shown in Figure 3.22.

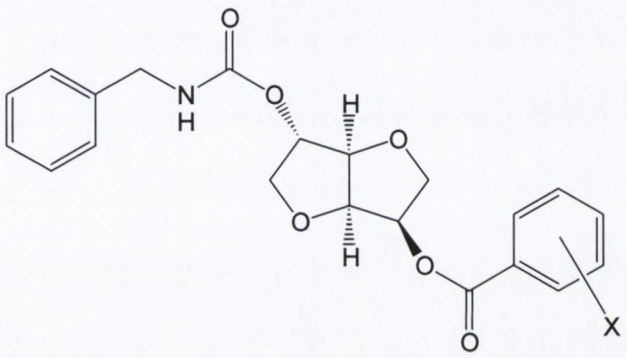


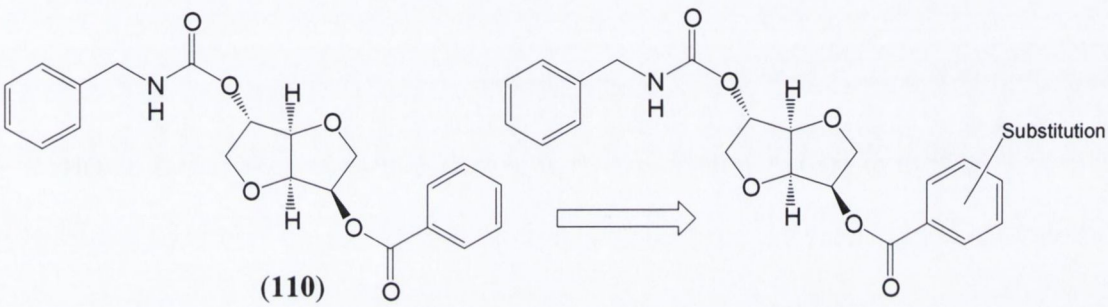
Figure 3.22

CHAPTER 4

SYNTHESIS OF LIBRARY-3 COMPOUNDS: SUBSTITUTED ARYL ESTER DERIVATIVES OF 2-BENZYL CARBAMATE 5-BENZOYL ISOSORBIDE

4.1 THE SYNTHESIS OF 2-BENZYL CARBAMATE 5-BENZOATE ESTERS

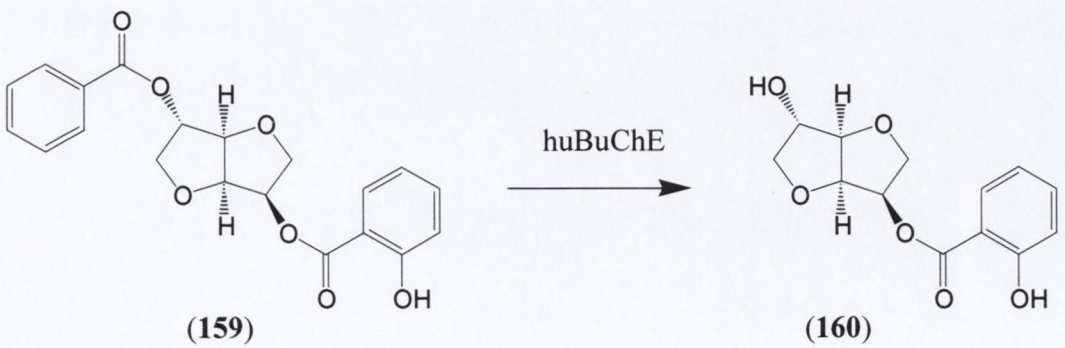
Despite the success in Chapter 3 of synthesising a series of compounds with potent and selective inhibition of huBuChE, we failed to improve upon the potency and selectivity of **110**, possessing an IC_{50} of 4.3 nM and a selectivity of approximately 24,000 for huBuChE. The logical next step was to investigate the effect of substitution on the potency and selectivity of 2-benzyl carbamate 5-benzoate esters (Figure 4.1).



(110)

Figure 4.1

We were especially interested in a 5-benzoate ester bearing an ortho –OH group (ie. A salicylic ester) as our research group had already shown that 2-benzoate 5-salicylic isosorbide **159** is hydrolysed at position-2 with exceptional rapidity[4, 111].



(159) **(160)**

Figure 4.2

The 2-aspirinate ester of 5-salicylic isosorbide **161** is the most successful aspirin prodrug discovered, is patent-protected by this research group[222] and is in preclinical development. Given the high affinity of huBuChE for esters of this type, it was hoped that compound **162** would emerge as a highly potent and selective inhibitor of huBuChE.

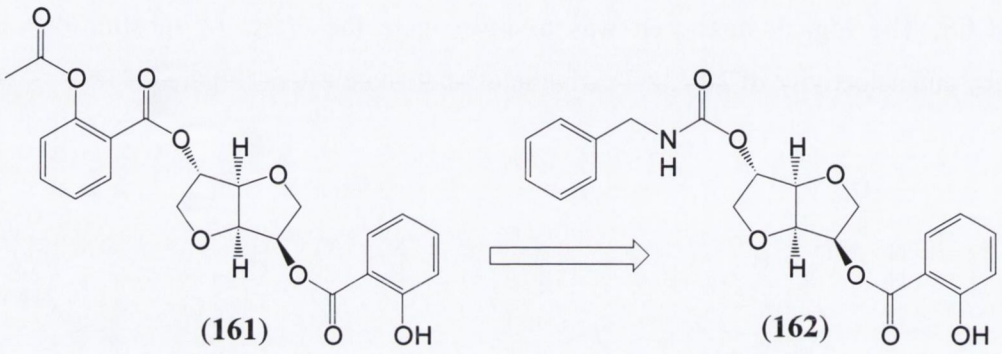
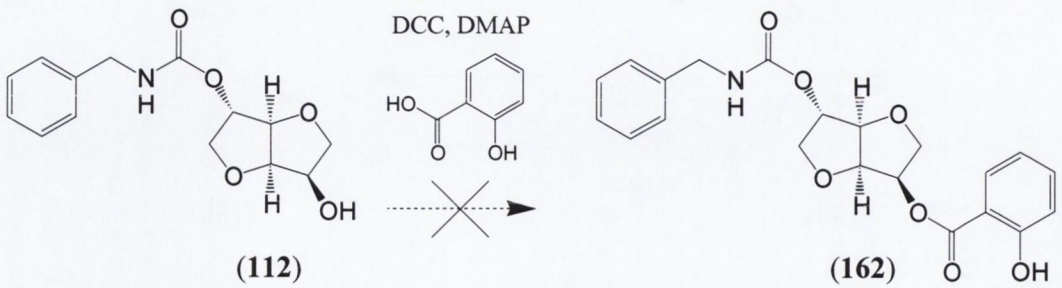


Figure 4.3

4.2 THE SYNTHESIS OF 2-BENZYL CARBAMATE 5-HYDROXYL BENZOATE ESTERS

The first approach investigated to the synthesis of 2-benzyl carbamate 5-salicylate isosorbide **162**, involved the direct esterification of **112** with salicylic acid. However, this approach proved unsatisfactory, as the procedure gave rise to a number of complex products due to the competition between the –OH groups of **112** and salicylic acid.



Scheme 4.1

Therefore, a protection strategy was adopted, which involved the introduction of a benzyl protected hydroxyl benzoate ester at position-5 of **112**, followed by the reduction, to give 2-benzyl carbamate 5-hydroxyl benzoate isosorbide esters.

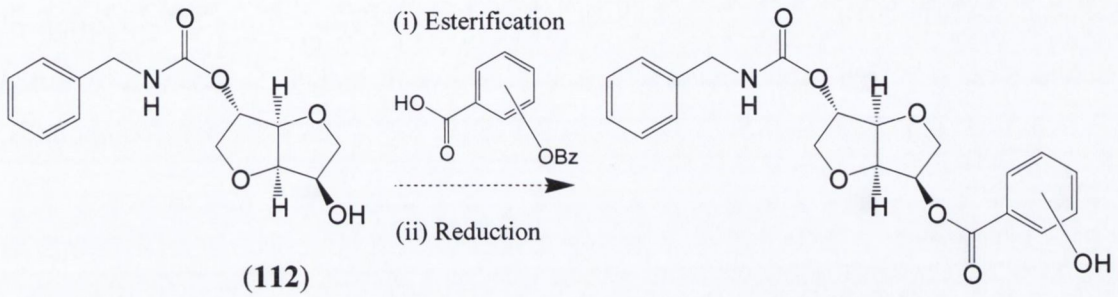
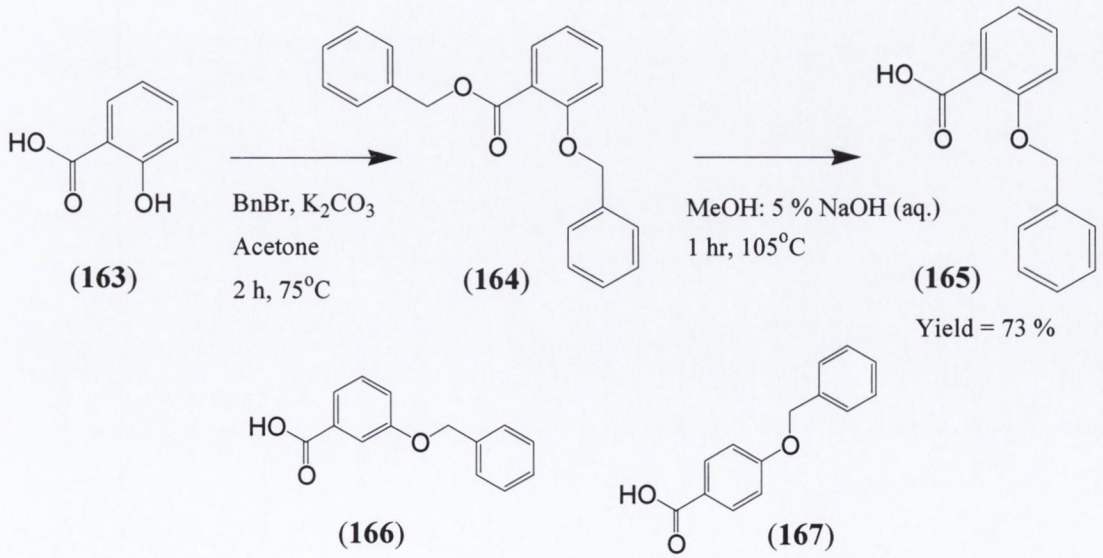


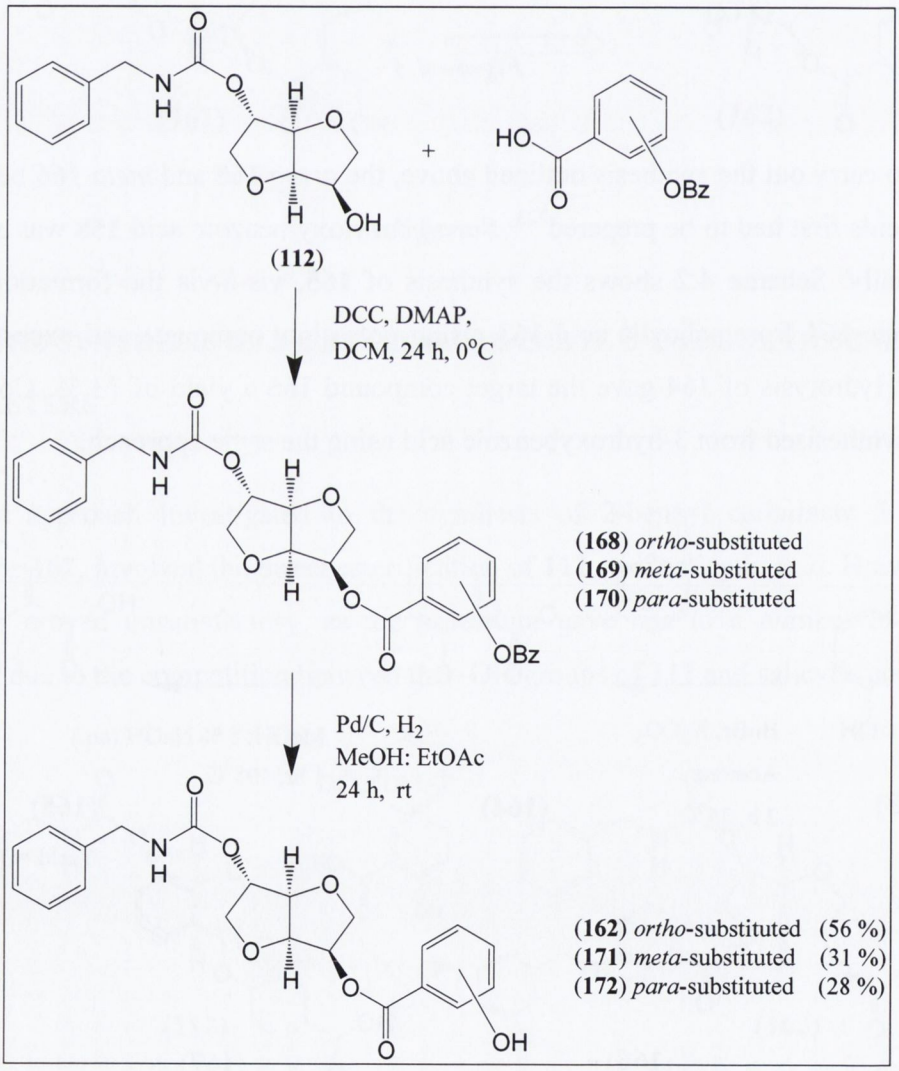
Figure 4.4

In order to carry out the synthesis outlined above, the *ortho* **165** and *meta* **166** benzyloxy benzoic acids first had to be prepared[223]. *Para*-benzyloxy benzoic acid **158** was available commercially. Scheme 4.2 shows the synthesis of **165,** vis-à-vis the formation of the intermediate **164** from salicylic acid **163**, using potassium carbonate and excess benzyl bromide. Hydrolysis of **164** gave the target compound **165** a yield of 73 %. Compound **166** was synthesised from 3-hydroxybenzoic acid using the same approach.



Scheme 4.2

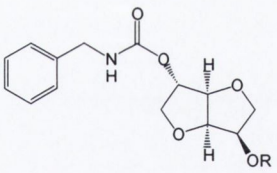
The *ortho*-, *meta*- and *para*- hydroxyl substituted benzoate esters were synthesised by firstly reacting **112** with *ortho*, *meta* or *para* benzyloxy benzoic acid using the acylating agent DCC, in the presence of DMAP, in a solution of chloroform to give the benzyloxy substituted benzoate ester compounds **168**, **169** and **170**. These compounds were then reduced using 10 % palladium on activated carbon and hydrogen gas in a solution of methanol and ethyl acetate to remove the substituted benzyl group[224], giving a hydroxyl group substituted at the ortho-, meta- and para- position of the respective compounds, **162**, **171** and **172**.

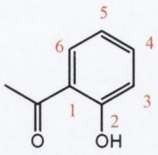


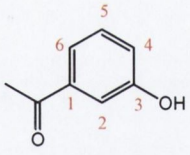
Scheme 4.3

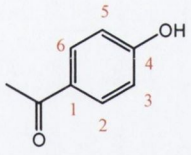
As with the synthesis of the coumarin product **127** in Chapter 3, the DCC reactions again proved problematic, insofar as the reaction mixtures contained a number of impurities including the characteristic urea by-product. Purification of compounds **168**, **169** and **170** included filtration using filter paper and acro discs, extraction with water and DCM, and finally separation by flash chromatography.

The intermediate compounds **168**, **169** and **170** were not fully characterised but NMR analyses were performed to ensure that the DCC coupling reactions had been successful. The successful synthesis of these compounds was indicated by the ^{1}H spectra, which detected 14 signals in the aromatic region and a two proton signal at 5.1 ppm relating to the benzyloxy CH_2 group. The ^{13}C DEPT spectra showed four CH_2 signals relating to IsC-1 and IsC-6, the CH_2 group of the carbamate ester and the CH_2 group of the benzyloxy group. The ^{1}H NMR analysis of the hydroxyl-substituted benzoate esters **162**, **171** and **172** gave similar spectra, except for characteristic chemical shifts and splitting in the aromatic region between 6.8 and 8.0 ppm, which are summarised in Table 4.1. They each gave nine proton signals in this region. The CH_2 group signal of the benzyloxy group was absent in the ^{1}H and ^{13}C spectra of each compound.









	162	171	172
Position -2	-	7.55 (singlet)	7.93 (doublet with H-6)
Position -3	7.02 (doublet)	-	6.86 (doublet with H-5)
Position -4	7.51 (multplet)	7.05 (double doublet)	-
Position -5	6.93 (triplet)	7.14 (broad singlet)	6.86 (doublet with H-3)
Position -6	7.89 (double doublet)	7.59 (doublet)	7.93 (doublet with H-2)

Table 4.1

4.3 THE SYNTHESIS OF 2-BENZYL CARBAMATE 5-NITRO, NITROSO AND AMINO BENZOATE ESTERS

The approach to the synthesis of 5-nitro and amino-benzoate esters is presented in Figure 4.5. The 5-nitro substituted esters were synthesised from **112** and nitrobenzoyl acid chlorides. The reduction of the nitro-benzoate esters gave the amino-benzoate esters.

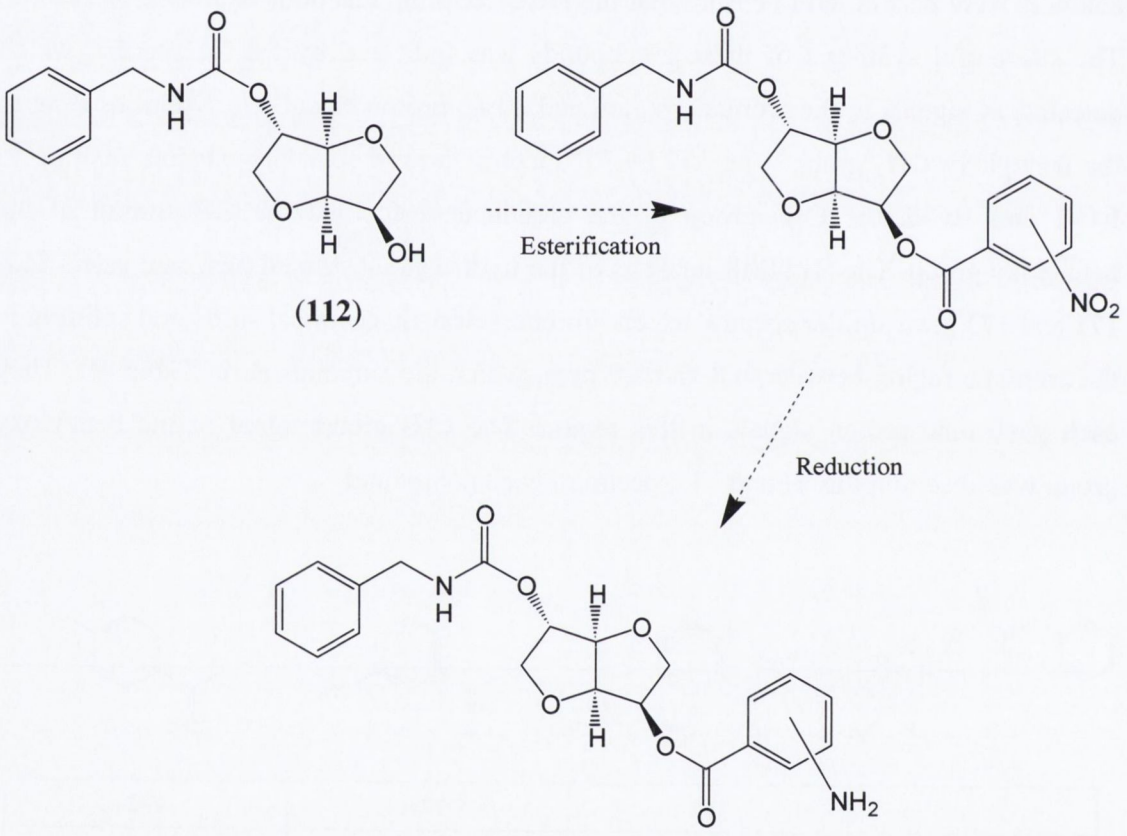
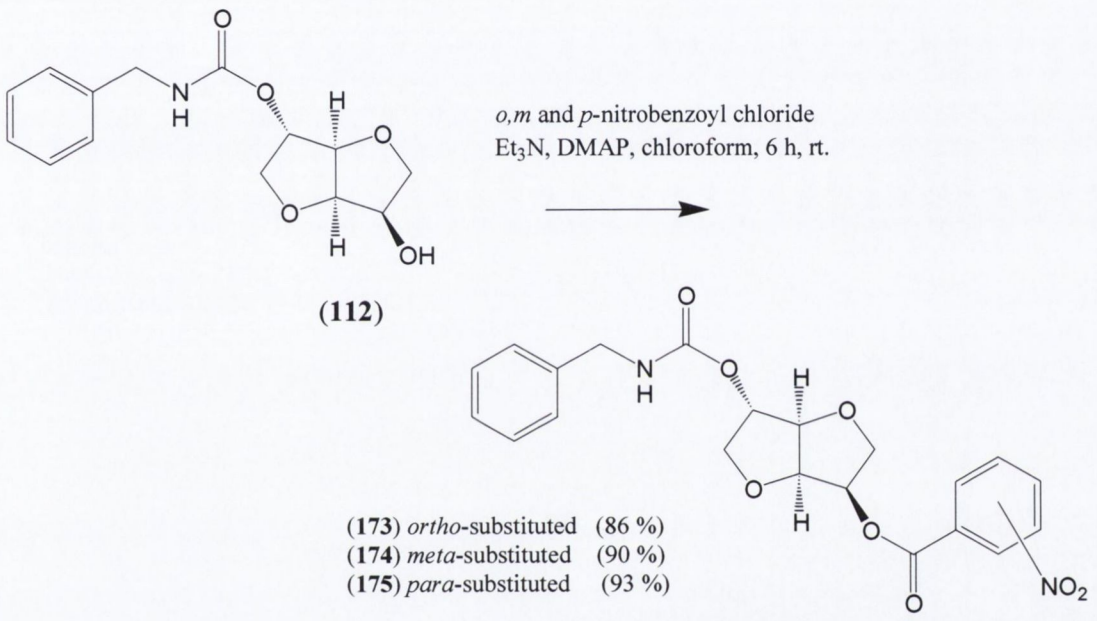


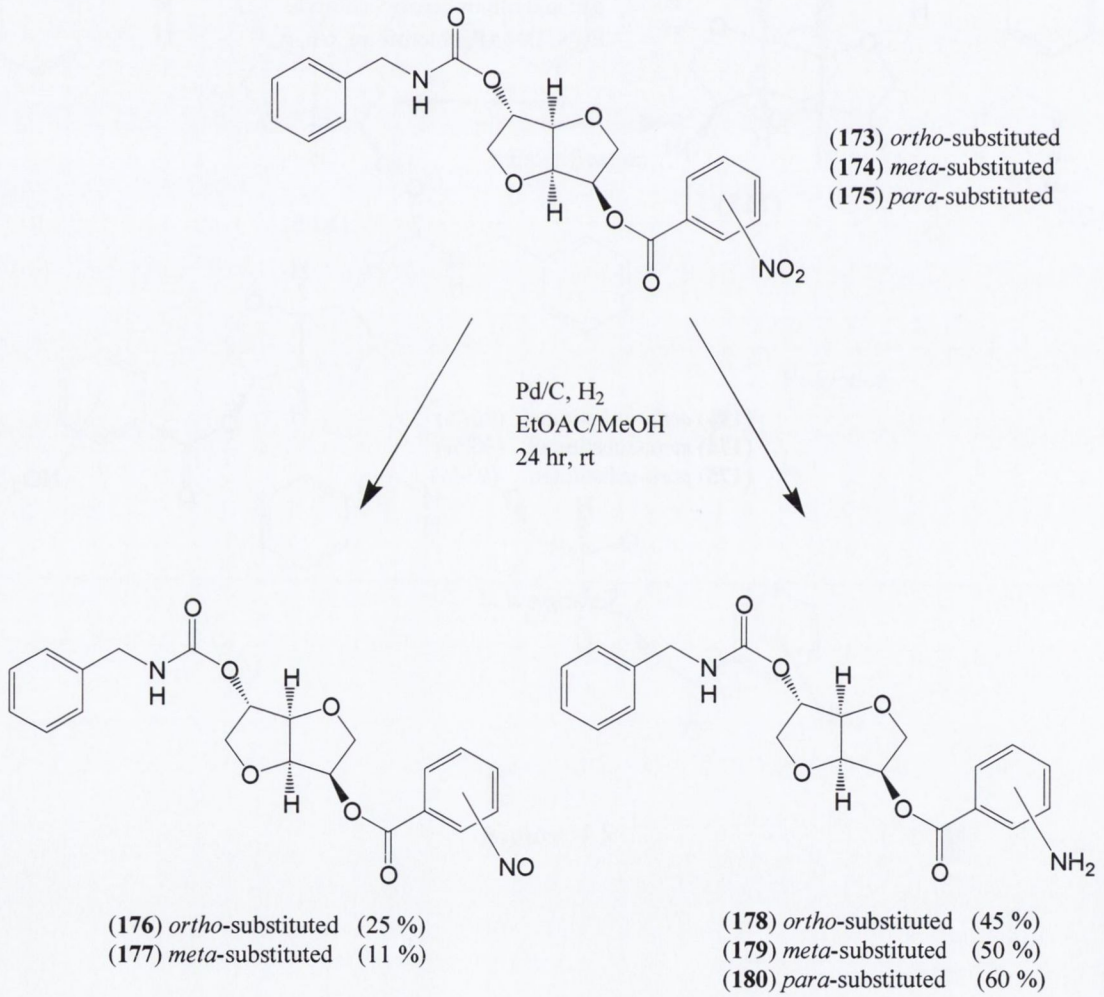
Figure 4.5

The nitro-substituted compounds **173**, **174** and **175** were synthesised from **112** with the relevant ortho, meta or para substituted nitrobenzoyl chloride in the presence of triethylamine and DMAP[225, 226] in chloroform. The resulting crude nitro-substituted benzoate esters were recrystallised from hot methanol to give the target compounds as white crystalline compounds, except for the ortho-substituted nitro-benzoate ester, which resisted recrystallisation and was given as an oil.



Scheme 4.4

From the nitro-substituted benzoate esters it was possible to synthesise the amino derivatives of these compounds[227, 228], where the nitro group was reduced to an amine group using 10 % palladium on activated carbon and hydrogen gas in a solution of ethyl acetate and methanol. TLC analysis upon completion of the reduction reactions showed that two products were obtained in the reduction of the ortho and meta-substituted benzoate esters, whilst only one product was obtained in the reduction of the para-substituted benzoate ester. The reaction products were isolated using flash chromatography.



Scheme 4.5

Compounds **176** to **180** were analysed by ^{1}H and ^{13}C NMR spectroscopy with the products of each reduction giving very similar ^{1}H and ^{13}C spectra to the respective nitro-substituted reactant, with only some slight differences observed in the aromatic regions. The successful synthesis of **178** and **179** was confirmed by high-resolution mass spectrometry (HRMS) (see Table 4.2), which gave m/z ratios of 421.1376 for each, which corresponded to the molecular mass of the expected amino compounds **178** and **179**. HRMS analyses of the secondary products suggested that some of the ortho and meta nitro-benzoate esters had been reduced to the nitroso derivatives **176** and **177**.

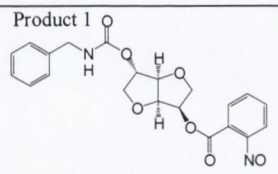
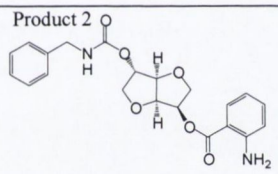
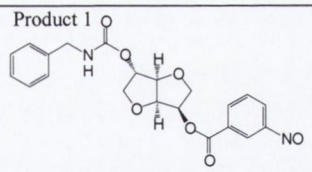
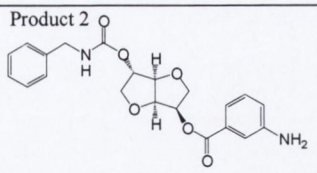
No	Compound	Mass Required	Mass Found
176	Product 1 	435.1168	435.1270
178	Product 2 	421.1393	421.1376
177	Product 1 	435.1168	435.1518
179	Product 2 	421.1393	421.1376

Table 4.2

4.4 BIOLOGICAL TESTING OF SUBSTITUTED BENZOATE ESTER INHIBITORS

4.4.1 THE huBuChE AND AChE INHIBITION OF SUBSTITUTED BENZOATE ESTER INHIBITORS

The effects of substitution on the aryl esters were investigated using the Ellman assay procedure employed in earlier chapters. From the results in Table 4.3, it is clear that all compounds in this series are excellent inhibitors of huBuChE and poor inhibitors of AChE. As indicated already, there were high hopes that **162** would transpire to be a good inhibitor of huBuChE, and in the event, **162** gave an exceptional IC_{50} value of 0.179 nM, which exceeded expectations. This compound also offered excellent selectivity of huBuChE over AChE.

Table 4.3: % huBuChE and AChE inhibition of substituted benzoate esters*

No.	Name	% huBuChE 100 nM	IC_{50} (nM) huBuChE	% AChE 100μM	IC_{50} (μM) AChE
162	5-(salicyloyl)	99.8	0.179 (0.151 to 0.210)	62.6	10.40 (9.82 to 10.97)
171	5-(*m*-hydroxy benzoyl)	99.2	16.34 (14.28 to 18.69)	0	-
172	5-(*p*-hydroxy benzoyl)	98.5	323.38 (249.46 to 419.21)	0	-
173	5-(*o*-nitro) benzoate	99.3	18.04 (15.40 to 21.13)	0	-
174	5-(*m*-nitro benzoyl)	96.1	32.00 (27.61 to 37.09)	0	-
175	5-(*p*-nitro benzoyl)	99.1	117.00 (100.54 to 136.16)	58.7	-
178	5-(*o*-amino benzoyl)	98.6	27.43 (24.09 to 31.23)	0	-
179	5-(*m*-amino benzoyl)	98.1	85.73 (72.50 to 101.38)	0	-
180	5-(*p*-amino benzoyl)	99.4	297.06 (251.18 to 351.33)	98.1	-
176	5-(*o*-nitroso benzoyl)	99.7	38.80 (33.24 to 45.30)	0	-

* Values shown are the mean of three readings. The IC_{50} results and 95 % confidence intervals, which are displayed in brackets, were calculated using GraphPad Prism software version 4.02. [S] 0.5 mM, [DTNB] 0.5mM.

Table 4.4 shows the inhibition data for **162**, which is typical of the data generated for each compound. The inhibition curve was generated by GraphPad Prism software version 4.02.

Table 4.4: huBuChE inhibition data for compound 162

Conc. (pM)	Log 10 Conc. (nM)	Rep1 % Inhibition	Rep2 % Inhibition	Rep3 % Inhibition	Mean % Inhibition	SD	95% Confidence
100,000,000	8	97.84424	96.6547	100.1246	98.2078	1.7633	1.9953
10,000,000	7	98.6478	96.3011	99.6573	98.2021	1.7219	1.9485
1,000,000	6	100.0685	95.5069	98.4343	98.0032	2.3111	2.6153
100,000	5	97.39726	99.3469	93.7822	96.8421	2.8236	3.1951
10,000	4	95.87312	93.7771	98.6326	96.0943	2.4353	2.7557
1,000	3	88.2457	84.4432	90.8211	87.8367	3.2086	3.6308
100	2	34.2521	31.5343	38.3121	34.6995	3.4110	3.8598
10	1	10.7483	8.58529	8.2344	9.1893	1.3615	1.5406
1	0	0.2587	3.1118	5.9099	3.0935	2.8256	3.1975

Compound **162**: 2-benzyl carbamate 5-salicylate isosorbide

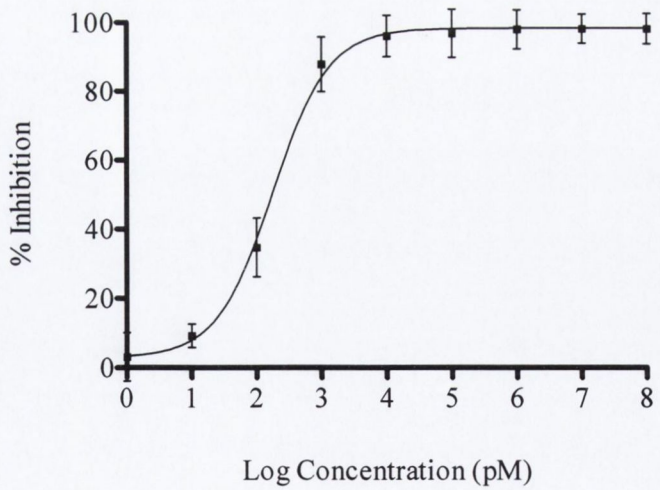


Table 4.5 shows how compound **162** compares with established BuChE inhibitors and with other inhibitors, synthesised during this project. It is significantly more potent than the other leading BuChE inhibitors in the field. It is 16.6 and 50 fold more potent than bambuterol and MF-8422 respectively, which were the two specific inhibitors of BuChE

used in the influential studies of ACh in 'knockout' mice[64, 65] (refer to Section 1.4). It is also 33.3 fold more potent than phenylethylcymserine, which is the leading BuChE inhibitor analogue of the quintessential cholinesterase inhibitor, physostigmine, and approximately 5400 fold more potent than *iso*-OPMA[6]. Compound **162** is also 200 and 260 fold more potent than the AD therapeutic agents, rivastigmine and tacrine[5]. Compound **162** is easily the most potent and selective isosorbide-based inhibitor. It is approximately 24-fold more potent than the previous lead compounds **110** and with selectivity of approximately 60,000 this compound represents the most selective huBuChE ever reported.

Table 4.5 Selectivity of Library-4: substituted benzoate esters

No.	Name	IC_{50} (nM) huBuChE	IC_{50} (μM)* AChE	Selectivity
162	5-(salicyloyl)	0.18	10.4	57,777
171	5-(*m*-hydroxy benzoyl)	16.3	100	6,135
172	5-(*p*-hydroxy benzoyl)	323.4	100	309
173	5-(*o*-nitro benzoyl)	18.0	100	5,556
174	5-(*m*-nitro benzoyl)	32	100	3,125
175	5-(*p*-nitro benzoyl)	117.0	60♦	855
178	5-(*o*-amino benzoyl)	27.4	100	3,650
179	5-(*m*-amino benzoyl)	85.7	100	1,167
180	5-(*p*-amino benzoyl)	297.1	60♦	202
176	5-(*o*-nitroso benzoyl)	38.8	100	2577
110	5-*O*-(benzoyl)	4.3	100	23,255
121	5-(cyclopentylcarbonyloxy-)	5.7	56.2	9,860
124	5-(*p*-phenyl-phenylcarbonyloxy-)	12.3	51.6	4,195
122	5-*O*-(1-naphthoyl)	28.2	37.6	1,333
	Bambuterol[6]	3	30	10,000
	MF-8422[6]	9	100	11,111
	Phenylethylcymserine[6]	6	30	5,000
	Physostigmine[6]	16	0.28	0.57
	iso-OMPA[6]	980		35
	Rivastigmine[6]	37	4150	112
	Tacrine[6]	47	190	4

* A value of 100 μM was used to calculate the selectivity of compounds that showed less than 50 % inhibition of AChE at this concentration.

♦ A value of 60 μM was used to calculate the selectivity of compounds that showed less than 50 % inhibition of AChE at this concentration.

4.4.2 MOLECULAR MODELLING OF COMPOUND 162

We cannot be certain of the inhibitor-enzyme interactions that make **162** more efficient in inhibiting huBuChE compared to any other inhibitors discussed, but from molecular modelling studies it can be shown that the hydroxyl group, substituted at the ortho position of the aryl ester, may form a hydrogen bond with the Aspartic acid 70 at the peripheral site of the active site gorge (yellow arrow in Figure 4.6). It is also possible that the hydroxyl group of the ester forms a hydrogen bond with Tyrosine 332 (green arrow in Figure 4.7). These interactions, which have not been observed in the molecular modelling of other potent isosorbide inhibitors, may facilitate extra binding of the ester portion of the molecule to the enzyme, thus giving greater inhibition. It also may be the case that hydrogen bonding between the substituted hydroxyl group and the carbonyl of the ester (green arrow in Figure 4.6) helps to stabilise the structure of the ester portion by limiting the degree of rotation or movement of the aryl ring.

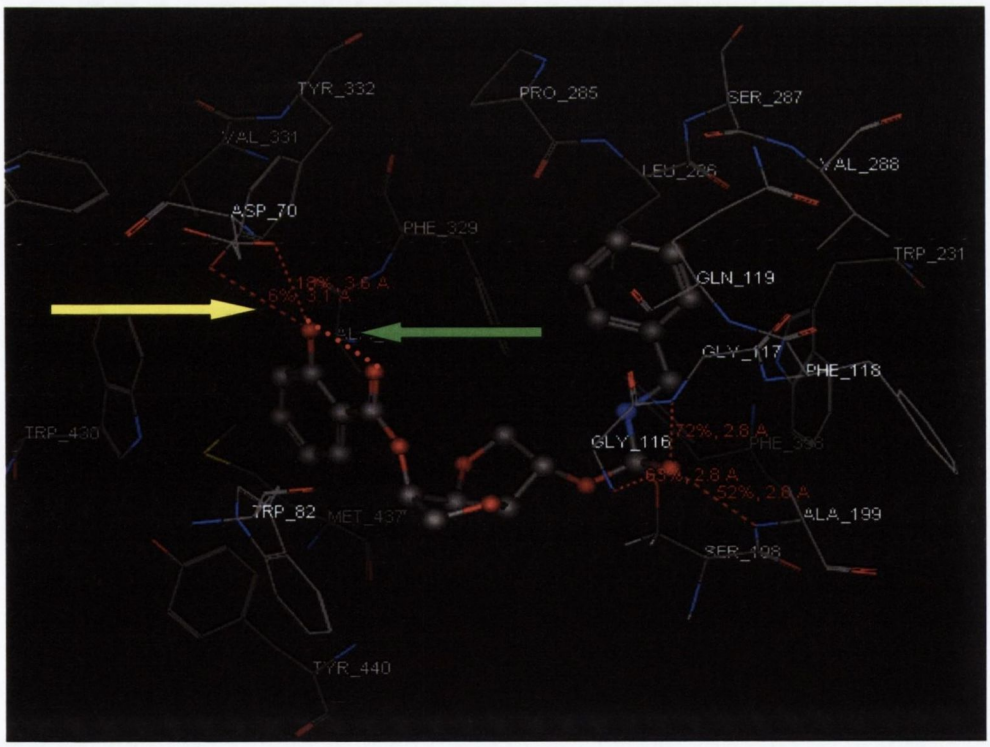


*Figure 4.6: Intramolecular and intermolecular interactions of **162** with BuChE*

It can be shown that the aryl ring of the ester fits exceptional well within the active site gorge and aligns itself along the side of the gorge, next to Tryptophan 82 (yellow arrow in Figure 4.7). As with other carbamate inhibitors, the carbonyl bond of the carbamate group forms a tetrahedral intermediate with the serine 198 of the catalytic triad[6] (red arrow). The interactions between the oxygen atom of the carbonyl bond and the three amino residues, Alanine 199, Glycine 116 and 117, at the so-called 'oxyanion hole can also be shown (orange arrow). The benzyl group also fits well into the active site and is shown to position itself between Valine 288 and Leucine 286 of the acyl pocket (purple arrow).

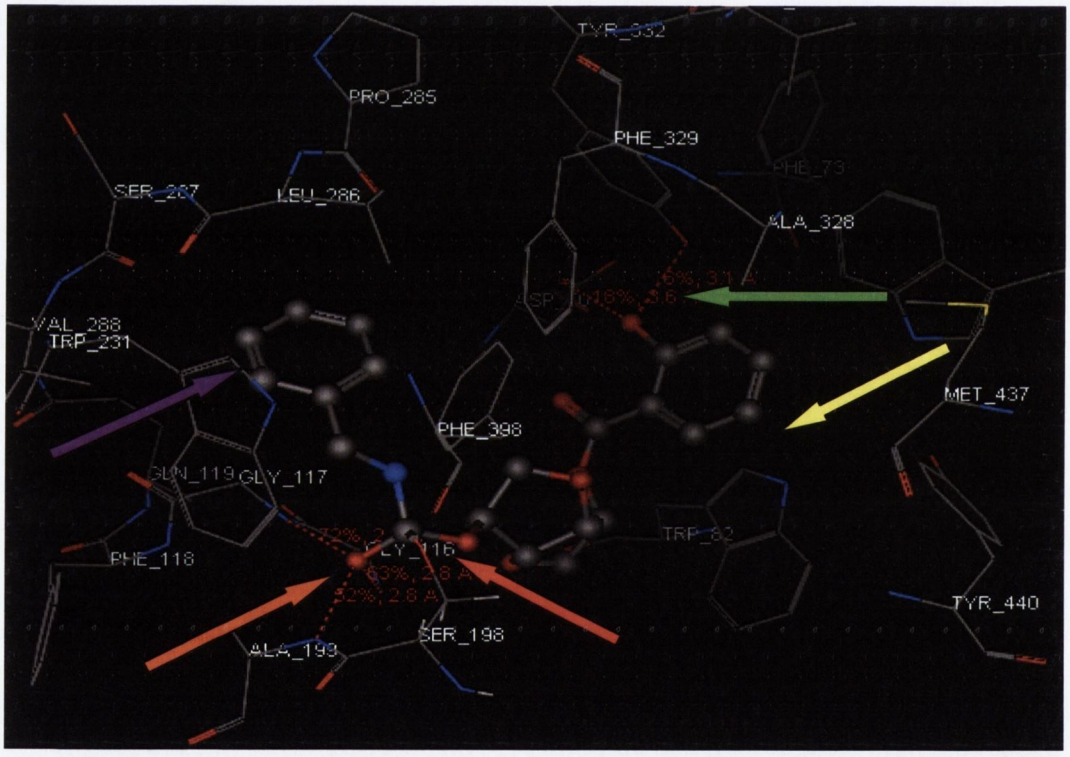


*Figure 4.7: Intermolecular interactions of **162** with BuChE*

All pictures were created using Molecular Operating Environment (MOE) – Chemical Computing Group, Montreal, Canada. Structures were prepared by minimisation of the ligand and protein from a starting structure based on the crystal structure of the transition state analogue TMTFA (tri-methyl tri-fluoro acetophenone) in AChE[229].

4.4.3 THE SAR OF NITRO, AMINO AND HYDROXYL ESTERS

The SAR information presented in Table 4.3 showed some interesting trends and features relating to specific sub-groups within the overall set of compounds. For example, with respect to the orientation of each of the substituents, the huBuChE inhibition activity decreases in the order of *ortho-*, *meta-*, then *para-* substitution, i.e. the IC_{50} results of the *ortho-*, *meta-* and *para-* substituted hydroxyl esters is 0.18, 16.34 and 323.38 nM respectively. This trend is mirrored in the nitro-substituted esters, whose IC_{50} values are 18.04, 32.0 and 117.0 nM respectively and the amino substituted esters, whose IC_{50} values are 27.43, 85.75 and 247.06 respectively.

In the case of **162**, it is possible that the high level of inhibition was due to either intramolecular hydrogen bonding or intermolecular bonding with the enzyme. ^{1}H NMR indicates the existence of a hydrogen bond in **162** between the salicylate group and the carbonyl group of the ester. It is possible that this may promote a favourable inhibitory conformation of the compound when in the active site gorge, however, if this is the case, one is forced to wonder why **178**, bearing *o*-amino group does not seem to act in the same way. It was surprising that the amino compounds gave results that were so different to the hydroxyl compounds, given that both groups contain electronegative atoms (i.e., O and N) and both groups can act as hydrogen bond donators and acceptors. Of course, the amino group has the capacity to ionise, however, given the pKa of the anilide (~4.6) and the assay buffer pH (8), it would be surprising if the protonated form played a role in the binding of the inhibitor to the enzyme. It was also surprising that the amino compounds offered less inhibition than the nitro compounds, which can only act as hydrogen bond acceptor.

It may also be possible that the –OH group of **162** interacts *inter*molecularly with the enzyme. One possibility for this, as already shown in Figure 4.6, is the interaction between the -OH of **162** and Asp 70, giving **162** greater binding to the enzyme and therefore greater inhibition. It is however unclear, if that is the case, why the *m*-hydroxyl substituted **171** cannot make a similarly reinforcing interaction. Furthermore, it is not

clear why the compounds of this class, except for **162**, exhibit less inhibition than that of the unsubstituted benzoate **110**.

Within the scope of this thesis, it is clear that the discovery of **162** represents a landmark achievement in the pursuit of the development of potent and selective huBuChE inhibitors using isosorbide as a building block. With an IC_{50} of 0.18 nM and a selectivity of approximately 60,000 for huBuChE over AChE, this inhibitor is the most potent and selectivity inhibitor of huBuChE ever reported. It is important to note that the degree of potency is based on biological testing *in vitro* and the pharmacological and toxicological properties of this, and all other huBuChE inhibitors of this project, are as yet unknown.

Outside the scope of this thesis, this compound will be carried forward by our broader research group, who will investigate the stability and pharmacological toxicological properties of this inhibitor and other isosorbide inhibitors *in vivo* and explore the ability of these inhibitors to act in a therapeutic sense. With a level of potency approximately 200 times that of established AD therapeutics, clearly **162** holds the potential to operate in a therapeutic setting. Ultimately, further research will determine whether these inhibitors can deliver on their potential.

At this stage of our research, it was clear that the salicylate was the optimum group at position-5. Therefore, we decided to focus on modifying the carbamate group in position-2, whilst retaining the salicylate group at position-5. The design of our inhibitors for the following section is shown in Figure 4.8. We were especially interested in the synthesis of secondary carbamates, since all others investigated in this project and in the wider program, were up to this point unsubstituted carbamates.

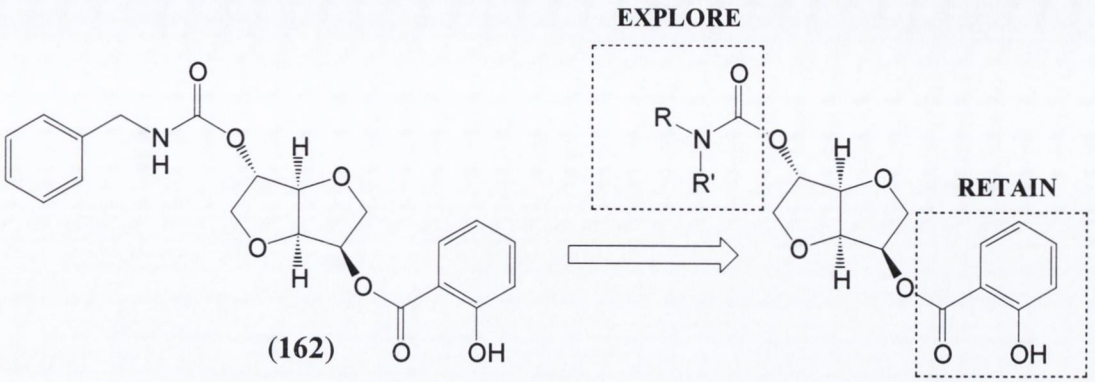


Figure 4.8

4.5 THE SYNTHESIS OF 2-CARBAMATE 5-SALICYLIC ESTERS OF ISOSORBIDE

4.5.1 INTRODUCTION

The synthetic approach to making these compounds is outlined in Figure 4.9. This approach used the 2-tBDMS protected isosorbide **145,** as the starting material. Into this structure was incorporated a *o*-benzyloxy protected salicylate group at position-5. Cleavage of the tBDMS protecting group allowed the introduction of several carbamate groups into position-2. Finally, the reduction of the benzyl group to give the salicylate ester afforded the desired series of alternative carbamate salicylic esters.

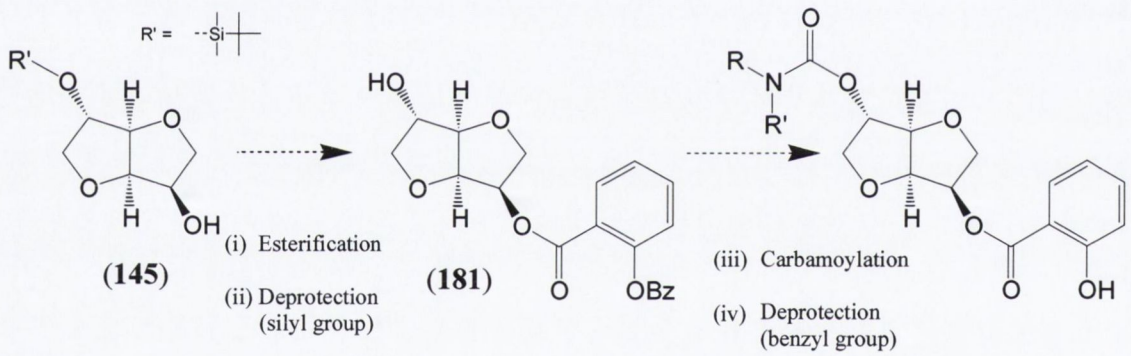
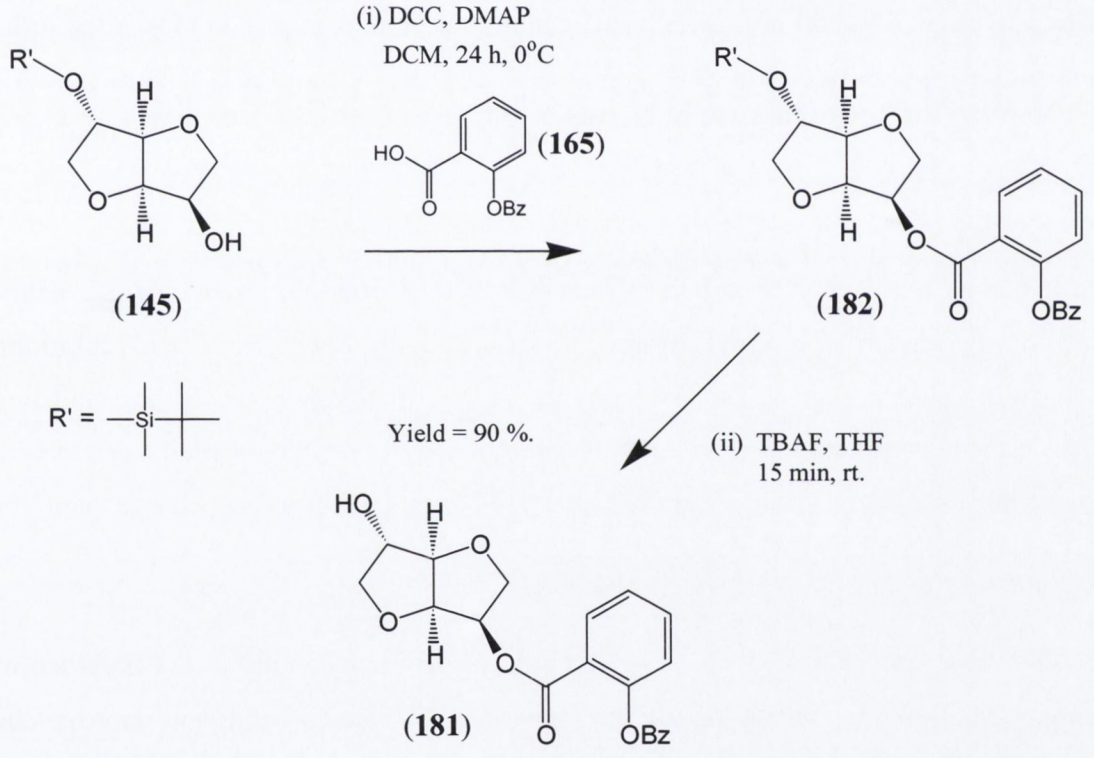


Figure 4.9

4.5.2 THE SYNTHESIS OF 5-BENZYLOXY BENZOATE ISOSORBIDE 181

The first two steps of Figure 4.9 were designed to synthesise **181**, which could be used as a substrate to prepared a series of compounds whose composition would vary depending on the type of carbamate ester at position-2. The first step of this approach worked very effectively with only one primary product being obtained. The first step worked best when excess alcohol was used in the reaction and, therefore, three mol. eq. of **145** was used to one mol. eq. of **165**.



Scheme 4.6

These factors allowed the reaction to proceed relatively quickly and efficiently. Upon completion of the reaction, the undesired urea by-product, which is sparingly soluble in DCM, was easily removed by filtration. The crude reaction product was purified by column chromatography using a mobile phase of 95:5 DCM: ethyl acetate. This mobile phase worked very effectively in separating the excess DCC reagent and remaining urea

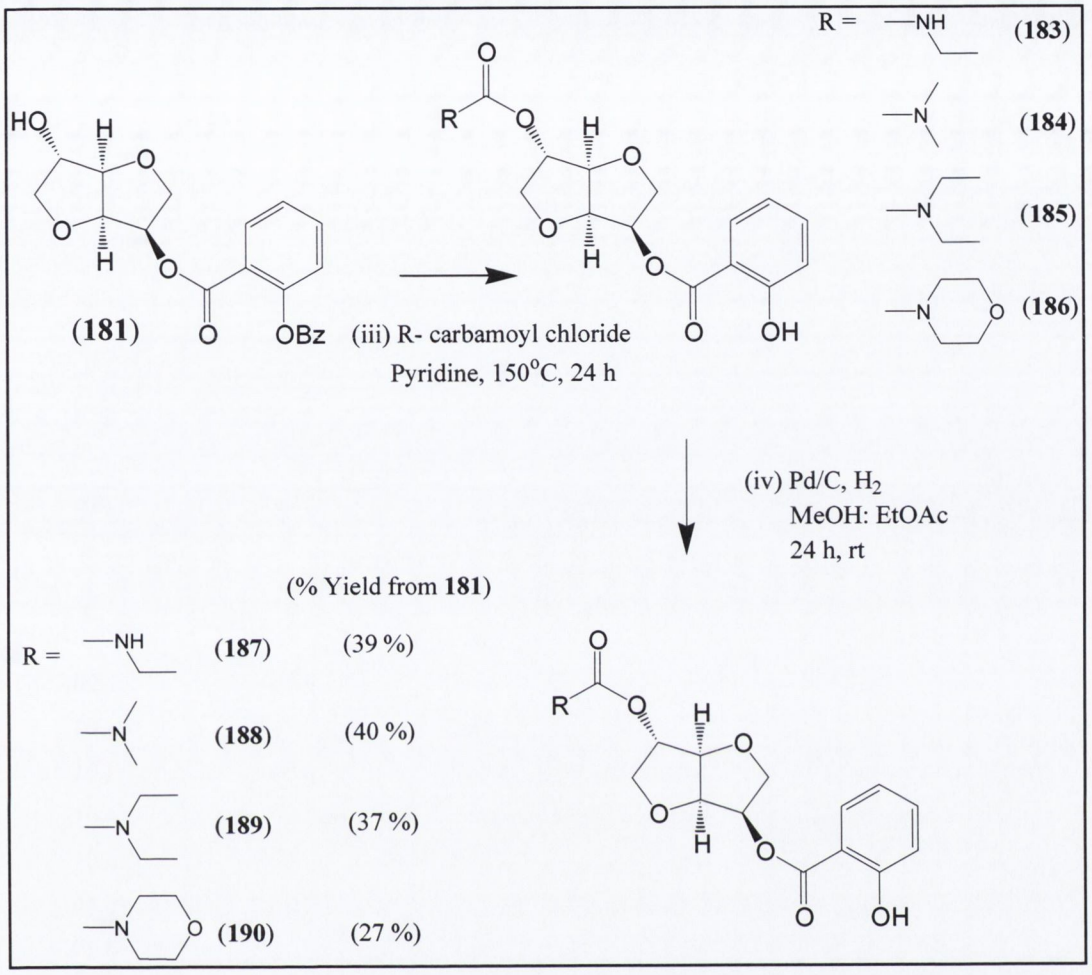
by-product from the **182** and also recovered the unreacted alcohol starting material **145.** Compound **181** was afforded by deprotection of the tBDMS-protecting group at position-2 with TBAF to give **181** as a clear oil with a yield of 90 %.

4.5.3 THE SYNTHESIS OF 2-CARBAMATE 5-SALICYLIC ESTERS OF ISOSORBIDE

The general procedure[230, 231, 232] in synthesising **188** to **190** (Scheme 4.7) involved the reaction of **181** with a relevant carbamoyl chloride in anhydrous conditions at a temperature of 150°C to give a series of intermediate compounds which were then reacted with 10 % palladium on activated carbon and hydrogen gas, to cleave the ortho substituted benzyl ether to afford the salicylic ester group at position-5. The mono-ethyl compound **187** was synthesised from **181** using ethyl isocyanate[233] as opposed to a carbamoyl chloride.

The reaction times of the carbamoyl reactions were often in excess of 24 hours, significantly longer than those observed when making esters from acid chlorides. Carbamoyl chlorides are much less reactive than acid chlorides due to the stabilising effect of the nitrogen atom, whose unbonded pair of electrons can be partially shared with the carbon of the carbonyl group, thus reducing its positive charge and its electrophilicity[234].

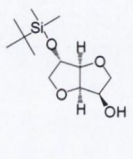
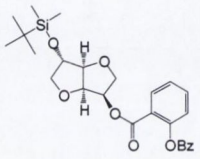
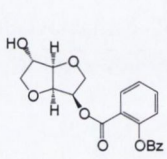
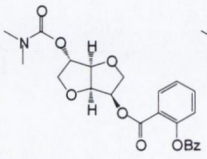
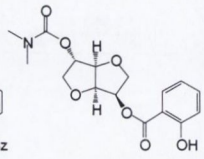
The dimethyl carbamate **184** was also prepared using triethylamine in the presence of excess DMAP in a solution of DCM. However, this method did not improve the efficiency of the reaction. The purification of the morpholine **190** proved problematic as the intermediate reaction product **186**, the carbamoyl chloride reagent and the starting material **181** all had similar Rf values on TLC. Purification by column chromatography therefore proved difficult and the product and reactants were finally separated using a non-polar mobile phase of 4:1 hexane: diethyl ether.


R = —NH (183)
—N (184)
—N (185)
—N O (186)
HO
H
O
H
O
O
OBz
(181)
(iii) R- carbamoyl chloride
Pyridine, 150°C, 24 h
R
O
H
O
OH
(iv) Pd/C, H2
MeOH: EtOAc
24 h, rt
(% Yield from 181)
R = —NH (187) (39 %)
—N (188) (40 %)
—N (189) (37 %)
—N O (190) (27 %)

Scheme 4.7

4.5.4 THE NMR OF 2-CARBAMATE 5-SALICYLIC ESTERS OF ISOSORBIDE

All final compounds in this series were fully characterised by NMR, IR, MS and elemental analysis (see experimental section). NMR analysis proved particularly useful in monitoring the progress of this multi-step procedure.

	145	182	181	184	188
$(CH_3)_2$	0.10	0.10	-	-	-
$(CH_3)_3$	0.89	0.90	-	-	-
OH	2.78	-	2.90	-	10.59
$-N(C\underline{H}_3)_2$	-	-	-	2.90	2.90
IsH-6	3.52	3.80	3.96	3.86	4.01
IsH-6'	3.87	4.04	3.84	3.99	4.01
IsH-1	3.87	3.80	3.84	3.99	4.01
IsH-1'	3.87	3.88	3.84	3.99	4.01
IsH-5	4.31	5.35	5.35	5.35	5.40
IsH-2	4.31	4.28	4.26	5.12	5.19
IsH-3	4.31	4.39	4.42	4.56	4.56
IsH-4	4.62	4.72	4.95	4.96	4.99
CH_2		5.18	5.18	5.18	-
ArH_2-3	-	7.02	7.02	7.02	7.00
ArH_2-5	-	7.02	7.02	7.02	6.91
ArH_2-4	-	7.33	7.33	7.33	7.48
ArH_1	-	7.45	7.45	7.45	-
ArH_2-5	-	7.90	7.90	7.90	7.87

Table 4.6

4.5.5 BIOLOGICAL TESTING OF 2-CARBAMATE 5-SALICYLIC ESTERS OF ISOSORBIDE

From Table 4.7, we can see that all inhibitors of this group gave good inhibition at 100 μM. Compounds **189** and **190** were moderate inhibitors of huBuChE with IC_{50} values of 2609 and 1057 nM respectively, while **187** and **188** showed excellent inhibition with IC_{50} values of 9.61 and 6.40 respectively.

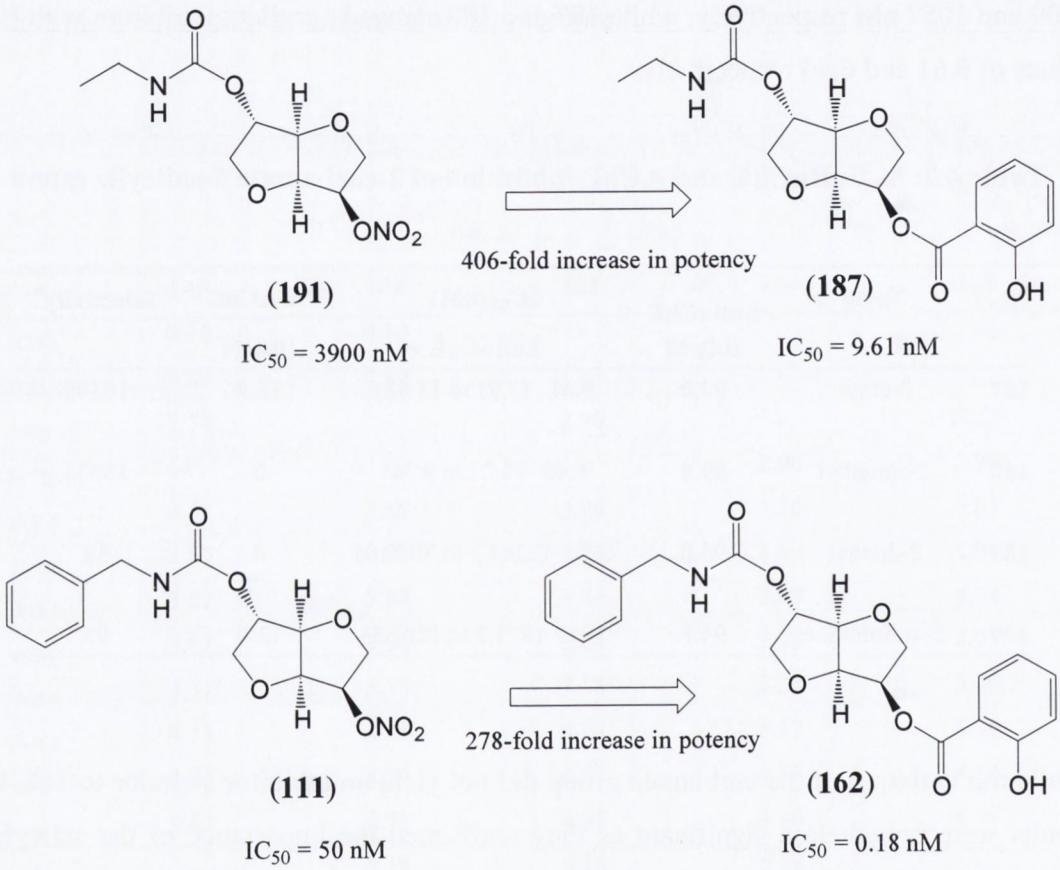
Table 4.7: % huBuChE and AChE inhibition of 2-carbamate 5-salicylic esters

No.	Name	% huBuChE 100μM	IC_{50} (nM) huBuChE	% AChE 100μM	Selectivity*
187	2-ethyl	97.0	9.61 (7.91 to 11.68)	15.9	10406
188	2-dimethyl	99.8	6.40 (4.21 to 9.74)	0	15625
189	2-diethyl	95.0	2609 (2204.3 to 3089.0)	0	38
190	2-morpholine	94.8	1057 (871.2 to 1282.3)	12.0	95

While the variation of the carbamate group did not yield an inhibitor superior to **162**, the results were nonetheless significant as they reaffirmed the importance of the salicylate group at position-5 in conferring potency with respect to huBuChE. The most significant result of this subset was the ethyl carbamate **187**. The initial investigative work into group 1 compounds, carried out by our broader research group, showed that an ethyl carbamate derivative of IS-5-MN **191** gave an IC_{50} value of only 3900 nM. This contrasts considerably with the IC_{50} value of 9.61 nM for **187** and demonstrates that replacement the nitrate group of **191** with a salicylate group causes a 400-fold increase in potency.

* A value of 100 μM was used to calculate the selectivity of compounds that showed less than 50 % inhibition of AChE at this concentration. Values shown are the mean of three readings. The IC_{50} results and 95 % confidence intervals, which are displayed in brackets, were calculated using GraphPad Prism software version 4.02. [S] 0.5 mM, [DTNB] 0.5mM.

This mirrors previously reported results, where the replacement of the nitrate group in position-5 of **111** with a salicylate group, as in **162**, gives an approximate 300-fold increase in potency.



(191) IC_{50} = 3900 nM

(187) IC_{50} = 9.61 nM

(111) IC_{50} = 50 nM

(162) IC_{50} = 0.18 nM

Figure 4.10

4.6 SUMMARY

In this chapter, we aimed to investigate the effects of substitution on the aryl ester at position-5 in conferring potency on isosorbide-based inhibitors. We had hoped that **162** would transpire to be a highly potent inhibitor of huBuChE due to the exceptional affinity salicylate ester prodrugs of isosorbide had shown towards huBuChE. Subsequently, **162** surpassed all expectations and not only emerged as the most potent inhibitor in this project, but also the most potent and selective inhibitor for huBuChE ever reported with an IC_{50} of 0.18 nM and a selectivity for huBuChE of approximately 60,000.

The other compounds in this series, i.e. the nitro, amino and hydroxyl substituted benzoate esters, also proved to be excellent inhibitors with IC_{50} values ranging between 18.0 and 323.4 nM.

We also investigated the modification of the carbamate group at position-2, whilst retaining the salicylate group at position-5. In this work, we also obtained compounds offering excellent inhibition of huBuChE, but more importantly these compounds emphasised the importance of the salicylate group at position-5 by showing the amplification of potency in compounds possessing the salicylate group at position-5.

In Chapter 5, it was decided to look at the importance of the stereochemistry of isosorbide-based inhibitors and assess the inhibitory activity of the stereoisomers of compound **110**.

CHAPTER 5

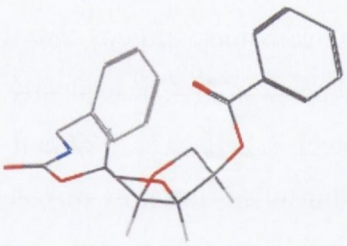
THE STEREOCHEMISTRY OF ISOSORBIDE-BASED INHIBITORS OF huBuChE

5.1 INTRODUCTION

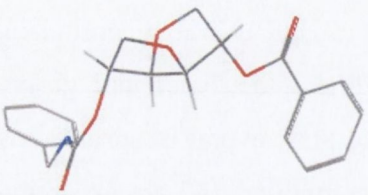
The objective of Chapters 2 to 4 was to describe the synthesis of potent and selective inhibitors of huBuChE by optimising of the type of carbamate groups substituted at position-2 and the functional groups substituted at position-5. Consequently, it was discovered that several isosorbide-based compounds such as **110**, **121**, **122** and **124** and the lead compound **162** are exceptional inhibitors of huBuChE in terms of potency and selectivity.

Having achieved these goals, we then aimed to examine the role of stereochemistry of isosorbide-based inhibitors. In order to demonstrate the significance of the stereochemistry experimentally, it was proposed to synthesise three stereoisomers of **110**. It has already been reported that the IC_{50} of **110** is 4.3 nM. It was predicted from the outset of the synthesis of these isomers, that they would be less effective in terms of the inhibition of huBuChE.

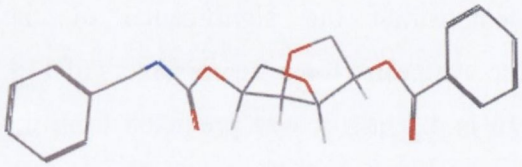
Figure 5.1 shows the 3D and 2D depictions of **110** and its three stereoisomers. The 3D drawings show the local minimum energy conformations of each compound (drawings were generated using Molecular Operating Environment (MOE-Mmff94aX.ff) and kindly supplied by Dr. J.F. Gilmer, Trinity College Dublin). From Figure 5.1, it is clear that the structure of the isoiodide derivative **193** deviates most from that of the inhibitor **110** and therefore was expected to be the least potent isomer of this class. The structures of both **192** and **194** are also noticeable quite different to **110**, however, the oxygen atom of the carbamate appears to be in approximately the same orientation as that in **110**, suggesting that these two compounds may give better huBuChE inhibition than **193**. The three isomers, when synthesised, were analysed by the Ellman method to ascertain their biological activity, the results of which are discussed later in the chapter.



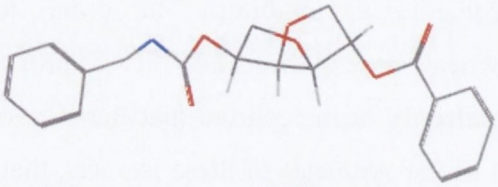
(110)



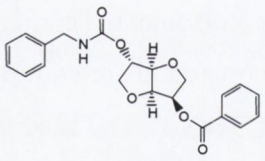
(193)



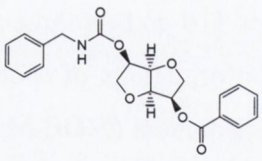
(192)



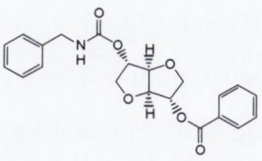
(194)



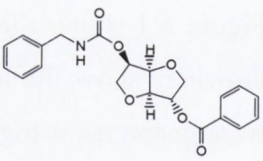
(110)



(192)



(193)

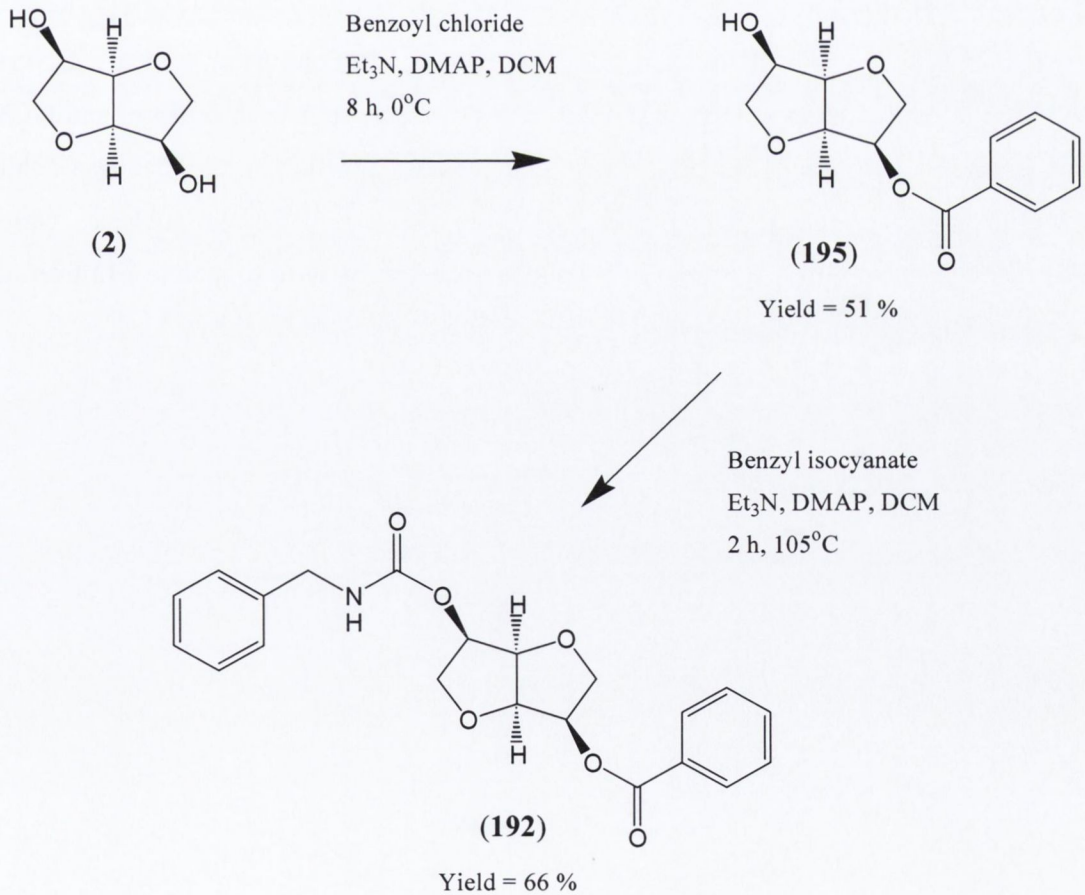


(194)

Figure 5.1: Local minimum energy conformations for the isomeric carbamate ester compounds

5.2 THE SYNTHESIS OF 2-(BENZYLAMINOCARBONYLOXY-)-5-O-BENZOYL-1,4:3,6-DIANHYDRO-D MANNITOL

The approach to the synthesis of **192** used commercially available isomannide **2** as the starting material (Scheme 5.1). The first step involved esterifying one of the equivalent hydroxyl groups of isomannide **2** using benzoyl chloride in the presence of triethylamine and DMAP at 0°C. The reaction yielded two products, which were separated by flash chromatography. NMR analysis showed the primary product to be the mono-substituted product **195**. The synthesis of **192** was achieved by the reaction of **195** with benzyl isocyanate in the presence of triethylamine and DMAP in a solution of DCM at 105°C.



Scheme 5.1

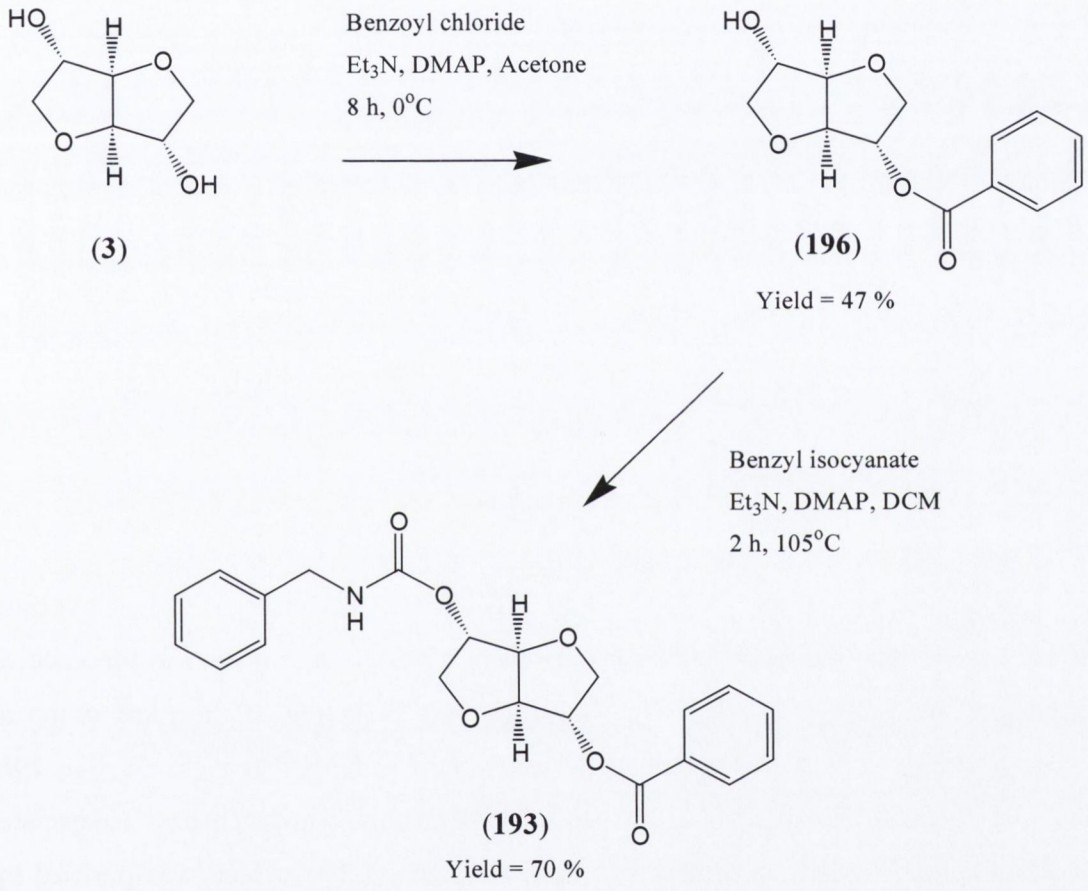
NMR analysis showed that the reaction product was impure due to additional spurious peaks in the ^{1}H spectrum. Further TLC analysis showed that the reaction product contained another component. The presence of two products was only observed when a polar mobile phase was used and the TLC plate was stained with vanillin solution. Using this staining solution, one product, which did not show up under UV light, turned a dark brown colour. The other product, which did absorb UV light, remained clear when stained with vanillin.

As the two products had similar Rf values, it was quite difficult to separate them using flash chromatography. Also, as the solid mixture was poorly soluble in non-polar solvent, any attempt to separate the mixture by column chromatography resulted in the mixture crystallising on column.

The two products were finally separated by recrystallisation using toluene. Several solvents, including, ethanol, methanol, ethyl acetate, diethyl ether, chloroform and DCM were used in attempting this procedure. The isolated purified product was a white crystalline solid, which was analysed by NMR, IR and elemental analysis, which confirmed that is was **192**. A prep TLC was also successfully used to isolate **192** from its impurities but in a much smaller yield than in the method described above.

5.3 THE SYNTHESIS OF 2-(BENZYLAMINOCARBONYLOXY-)-5-*O*-BENZOYL-1,4:3,6-DIANHYDRO-L-IDITOL

Compound **193** was synthesised from isoiodide **3** (Scheme 5.2) using an approach similar to that used in the synthesis of **192**



Scheme 5.2

5.4 THE SYNTHESIS OF 2-O-BENZOYL-5-(BENZYLAMINOCARBONYLOXY-1,4:3,6-DIANHYDRO-D GLUCITOL

After synthesising the endo-endo orientated isomer **192** from isomannide **2** and the exo-exo isomer **193** from isoiodide **3**, we then sought to examine the effect of switching the orientation of the two groups in **110,** to the alternative structure **194**, bearing an endo-carbamate and an exo-ester, as shown in Figure 5. 2

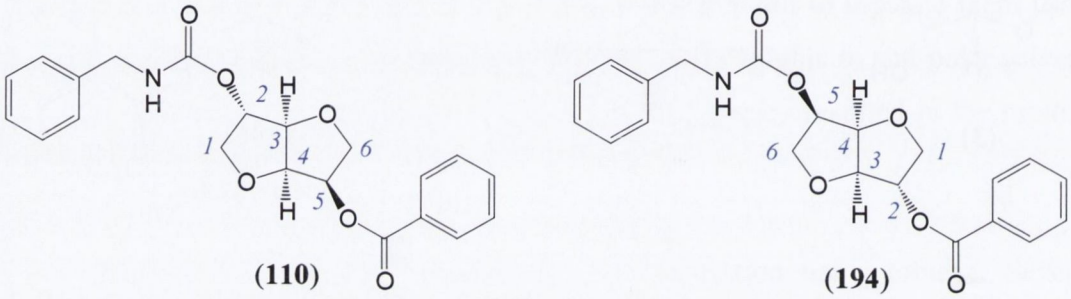
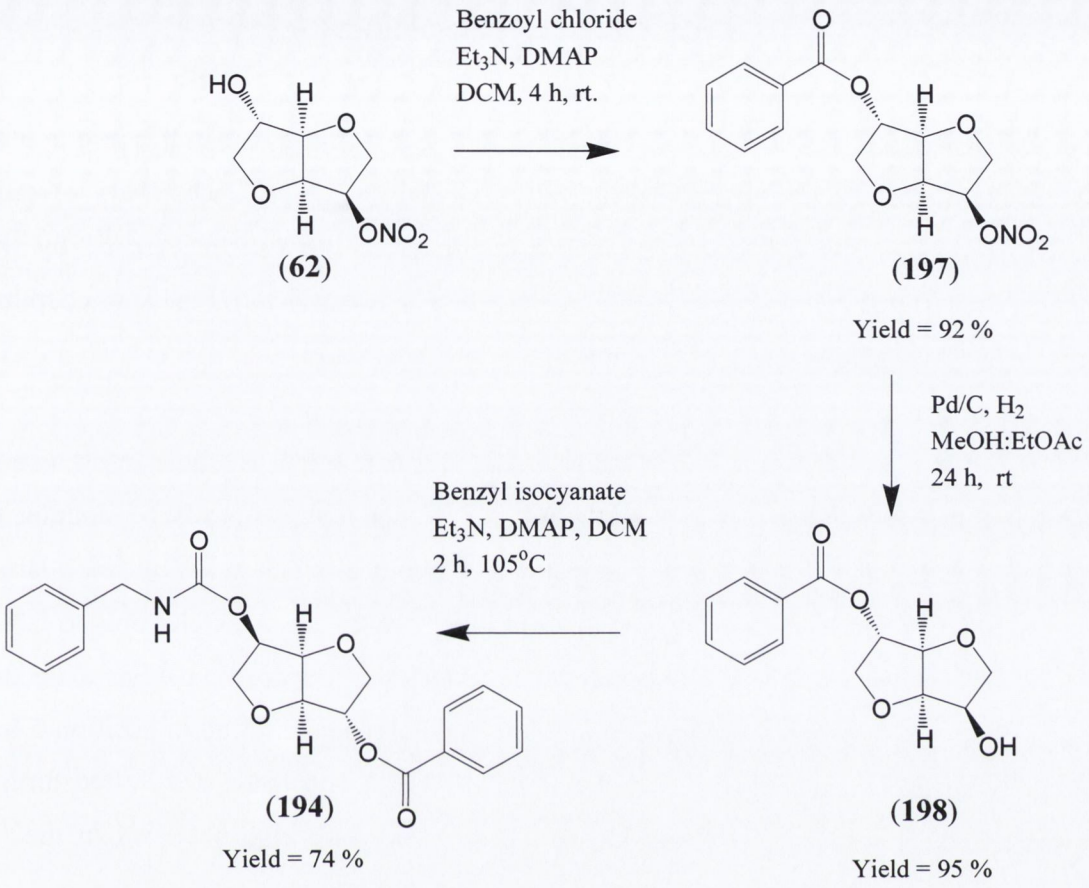


Figure 5.2

The approach to **194** used IS-5-MN **62** as starting material (Scheme 5.3). A benzoyl ester was introduced into position-2 of IS-5-MN, using benzoyl chloride in the presence of triethylamine and DMAP. This was followed by the reduction of the nitro group at position-5 using 10 % palladium on activated carbon and hydrogen gas to give **198**. Finally, the benzyl carbamate was introduced into position-5 using benzyl isocyanate, triethylamine and DMAP as in previous examples, giving **194**, which was purified by flash chromatography to give the target compound in a yield of 74 %.


Benzoyl chloride
Et3N, DMAP
DCM, 4 h, rt.
(62)
(197)
Yield = 92 %
Pd/C, H2
MeOH:EtOAc
24 h, rt
Benzyl isocyanate
Et3N, DMAP, DCM
2 h, 105°C
(194)
Yield = 74 %
(198)
Yield = 95 %

Scheme 5.3

5.5 THE NMR ANALYSIS OF THE STEREOISOMERS OF 110

The NMR spectra of the three isomers all proved to be different to each other and unique to each individual isomer analysed. This indicated the unique stereochemistry of each isomer compound, whose stereochemical structure could be further verified by the characteristic signal splitting in each ^1H spectrum and substantiated by the spin coupling patterns demonstrated by H-H COSY and C-H COSY spectra.

The ^1H and H-H COSY spectra of **194** (Figure 5.3) exhibits the usual signal splitting and coupling patterns associated with isosorbide-based compounds, where IsH-3 couples to IsH-4 and occurs as a doublet. IsH-4 couples to IsH-3 ands IsH-5 and is a triplet. IsH-5 couples to IsH-4 and IsH-6 and is given as a multiplet, whilst IsH-2 couples only to IsH-1 and is a broad singlet. The presence of the benzoyl group is position-2 has a greater deshielding effect on IsH-1 and IsH-2 than the benzyl carbamate group in position-5 has on IsH-5 and IsH-6 and subsequently the signals if IsH-1 and IsH-2 are shifted further downfield than those of IsH-5 and IsH-6. These effects are also observed in the ^1H spectra of the other isomers.

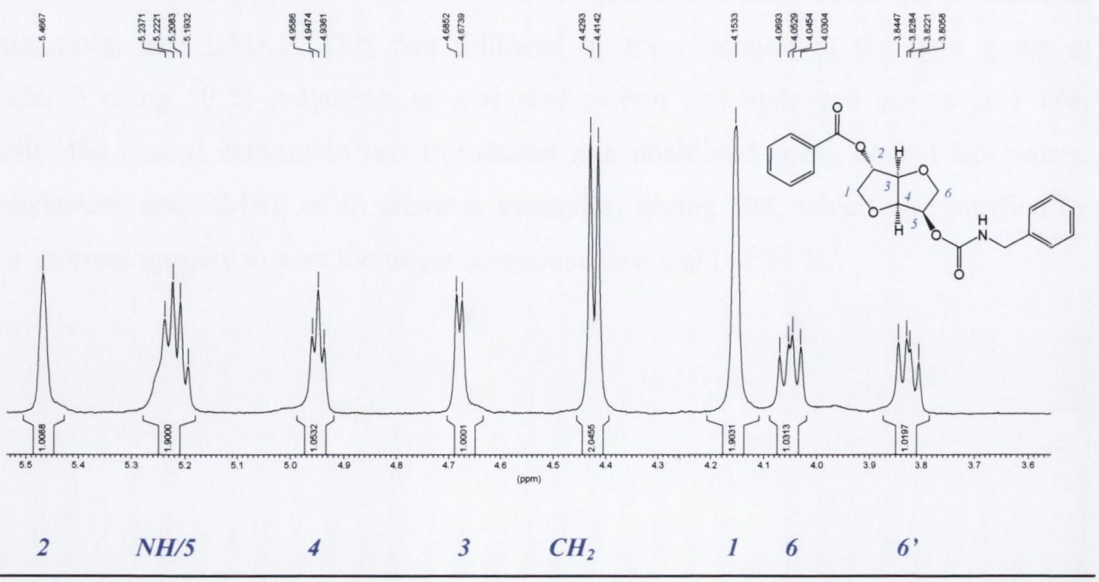


Figure 5.3

The ^{1}H spectrum of **192** (Figure 5.4) exhibits a number of differences compared **194**. Firstly, the signal for IsH-3 is a triplet as opposed to a doublet. This is due to the endo-orientation of the benzyl carbamate group in position-2 and where IsH-2 is, accordingly, in an exo-orientation. IsH-3 couples to IsH-2 and IsH-4 giving a triplet in the same way that IsH-4 couples to IsH-3 and IsH-5, all of which are in an exo-orientation. Similarly, IsH-2 mirrors the splitting pattern of IsH-5. IsH-2, which is given as a broad singlet or doublet in isosorbide based compounds, is here given as a multiplet due to its coupling with IsH-1 and IsH-3, in the same manner as IsH-5 couples to IsH-4 and IsH-6.

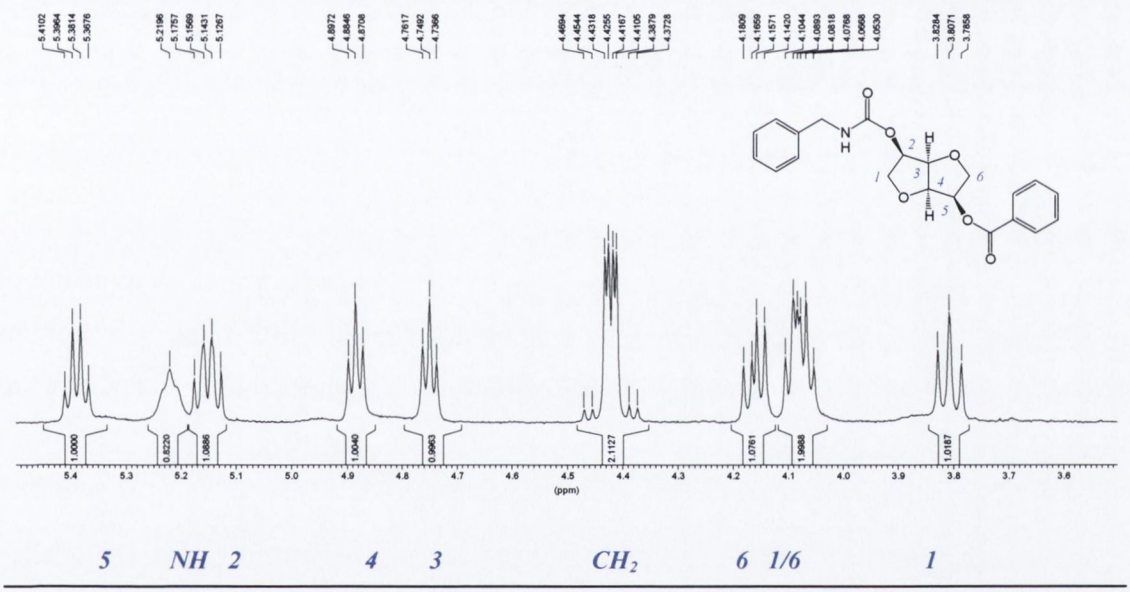


Figure 5.4

The ^{1}H spectrum of **193** (Figure 5.5) is noticeably quite different to the ^{1}H spectra of the other isomers with an obvious decrease in coupling and splitting exhibited by each ^{1}H signal. This reflects the exo-orientation of the functional groups substituted at positions-2 and 5 and the corresponding endo-orientation of the H atoms in these positions, IsH-2 couples only to IsH-1 and IsH-5 couples only to IsH-6 giving broad signals in each case. Unlike the other isomers, IsH-3 and IsH-4 occur as one singlet.

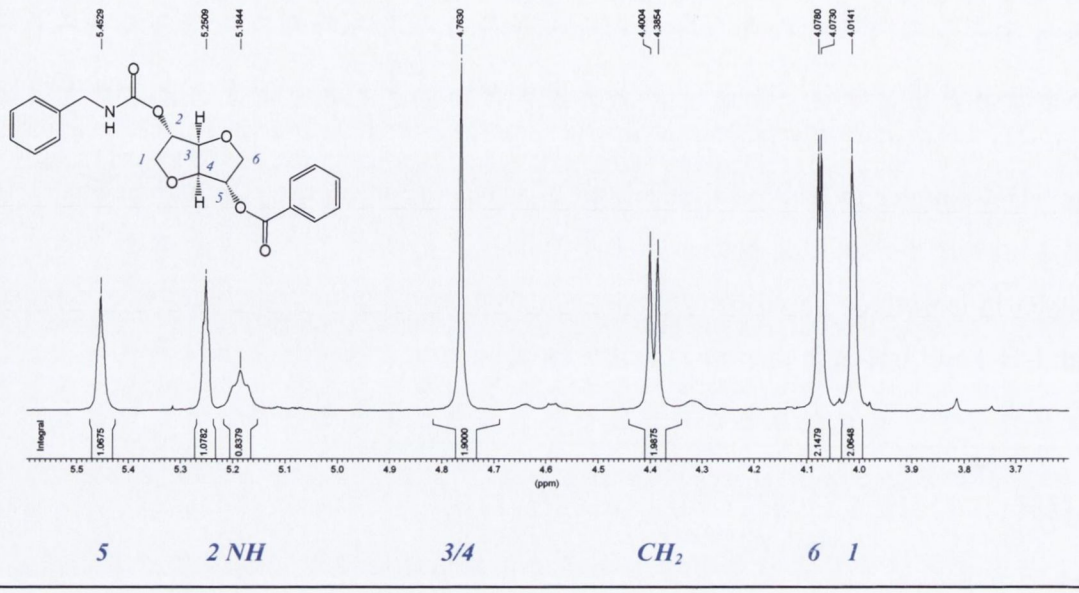
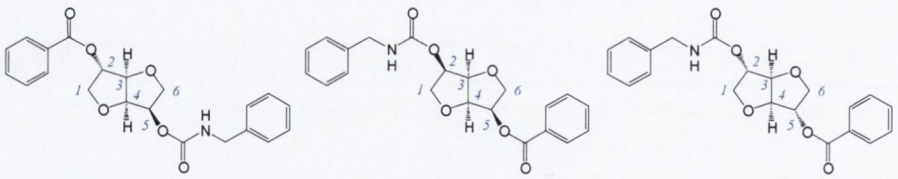


Figure 5.5

The[13]Cand C-H COSY spectra of the three isomers also showed the distinctive nature of each individual isomer by the demonstrating different chemical shifts of the carbon atoms in each compound. The positioning of each carbon in each compound is summarised in Table 5.1.



	194	192	193
IsC-6	69.73	70.72	72.41
IsC-1	73.22	69.67	72.67
IsC-5	73.99	74.01	77.95
IsC-2	78.12	73.53	77.99
IsC-4	80.78	80.35	85.45
IsC-3	85.44		85.28

Table 5.1

5.6 BIOLOGICAL TESTING OF CHAPTER 5 COMPOUNDS AND CONCLUSION

As expected the three isomers synthesised of **110** proved to be poor inhibitors huBuChE, even at a concentration of 100 μM and all gave less than 50 % at 80 μM. They gave good inhibition of AChE at 100 μM, however gave less than 50 % inhibition at 60 μM.

Table 5.2: % huBuChE and AChE inhibition stereoisomers of 110

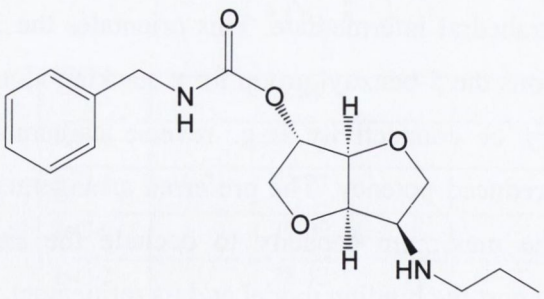
No.	Name	% huBuChE 100μM	% AChE 100μM
192	2-benzoyl carbamate 5-benzoyl isomannide	66.8	92.3
193	2-benzoyl carbamate 5-benzoyl isoiodide	41.7	89.7
194	2-benzoyl 5-benzyl carbamate isosorbide	50.6	81.7

From these results we can surmise that their unique stereochemistry allows isosorbide-based inhibitors to enter the active site gorge of huBuChE and to orientate the functional groups substituted at position-2 and 5 towards the catalytic triad and other amino acid residues within the active site. This would also seem to be suggested by the results of molecular modelling. Modelling has consistently indicated that the isosorbide group sits snugly in the bottom of the gorge in the tetrahedral intermediate. This orientates the 2-carbamate group into the acyl site and positions the 5-benzoyl group for π stacking along Trp 82. Minor changes to stereochemistry or connectivity (e.g. reverse carbamate compounds) disrupt the binding leading to reduced potency. The preferred arrangement (e.g. compounds **110** or **162**) also has the maximum capacity to occlude the site, excluding water. The results here serve to support the binding model and its refinement.

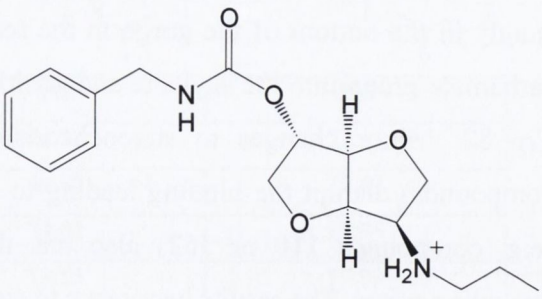
5.7 FUTURE WORK

As previously mentioned in section 4.4.3, the future progression of this project in a broader context lies in the investigation of the stability and the pharmacological and toxicological properties of the huBuChE inhibitors described in this thesis *in vivo*.

The extent of the work covered in this thesis describes several structural manipulations of isosorbide and the generation of a considerable range and variety of huBuChE inhibitors. Nevertheless this work did not exhaust the scope for the synthesis of new isosorbide-based inhibitors of huBuChE. In a synthetic context, it would be worth considering the chemical synthesis of the amino compound **199**. The presence of an amino group is characteristic of most established huBuChE inhibitors[6]. It has been reported that established huBuChE inhibitors such as rivastigmine and physostigmine, whose pKa values are 8.85[235] and 8.10[236] respectively, can become protonated at physiological pH and acquire a structure which mimics that of choline. Molecular modelling studies suggest that the protonated form of huBuChE inhibitors may interact with the aspartic acid 70 amino residue at the peripheral site of the enzyme and also may form a cation π interaction with the tryptophan 82 amino acid residue within the active site gorge. This type of compound may also offer superior water solubility than previously described inhibitors. These factors offer the precedent and rationale for the synthesis of **199**.



(199)



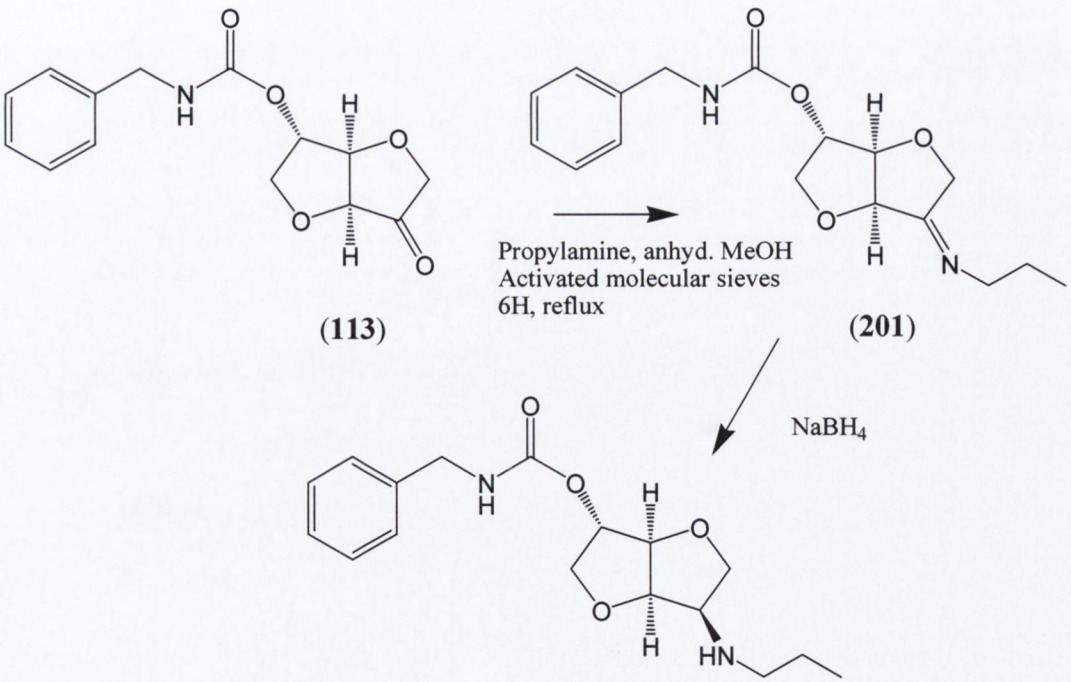
(200)

Figure 5.6

The synthesis of **199** could be achieved by reacting the ketone **113** with a primary amine, such as propylamine, as shown in Scheme 5.4, under anhydrous conditions to form the imine **201**.

Activated molecular sieves would have to be included in the reaction mixture to eliminate the water produced, which could interfere with the progression of the reaction. The reduction of the imine to the desired amino compound could be achieved using sodium borohydride.

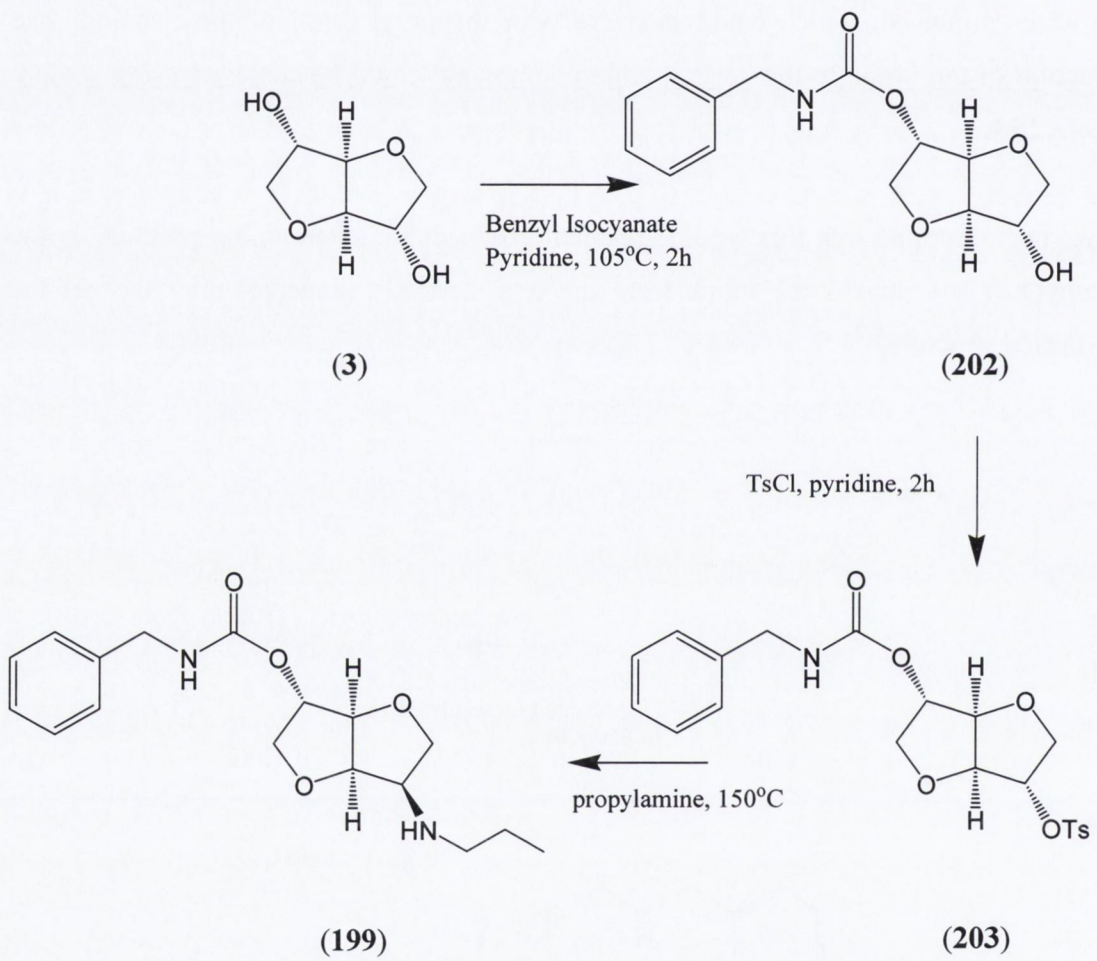
It has been reported that this type of reaction produces the endo-amino product, as the borohydride ion attacks the imine from the less sterically hindered outer face of the isosorbide structure[137].



(113) **(201)** **(199)**

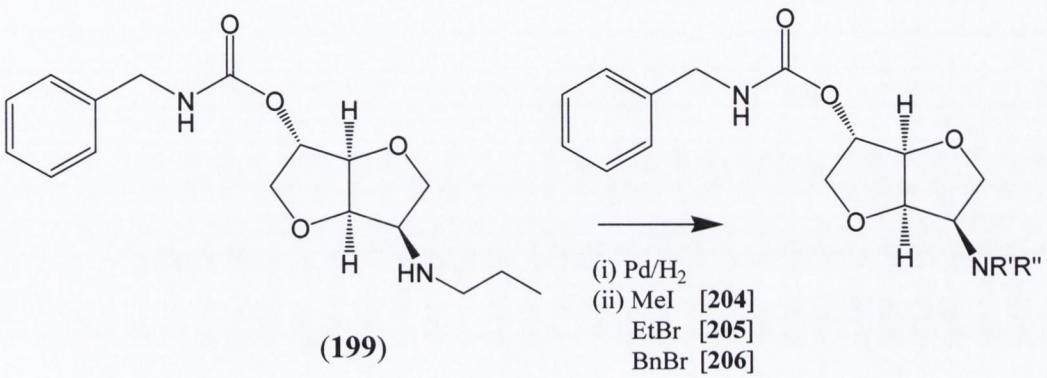
Scheme 5.4

Alternatively, **199** could be synthesised from isoiodide **3** by carbamylating one of the equivalent hydroxyl groups using benzyl isocyanate and introducing a tosyl group into the alternative position using p-toluene sulfonyl chloride. The amino function could then be introduced by direct reaction with a primary amine[126] (Scheme 5.5).



Scheme 5.5

Finally, the amine could be reduced using 10 % palladium on activated carbon to the primary amine at position-5, which could then be used to prepare several different types of amino analogues by reaction with a range of reagents. Scheme 5.6 shows a number of examples of this type.



(199)

R' = Me, R" = Me **(204)**
R' = Et, R" = Et **(205)**
R' = H, R" = Bz **(206)**

Scheme 5.6

CHAPTER 6

EXPERIMENTAL METHODS

6.1 MATERIALS

IS-5-MN was obtained from Sifa Ltd., Shannon Industrial Estate, Shannon, Co. Clare, Ireland. All other chemicals and reagents were obtained from Sigma-Aldrich Ltd. Multi-well pipette fillers, pipette tips and 96-well plates were obtained from Brand. Human plasma was sampled from healthy volunteers at the student Health Centre, Trinity College Dublin.

6.2 APPARATUS

Uncorrected melting points were obtained using a Gallenkamp melting point apparatus. Infra-red (IR) spectra were obtained using a Perkin Elmer 205 FT Infrared Paragon 1000 spectrometer. Band positions are given in cm^{-1}. Solid samples were obtained by KBr discs and oils were analysed as neat films on NaCl plates. ^{1}H and ^{13}C spectra were recorded at 27°C on a Brucker DPX 400 MHz FT NMR spectrometer (400.13 MHz ^{1}H, 100.61 MHz ^{13}C) or a Brucker AV600 (600.13 MHz ^{1}H, 150.6 MHz ^{13}C) in either $CDCl_3$ or $(CD_3)_2CO$ with tetramethylsilane (TMS) as internal standard. In $CDCl_3$, ^{1}H spectra were assigned relative to the TMS peak at 0.0 ppm and ^{13}C spectra were assigned relative to the middle $CDCl_3$ triplet at 77.00 ppm. In $(CD_3)_2CO$, ^{1}H spectra were assigned relative to the $(CD_3)_2CO$ peak at 2.05 ppm and ^{13}C spectra were assigned relative to the $(CD_3)_2CO$ at 29.5 ppm. Coupling constants were reported in Hertz (Hz). HRMS was performed using a Micromass Mass Spectrophotometer with electrospray ionisation at the Department of Chemistry, Trinity College Dublin. Elemental analyses were performed at the Microanalytical Laboratory, Department of Chemistry, University College Dublin. Flash chromatography was performed on Merck Kieselgel (particle size: 60 mm). Thin layer chromatography (TLC) was performed on silica gel Merck F-254 plates. Compounds were visually detected by UV absorbance at 254 nm or with potassium permanganate or vanillin staining solution. Enzyme activity and inhibition assays were performed using an Anthos bt2 plate reader with UV detection at 412 nm.

6.3 SYNTHESIS

2-O-p-*Toluenesulfonyl-1,4:3,6-dianhydro-D-mannitol* **91**
(2-Tosylate isomannide)

Isomannide **2** (146.14 g/mol, 68.43 mmol, 10 g) was dissolved in pyridine (100 ml) and cooled to 0°C in an ice bath. *p*-Toluenesulfonyl chloride (mol. wt. 190.5 g/mol, 68.43 mmol, 13.04 g) was added. The reaction mixture was stirred for 24 hours and allowed to heat up to room temperature. The reaction was monitored by TLC (3:1 ethyl acetate: hexane) to observe the disappearance of the *p*-toluenesulfonyl chloride. The pyridine was evaporated under high vacuum yielding a brown oil which was diluted with 100 ml of DCM and washed with 1M HCl (100 ml), 5 % $NaHCO_3$ (100 ml), saturated brine solution (100 ml) and dried with anhydrous sodium sulphate (10 g). Purification by column chromatography, using hexane and ethyl acetate (3:1) as eluent, gave a white solid, which was recrystallised from hot ethyl acetate and hexane to give the title compound as a white crystalline product (12.02 g, 58.5 %). Mol. wt. 300.33 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 2.46 (s, 3H, CH_3), 2.57 (d, 1H, J = 8.53 Hz, OH), 3.55 (dd, 1H, J = 7.53 and 9.54 Hz, IsH-6), 3.79 (dd, 1H, J = 7.53 and 9.54 Hz, IsH-1), 3.97 (dd, 1H, J = 6.53 and 9.54 Hz, IsH-6'), (dd, 1H, J = 7.13 and 9.54 Hz, IsH-1'), 4.30 (m, 1H, IsH-5), 4.44 (t, 1H, J = 5.02 Hz, IsH-4), 4.50 (t, 1H, J = 4.77 Hz, IsH-3), 4.92 (m, 1H, IsH-2), 7.37 (d, 2H, J = 8.53, ArH-3/ArH-5), 7.84 (d, 2H, J = 8.54, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 21.26 (CH_3), 69.60 (IsC-1), 71.88 (IsC-2), 73.53 (IsC-6), 77.97 (IsC-5), 79.59 (IsC-3), 80.95 (IsC-4), 127.56 (ArC-2/ArC-6), 129.48 (ArC-3/ArC-5), 132.56 (ArC-1), 144.86 (ArC-4).

2-Deoxy-2-azido-1,4:3,6-dianhydro-D-glucitol **89**
(2-Azido isosorbide)

2-O-*p*-Toluenesulfonyl-1,4:3,6-dianhydro-D-mannitol **91** (mol. wt. 300.33 g/mol, 33.30 mmol, 10 g) was dissolved a 20 ml. of DMSO. Excess sodium azide (mol. wt. 65.01 g/mol, 133.20 mmol, 8.66 g) was added forming a slurry, which was heated to 125°C and

stirred for 24 hours. The reaction was monitored by TLC (3:1 ethyl acetate: hexane) to check for the disappearance of the starting material. The mixture was diluted with ethyl acetate (200 ml) was washed with three equal volumes of water and dried over anhydrous sodium sulphate (10 g). The solution was filtered into a round bottom flask and was evaporated under vacuum giving a brown oil. Purification by column chromatography, using hexane and ethyl acetate (3:1) as eluent, yielded an off-white solid, which was recrystallised from hot ethyl acetate and hexane to afford 3.62 g of the title compounds as a white crystalline product (63.5 %). Mol. wt. 171.15 g/mol. **^{1}H-NMR δ** ($CDCl_3$): 3.63 (dd, 1H, J = 5.46 and 10.02 Hz, IsH-6), 3.89 (dd, 1H, J = 6.15 and 10.24 Hz, IsH-1), 3.97 (dd, 1H, J = 3.41 and 9.56 Hz, IsH-6'), 4.09 (m, 2H, IsH-1', IsH-2), 4.34 (q, 1H, J = 5.46 and 10.93 Hz, IsH-5), 4.50 (d, 1H, J = 4.08 Hz, IsH-3), 4.65 (t, 1H, J = 4.78 Hz, IsH-4). **^{13}C-NMR ppm** ($CDCl_3$): 65.74 (IsC-2), 71.65 (IsC-5), 72.21 (IsC-1), 73.39 (IsC-6), 81.52 (IsC-4), 85.74 (IsC-3).

2-Deoxy-2-(t-butyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **93**
(2-BOC isosorbide)

2-Deoxy-2-azido-1,4:3,6-dianhydro-D-glucitol **89** (mol. wt. 171.15 g/mol, 5.84 mmol, 1 g) was dissolved in ethyl acetate (5 ml) in a 50 ml round bottom flask. A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas. Di-*tert*-butyl dicarbonate (mol. wt. 218.25 g/mol, d 0.8659 g/ml, 6.42 mmol, 1.40 g, 1.62 ml) and stirred for 24 hours. The progress of the reaction was monitored by TLC (3:1 ethyl acetate: hexane) to observe the disappearance of the starting material. The reaction mixture was filtered through cotton wool to remove the catalyst. The solvent was evaporated under vacuum and the crude product was dissolved in DCM and purified by column chromatography, using hexane and ethyl acetate (1:1) as eluent, which gave a brown crystalline solid, which was recrystallised from hot ethyl acetate and hexane to afford the target compound as a white crystalline product (1 g, 70 %). M.pt 83°C, mol. wt. 245.27 g/mol. IR_{vmax} (KBr): 1087.0 (C-O-C) 1171.1, 1253.1 (C(O)OR), 1694.1 (C=O), 1531.6 and 2876.8 (aliphatic CH), 3324.0 (OH) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 1.45

(s, 9H, $(CH_3)_3$), 2.73 (d, 1H, J = 6.14 Hz, OH), 3.62 (dd, 1H, J = 5.46 and 9.56 Hz, IsH-6), 3.83-3.92 (m, 2H, IsH-6', IsH-1), 3.96 (dd, 1H, J = 6.14 Hz, IsH-1'), 4.23 (s, 1H, IsH-2), 4.30 (m, 1H, IsH-5), 4.41 (d, 1H, J = 4.1 Hz, IsH-3), 4.55 (t, 1H, J = 4.78 Hz, IsH-4), 4.87 (s, 1H, NH). **^{13}C-NMR ppm** ($CDCl_3$): 28.27 ($(CH_3)_3$), 57.60 (IsC-2), 72.11 (IsC-5), 73.59 (IsC-1), 73.81 (IsC-6), 79.60 ($(CH_3)_3C$), 81.45 (IsC-4), 86.92 (IsC-3), 154.50 (CO). $C_{11}H_{19}NO_5$ requires C, 53.87; H, 7.81; N, 5.71: found C, 53.54; H, 7.72; N, 5.95.

The procedure for the preparation of esters follows the method outlined in the example below:

*2-Deoxy-2-(*t*-butyloxycarbonylamino-) 5-O-benzoyl-1,4:3,6-dianhydro-D-glucitol* **97**
(2-BOC 5-benzoyl isosorbide)

2-Deoxy-2-(*t*-butyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **93** (mol. wt. 245.27 g/mol., 0.8154 mmol, 200 mg) was dissolved in DCM (10 ml). Triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 0.90 mmol, 91 mg, 0.13 ml), benzoyl chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 0.90 mmol, 126 mg, 0.11 ml) and DMAP (mol. wt. 122.17 g/mol, 0.18 mmol, 22 mg) were added to the reaction vessel and the mixture was stirred for six hours. The progress of the reaction was monitored by TLC (1:1, ethyl acetate: hexane). *Where necessary, the reaction was allowed to react for longer or heated under reflux to ensure the reaction went to completion.* DCM (10 ml) was added to the reaction vessel and the mixture was washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml) saturated brine solution (20 ml) and dried with anhydrous sodium sulfate (1 g). Purification by column chromatography, using hexane and ethyl acetate as eluent, yielded a white solid, which was recrystallised from hot ethyl acetate and hexane to afford the target compound as a white crystalline product 206 mg (72 %) M.pt 156°C, mol. wt. 349.38 g/mol. IR_{vmax} (KBr): 1094.1 (C-O-C) 1173.4, 1274.6 (C(O)OR), 1720.3 (C=O), 1518.7 and 2977.7 (aliphatic CH), 3338 (2°amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 1.46 (s, 9H, $(CH_3)_3$), 3.78 (d, 1H, J = 9.55 Hz, IsH-1), 3.96 (m, 2H, IsH-1', IsH-6), 4.07 (dd, 1H, J = 3.41 and 10.24 Hz, IsH-6), 4.25 (s, 1H, IsH-2), 4.45 (d, 1H, J = 4.77 Hz, IsH-3), 4.75 (d, 1H, J = 4.78 Hz, NH), 4.91 (t, 1H, J = 5.47 Hz, IsH-4), 5.41 (m, 1H, IsH-5), 7.47 (t, 2H, J = 7.86 Hz,

ArH-3/ArH-5), 7.60 (t, 1H, J = 7.51 Hz, ArH-4), 8.07 (d, 2H, J = 6.83 Hz, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 27.88 ($(CH_3)_3$), 56.71 (IsC-2), 70.72 (IsC-6), 72.81 (IsC-1), 74.05 (IsC-5), 79.59 ($(CH_3)_3\underline{C}$), 80.21 (IsC-4), 87.03 (IsC-3), 128.01 (ArC-3/ArC-5), 129.06 (ArC-2/ArC-6), 129.29 (ArC-1), 132.82 (ArC-4), 154.53 (-$\underline{C}$(O)C$(CH_3)_3$), 165.49 (-$\underline{C}$(O)Ar). $C_{18}H_{23}NO_6$ requires C, 61.88; H, 6.64; N, 4.01: found C, 62.03; H, 6.51; N, 3.78.

*2-Deoxy-2-(*t*-butyloxycarbonylamino-) 5-O-(*p*-nitro-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **98**
(2-BOC 5-*p*-nitro-benzoyl isosorbide)

Compound **98** was synthesised from 2-deoxy-2-(*t*-butyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **93** (mol. wt. 245.27 g/mol, 0.82 mmol, 200 mg) with *p*-nitrobenzoyl chloride (mol. wt. 185.57 g/mol, 0.90 mmol, 167 mg) as reagent, using the method outlined for the preparation of esters, as with compound **97**. Recrystallisation from hot ethyl acetate and hexane afforded 188 mg (59 %) of the title compound as a white crystalline product. M.pt 83°C, mol. wt. 394.38 g/mol. IR_{vmax} (KBr): 1092.7 (C-O-C) 1169.5, 1275.5 (C(O)OR), 1608.2 (C-NO_2) 1695.5, 1704.5 (C=O), 1529.6 and 2978.4 (aliphatic CH), 3329.1 (2°amine) cm^{-1} **^{1}H-NMR δ** ($CDCl_3$): 1.44 (s, 9H, $(CH_3)_3$), 3.77 (d, 1H, J = 9.56 Hz, IsH-1), 3.90 (dd, 1H, J = 4.09 and 9.56 Hz, IsH-1'), 3.97 (dd, 1H, J = 4.78 and 10.24 Hz, IsH-6), 4.10 (m, 1H, IsH-6'), 4.23 (s, 1H, IsH-2), 4.45 (d, 1H, J = 3.41 Hz, IsH-3), 4.88 (d, 2H, NH, IsH-4), 5.41 (m, 1H, IsH-5), 8.20 (d, 2H, J = 8.87 Hz, ArH-2/ArH-6), 8.30 (d, 2H, J = 8.87 Hz, ArH-3/ArH-5). **^{13}C-NMR ppm** ($CDCl_3$): 27.85 ($(CH_3)_3$), 56.63 (IsC-2), 70.52 (IsC-6), 72.84 (IsC-1), 74.98 (IsC-5), 79.60 ($(CH_3)_3\underline{C}$), 80.10 (IsC-4), 87.07 (IsC-3), 123.18 (ArC-3/ArC-5), 130.38 (ArC-2/ArC-6), 134.42 (ArC-1), 150.24 (ArC-4), 154.55 (-$\underline{C}$(O)C$(CH_3)_3$), 163.61 (-$\underline{C}$(O)Ar). $C_{18}H_{22}N_2O_8$ requires C, 54.82; H, 5.62; N, 7.10: found C, 54.57; H, 5.68; N, 6.91.

2-Deoxy-2-(t-butyloxycarbonylamino-) 5-O-(p-bromobenzoyl)-1,4:3,6-dianhydro-D-glucitol **99**
(2-BOC 5-*p*-bromo-benzoyl isosorbide)

Compound **99** was synthesised from 2-deoxy-2-(t-butyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **93** (mol. wt. 245.27 g/mol, 0.82 mmol, 200 mg) with *p*-bromobenzoyl chloride (mol. wt. 219.47 g/mol, 0.90 mmol, 178.9 mg) as reagent, using the method outlined for the preparation of esters. Re-crystallization from hot ethyl acetate and hexane afforded 282 mg (81 %) of the title compound as a white crystalline product. M.pt 156°C, mol. wt. 349.38 g/mol. IR_{vmax} (KBr): 1093.4 (C-O-C) 1172.8, 1271.7 (C(O)OR), 1722.8 (C=O), 1519.9 and 2977.6 (aliphatic CH), 3337.3 (2°amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 1.46 (s, 9H, $(CH_3)_3$), 3.77 (d, 1H, J = 9.56 Hz, IsH-1), 3.95 (m, 2H, IsH-1', IsH-6), 4.06 (dd, 1H, J = 3.41 and 10.24, IsH-6'), 4.24 (s, 1H, IsH-2), 4.45 (d, 1H, J = 4.09 Hz, IsH-3), 4.73 (s, 1H, NH), 4.89 (t, 1H, J = 5.12 Hz, IsH-4), 5.38 (m, 1H, IsH-5), 7.62 (d, 2H, J = 8.20 Hz, ArH-3/ArH-5), 7.94 (d, 2H, J = 8.20 Hz, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 27.87 ($(CH_3)_3$), 56.66 (IsC-2), 70.64 (IsC-6), 72.80 (IsC-1), 74.33 (IsC-5), 79.63 ($(CH_3)_3\underline{C}$), 80.16 (IsC-4), 87.05 (IsC-3), 127.95 (ArC-4), 128.02 (ArC-1), 130.79 (ArC-3/ArC-5), 131.38 (ArC-2/ArC-6), 154.75 (-$\underline{C}$(O)$C(CH_3)_3$), 164.77 (-$\underline{C}$(O)Ar). $C_{18}H_{22}BrNO_6$ requires C, 50.48; H, 5.18; N, 3.27: found C, 50.55, H, 4.88; N, 3.40.

2-Deoxy-2-(ethyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **94**
(2-Ethyl reverse carbamate isosorbide)

2-Deoxy-2-azido-1,4:3,6-dianhydro-D-glucitol **89** (mol. wt. 171.15 g/mol, 5.84 mmol, 1 g) was dissolved in ethyl acetate (5 ml) in a 50 ml round bottom flask. A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas. The progress of the reaction was monitored by TLC (3:1, ethyl acetate: hexane) to observe the disappearance of the starting material. The reaction mixture was filtered through cotton wool to remove the catalyst. The solvent was evaporated under vacuum giving a clear oil, which was diluted with DCM (5 ml). The solution was stirred and cooled to 0°C on an

ice bath. Ethyl chloroformate (mol. wt. 108.52 g/mol, d 1.135 g/ml, 6.43 mmol, 698 mg, 0.6145 ml) and triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 6.43 mmol, 649 mg, 0.89 ml) were added to the solution ,which was stirred for two hours. A hard white precipitate was formed which was dissolved in DMSO and washed with ethyl acetate: 1M HCl (1:1, 50 ml). The organic layer was collected and washed with a further two times with 1M HCl (2 x 25 ml), dried over sodium sulfate (2 g) and evaporated to dryness. The crude product was purified by column chromatography, using hexane and ethyl acetate (2:1) as eluent, to give 444 mg (35 %) of the target compound as a clear oil. Mol. wt. 217.22 g/mol. IR_{vmax} (NaCl): 1089.1 (C-O-C), 1266.5 (C(O)OR), 1703.9 (C=O), 1538.4 and 2998.6 (aliphatic CH), 3340.8 (OH) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 1.26 (m, 3H, CH_3), 2.81 (d, 1H, J = 5.46 Hz, OH), 3.62 (dd, 1H, J = 6.14 and 9.55 Hz, IsH-6), 3.83-3.94 (m, 2H, IsH-6', IsH-1), 3.97 (dd, 1H, J = 4.09 and 8.87 Hz, IsH-1'), 4.13 (m, 2H, CH_2), 4.22-4.35 (m, 2H, IsH-2, IsH-5), 4.43 (d, 1H, J = 3.41 Hz, IsH-3), 4.56 (t, 1H, J = 4.78 Hz, IsH-4), 5.16 (s, 1H, NH). **^{13}C-NMR ppm** ($CDCl_3$): 14.10 (CH_3), 57.51 (IsC-2), 60.73 (CH_2), 71.71 (IsC-5), 73.67 (IsC-1), 73.36 (IsC-6), 81.08 (IsC-4), 86.39 (IsC-3), 155.41 (CO).); $C_9H_{15}NO_5Na$ requires 240.0849; found 240.0050.

2-Deoxy-2-(ethyloxycarbonylamino-) 5-O-benzoyl-1,4:3,6-dianhydro-D-glucitol **100**
(2-Ethyl reverse carbamate 5-benzoyl isosorbide)

Compound **100** was synthesised from 2-deoxy-2-(ethyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **94** (mol. wt. 217.22 g/mol, 0.92 mmol, 200 mg) with benzoyl chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 1.01 mmol, 129 mg, 0.11 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane to gave 160 mg (54 %) of the target compound as a white crystalline product. M.pt 101°C, mol. wt. 321.33 g/mol. IR_{vmax} (KBr): 1093.4 (C-O-C), 1122.1, 1274.6 (C(O)OR), 1722.1 (C=O), 1537.6 and 2981.0 (aliphatic CH), 3327.8 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 1.27 (m, 3H, CH_3), 3.80 (d, 1H, J = 8.81 Hz, IsH-1), 3.97 (m, 2H, IsH-1, IsH-6), 4.03-4.23 (m, 3H, IsH-6', CH_2), 4.29 (s, 1H, IsH-2), 4.46 (d, 1H, J = 3.91 Hz, IsH-3), 4.91 (t, 1H, J = 5.38 Hz, IsH-4), 5.02 (d, 1H, J = 6.85 Hz, NH), 5.41 (q, 1H, J = 5.87 and 9.78 Hz, IsH-5), 7.47 (t, 2H, J = 7.34 Hz, ArH-3/ArH-5), 7.59 (t, 1H, J =

7.34 Hz, ArH-4), 8.09 (d, 2H, J = 7.83 Hz, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 14.12 (CH_3), 56.98 (IsC-2), 60.69 (CH_2), 70.75 (IsC-6), 72.72 (IsC-1), 74.03 (IsC-5), 80.21 (IsC-4), 86.92 (IsC-3), 128.01 (ArC-3/ArC-5), 129.01 (ArC-1), 129.29 (ArC-2/ArC-6), 132.82 (ArC-4), 155.40 (-C(O)OCH_2CH_3), 165.48 (-C(O)Ar). $C_{16}H_{19}NO_6$ requires C, 59.81; H, 5.96; N, 4.36: found C, 59.99; H, 6.03; N, 4.01.

*2-Deoxy-2-(ethyloxycarbonylamino-) 5-O-(*p*-nitro-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **101**
(2-Ethyl reverse carbamate 5-(*p*-nitro-benzoyl) isosorbide)

Compound **101** was synthesised from 2-deoxy-2-(ethyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **94** (mol. wt. 217.22 g/mol, 0.92 mmol, 200 mg) with *p*-nitrobenzoyl chloride (mol. wt. 185.57 g/mol, 1.01 mmol, 188 mg) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane to gave the title compound as a while crystalline product (227 mg, 67 %). M.pt 119°C, mol. wt. 366.32 g/mol. IR_{vmax} (KBr): 1092.5 (C-O-C), 1124.2, 1275.7 (C(O)OR), 1608.3 (C-NO_2), 1727.7 (C=O), 1529.9 and 2982.5 (aliphatic CH), 3331.9 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 1.27 (m, 3H, CH_3), 3.80 (d, 1H, J = 9.56 Hz, IsH-1), 3.92 (dd, 1H, J = 4.1 and 9.56 Hz, IsH-1'), 4.00 (dd, 1H, J = 4.78 and 10.24 Hz, IsH-6), 4.07-4.20 (m, 3H, IsH-6', CH_2), 4.30 (s, 1H, IsH-2), 4.48 (d, 1H, J = 4.09 Hz, IsH-3), 4.87-4.98 (m, 2H, NH, IsH-4), 5.44 (m, 1H, IsH-5), 8.25 (d, 2H, J = 8.87 Hz, ArH-2/ArH-6), 8.32 (d, 2H, J = 8.87 Hz, ArH-3/ArH-5). **^{13}C-NMR ppm** ($CDCl_3$): 14.10 (CH_3), 56.85 (IsC-2), 59.96 (CH_2), 70.59 (IsC-6), 72.73 (IsC-1), 74.93 (IsC-5), 80.08 (IsC-4), 86.96 (IsC-3), 123.20 (ArC-3/ArC-5), 130.39 (ArC-2/ArC-6), 134.37 (ArC-1), 150.27 (ArC-4), 155.27 (-C(O)OCH_2CH_3), 163.61 (-C(O)Ar). $C_{16}H_{18}N_2O_8$ requires C, 52.46; H, 4.95; N, 7.65: found C, 52.13; H, 4.66; N, 7.28.

2-Deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **95**
(2-Phenyl reverse carbamate isosorbide)

To a solution of 2-deoxy-2-azido-1,4:3,6-dianhydro-D-glucitol **89** (mol. wt. 171.15 g/mol, 5.84 mmol, 1 g) in ethyl acetate: methanol (1:1, 5 ml), was added a spatula tip-full

of 10 % palladium on activated carbon. Air was expelled from the reaction vessel and the mixture was kept under an atmosphere of hydrogen gas. The reaction was stirred for 24 hours and the progress of the reaction was monitored by TLC (3:1, ethyl acetate: hexane) to observe the disappearance of the starting material. The reaction mixture was filtered through cotton wool in order to remove the catalyst and he organic solvent was removed under vacuum giving a clear oil. The oil was diluted with dioxane (7.5 ml) and saturated $NaHCO_3$ (15 ml) and cooled to –20°C on a Dewer flask containing ice and acetone. Phenyl chloroformate (mol. wt. 156.57 g/mol, d 1.248 g/ml, 6.43 mmol, 1 g, 0.81 ml) was added dropwise to the reaction vessel over ten minutes and the mixture was stirred rapidly for a further ten minutes. The reaction was quenched by diluting the mixture with of 1M HCl (30 ml) and washing it with three portions of ethyl acetate (3 x 50 ml). The organic portions were collected and dried over sodium sulfate (2 g) and evaporated to dryness on a rotary evaporator. Purification by flash chromatography using a solvent gradient of hexane and ethyl acetate (3:1, 1:1, 1:2, 1:3) as eluent, gave a white solid, which was recrystallised from hot ethyl acetate and hexane to give the title product 1.3 g (84 %) as a white crystalline needles. M.pt 181°C, mol. wt. 265.26 g/mol. IR_{vmax} (KBr): 1089.0 (C-O-C), 1219.8, 1265.6 (C(O)OR), 1557.4 (benzene), 1698.5 (C=O), 2988.6 (aliphatic CH), 3291.8 (OH) cm^{-1}. **^{1}H-NMR** δ ($(CD_3)_2CO$): 3.51 (dd, 1H, J = 6.83 and 8.87 Hz, IsH-6), 3.81 (dd, 1H, J = 6.15 and 8.88 Hz, IsH-6'), 3.98 (m, 2H, IsH-1, IsH-1'), 4.15 (s, 1H, IsH-2), 4.26 (m, 1H, IsH-5), 4.50-4.65 (m, 2H, IsH-3, IsH-4), 7.07-7.25 (m, 3H, ArH-2/ArH-6, ArH-4), 7.37 (t, 2H, J = 7.85 Hz, ArH-3/ArH-5). **^{13}C-NMR ppm** ($(CD_3)_2CO$): 58.40 (IsC-2), 72.04 (IsC-5), 72.24 (IsC-1), 72.55 (IsC-6), 81.27 (IsC-4), 86.29 (IsC-3), 121.16 (ArC-2/ArC-6), 124.47 (ArC-4), 128.47 (ArC-3/ArC-5), 151.00 (ArC-1), 153.54 (CO). $C_{13}H_{15}NO_5$ requires C, 58.86; H, 5.70; N, 5.28: found C, 58.70; H, 5.62; N, 5.38.

2-Deoxy-2-(phenyloxycarbonylamino-) 5-O-benzoyl-1,4:3,6-dianhydro-D-glucitol **102**
(2-Phenyl reverse carbamate 5-benzoyl isosorbide)

Compound **102** was synthesised from 2-deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **95** (mol. wt. 265.26 g/mol, 0.75 mmol, 200 mg) with benzoyl

chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 0.83 mmol, 117 mg, 0.10 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane afforded the title compound as a white crystalline product 256 mg (93 %). M.pt 140°C, mol. wt. 369.37 g/mol. IR_{vmax} (KBr): 1091.9 (C-O-C), 1205.9 and 1274.9 (C(O)OR), 1542.5 (benzene), 1720.5 (C=O), 2952.0 (aliphatic CH), 3326.8 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($(CD_3)_2CO$): 3.89 (d, 1H, J = 9.56 Hz, IsH-1), 3.95-4.10 (m, 3H, IsH-1', IsH-6, IsH-6'), 4.22 (s, 1H, IsH-2), 4.62 (d, 1H, J = 4.10 Hz, IsH-3), 5.01 (t, 1H, J = 5.12 Hz, IsH-4), 5.46 (m, 1H, IsH-5), 7.07-7.27 (m, 3H, Ar_1H-2/ Ar_1H-6, Ar_1H-4), 7.38 (t, 2H, J = 7.85 Hz, Ar_1H-3/ Ar_1H-5), 7.56 (t, 2H, J = 7.85 Hz, Ar_2H-3/Ar_2H-5), 7.68 (t, 2H, J = 7.17 Hz, Ar_2H-4), 8.09 (d, 2H, J = 6.83 Hz, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($(CD_3)_2CO$): 57.68 (IsC-2), 70.34 (IsC-6), 72.31 (IsC-1), 74.30 (IsC-5), 80.42 (IsC-4), 86.78 (IsC-3), 121.16 (Ar_1C-2/Ar_1C-6), 124.48 (Ar_1C-4), 128.14 (Ar_2C-3/Ar_2C-5),128.63 (Ar_1C-3/Ar_1C-5), 128.94 (Ar_2C-2/Ar_2C-6), 129.63 (Ar_2C-1), 132.71 (Ar_2C-4), 151.00 (Ar1C-1), 153.57 (-C(O)OAr1), 164.74 (-C(O)Ar2). $C_{20}H_{19}NO_6$ requires C, 65.03; H, 5.18; N, 3.79: found C, 65.35; H, 5.03; N, 3.52.

*2-Deoxy-2-(phenyloxycarbonylamino-) 5-O-(*p*-nitro-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **103**
(2-Phenyl reverse carbamate 5-*p*-nitro-benzoyl isosorbide)

Compound **103** was synthesised from 2-deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **95** (mol. wt. 265.26 g/mol, 0.75 mmol, 200 mg) with *p*-nitrobenzoyl chloride (mol. wt. 185.57 g/mol, 0.83 mmol, 154 mg) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane to gave 250 mg (80 %) of a white crystalline product. M.pt 171°C, mol. wt. 414.37 g/mol. IR_{vmax} (KBr): 1091.1 (C-O-C), 1205.5 and 1275.6 (C(O)OR), 1529.6 (benzene), 1607.2 (C-NO_2), 1729.7 (C=O), 2925.9 (aliphatic CH), 3327.6 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.91 (d, 1H, J = 10.24 Hz, IsH-1), 3.95-4.06 (m, 2H, IsH-1, IsH-6), 4.13 (dd, 1H, J = 3.42 and 10.24 Hz, IsH-6'), 4.39 (m, 1H, IsH-2), 4.59 (d, 1H, J = 4.78 Hz, IsH-3), 4.99 (t, 1H, J = 5.12 Hz, IsH-4), 5.33 (d, 1H, J = 7.51 Hz, NH), 5.47 (m, 1H, IsH-5), 7.13 (d, 2H, J = 8.19 Hz, Ar_1H-2/Ar_1H-6), 7.23 (t, 1H, J = 7.17, Ar_1H-4), 7.37 (t, 2H, J = 7.70 Hz, Ar_1H-3/Ar_1H-5), 8.26 (d, 2H, J = 8.88 Hz, Ar_2H-2/Ar_2H-6), 8.33

(d, 2H, J = 8.88 Hz, Ar_2H-3/Ar_2H-5). **^{13}C-NMR ppm** ($CDCl_3$): 57.14 (IsC-2), 70.68 (IsC-6), 72.63 (IsC-1), 74.90 (IsC-5), 80.19 (IsC-4), 86.78 (IsC-3), 121.01 (Ar_1C-2/Ar_1C-6), 123.24 (Ar_2C-3/Ar_2C-5), 125.16 (Ar_1C-4), 128.93 (Ar_1C-3/Ar_1C-5), 130.41 (Ar_2C-2/Ar_2C-6), 134.35 (Ar_2C-1), 147.86 (Ar_2C-4), 150.30 (Ar_1C-1), 153.40 (-C(O)OAr_1), 163.63 (-C(O)Ar2). $C_{20}H_{18}N_2O_8$ requires C, 57.97; H, 4.38; N, 6.76: found C, 57.82; H, 4.50; N, 6.48.

*2-Deoxy-2-(phenyloxycarbonylamino-) 5-O-(*p*-bromo-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **104**
(2-Phenyl reverse carbamate 5-*p*-bromo-benzoyl isosorbide)

Compound **104** was synthesised from 2-deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **95** (mol. wt. 265.26 g/mol, 0.75 mmol, 200 mg) with *p*-bromobenzoyl chloride (mol. wt. 219.47 g/mol, 0.83 mmol, 182 mg) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane afforded 300 mg of the title compound as a white crystalline product (89 %). M.pt 126°C, mol. wt. 448.26 g/mol. IR_{vmax} (KBr): 1091.4 (C-O-C), 1205.4 and 1271.6 (C(O)OR), 1538.5 (benzene), 1724.3 (C=O), 2955.5 (aliphatic CH), 3328.4 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($(CD_3)_2CO$): 3.83-4.11 (m, 4H, IsH-6, IsH-6', IsH-1, IsH-1'), 4.19 (m, 1H, IsH-2), 4.59 (d, 1H, J = 4.78 Hz, IsH-3), 5.00 (t, 1H, J = 5.12 Hz, IsH-4), 5.45 (m, 1H, IsH-5), 7.07-7.25 (m, 3H, Ar_1H-2/Ar_1H-6, Ar_1H-4), 7.37 (t, 2H, J = 7.51 Hz, Ar_1H-3/Ar_1H-5), 7.75 (d, 2H, J = 8.87 Hz, Ar_2H-3/Ar_2H-5), 7.99 (d, 2H, J = 8.20 Hz, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($(CD_3)_2CO$): 57.62 (IsC-2), 70.31 (IsC-6), 72.31 (IsC-1), 74.62 (IsC-5), 80.40 (IsC-4), 86.78 (IsC-3), 121.15 (Ar_1C-2/Ar_1C-6), 124.48 (Ar_1C-4), 127.15 (Ar_2C-4), 128.63 (Ar_1C-3/Ar_1C-5), 128.76 (Ar_2C-1), 130.80 (Ar_2C-3/Ar_2C-5), 131.45 (Ar_2C-2/Ar_2C-6), 150.98 (Ar_1C-1), 153.56 (-C(O)OAr_1), 164.07 (-C(O)Ar_2). $C_{20}H_{18}BrNO_6$ requires C, 53.59; H, 4.05; N, 3.12: found C, 53.96; H, 4.22; N, 3.27.

2-Deoxy-2-(phenyloxycarbonylamino-) 5-O-(2,6-dichlorobenzoyl)-1,4:3,6-dianhydro-D-glucitol **105** (2-phenyl reverse carbamate 5-(2,6-dichloro-benzoyl) isosorbide)

Compound **105** was synthesised from 2-deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **95** (mol. wt. 265.26 g/mol, 0.75 mmol, 200 mg) with 2,6-dichlorobenzoyl chloride (mol. wt. 209.46 g/mol, 0.83 mmol, 174 mg) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane yielded 177 mg of the target compound as a white crystalline product (54 %). M.pt 204°C, mol. wt. 438.26 g/mol. IR_{vmax} (KBr): 1083.5 (C-O-C), 1148.4, and 1271.8 (C(O)OR), 1563.8 (benzene), 1698.0, 1740.8 (C=O), 2926.1 (aliphatic CH), 3317.0 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($(CD_3)_2CO$): 3.91-4.00 (m, 3H, IsH-6, IsH-6', IsH-1), 4.05 (dd, 1H, J = 5.46 and10.24, IsH-1'), 4.18 (m, 1H, IsH-2), 4.63 (d, 1H, J = 4.78 Hz, IsH-3), 5.02 (t, 1H, J = 5.12 Hz, IsH-4), 5.51 (m, 1H, IsH-5), 7.10-7.25 (m, 3H, Ar_1H-2/Ar_1H-6, Ar_1H-4), 7.34 (t, 2H, J = 7.51 Hz, Ar_1H-3/Ar_1H-5), 7.55 (s, 3H, Ar_2H-3, Ar_2H-4, Ar_2H-5). **^{13}C-NMR ppm** ($(CD_3)_2CO$): 57.61 (IsC-2), 69.91 (IsC-6), 72.48 (IsC-1), 75.52 (IsC-5), 80.19 (IsC-4), 86.82 (IsC-3), 121.18 (Ar_1C-2/Ar_1C-6), 124.50 (Ar_1C-4), 127.86 (Ar_2C-3/Ar_2C-5), 128.64 (Ar_1C-3/Ar_1C-5), 130.99 (Ar_2C-2/Ar_2C-6), 131.47 (Ar_2C-4), 132.73 (Ar_2C-1), 150.99 (Ar_1C-1), 153.59 (-C(O)OAr_1), 163.00 (-C(O)Ar_2). $C_{20}H_{17}Cl_2NO_6$ requires C, 54.81; H, 3.91; N, 3.20: found C, 55.03; H, 4.11; N, 3.40.

2-deoxy-2-(phenyloxycarbonylamino-) 5-O-(o-methyl-benzoyl)-1,4:3,6-dianhydro-D glucitol **106** (2-phenyl reverse carbamate 5-toluoyl isosorbide)

Compound **106** was synthesised from 2-deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **95** (mol. wt. 265.26 g/mol, 0.75 mmol, 200 mg) with *o*-toluoyl chloride (mol. wt. 154.6 g/mol, d 1.185 g/ml, 0.83 mmol, 128 mg, 0.11 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane to afforded the title compound as a white crystalline product 194 mg (67 %). M.pt 96°C, mol. wt. 383.39 g/mol. IR_{vmax} (KBr): 1092.5 (C-O-C), 1143.8, 1206.1 and 1257.9 (C(O)OR), 1494.1 (CH_3), 1557.4 (benzene), 1691.0, 1715.5 (C=O), 2929.8 (aliphatic CH), 3326.0 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($(CD_3)_2CO$): 2.61 (s, 3H, CH_3), 3.85-

4.05 (m, 4H, IsH-6, IsH-6', IsH-1, IsH-1'), 4.20 (m, 1H, IsH-2), 4.62 (d, 1H, J = 4.78 Hz, IsH-3), 4.99 (t, 1H, J = 5.12 Hz, IsH-4), 5.41 (m, 1H, IsH-5), 7.07-7.25 (m, 3H, Ar_1H-2/Ar_1H-6, Ar_1H-4), 7.28-7.43 (m, 4H, Ar_1H-3/Ar_1H-5, Ar_2H-3/Ar_2H-5), 7.49 (m, 1H Ar_2C-4), 7.95 (d, 1H, J = 8.19 Hz, Ar_2H-6). **^{13}C-NMR ppm** ($(CD_3)_2CO$): 20.33 (CH_3), 57.76 (IsC-2), 70.20 (IsC-6), 72.25 (IsC-1), 74.17 (IsC-5), 80.28 (IsC-4), 86.76 (IsC-3), 121.17 (Ar_1C-2/Ar_1C-6), 124.48 (Ar_1C-4), 125.38 (Ar_2C-5), 128.63 (Ar_1C-3/Ar_1C-5), 129.08 (Ar_2C-1), 129.91 (Ar_2C-3), 131.16 (Ar_2C-6), 131.64 (Ar_2C-4), 139.42 (Ar_2C-2), 150.00 (Ar_1C-1), 153.58 (-C(O)OAr_1), 164.80 (-C(O)Ar_2). $C_{21}H_{21}NO_6$ requires C, 65.79; H, 5.52; N, 3.65: found C, 65.38; H, 5.45; N, 3.78.

2-Deoxy-2-(phenyloxycarbonylamino-) 5-O-(o-methyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **107** *(2-phenyl reverse carbamate 5-methyloxy isosorbide)*

Compound **107** was synthesised from 2-deoxy-2-(phenyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **95** (mol. wt. 265.26 g/mol, 0.75 mmol, 200 mg) with *o*-anisoyl chloride (mol. wt. 170.59 g/mol, 0.83 mmol, 129 mg) as reagent, using the method outlined for the preparation of esters. Purification by column chromatography yielded 112 mg of the title compound as a clear oil (37 %). Mol. wt. 399.39 g/mol. IR_{vmax} (NaCl): 1091.5 (C-O-C), 1140.7, 1205.8 and 1259.2 (C(O)OR), 1558.4 (benzene), 1699.7, 1725.1 (C=O), 2928.8 (aliphatic CH), 3324.2 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.89-3.97 (m, 4H, IsH-1, CH_3), 3.98-4.10 (m, 3H, IsH-1', IsH-6, IsH-6'), 4.37 (m, 1H, IsH-2), 4.58 (d, 1H, J = 4.09 Hz, IsH-3), 4.97 (t, 1H, J = 5.12 Hz, IsH-4), 5.34 (d, 1H, J = 7.51 Hz, NH), 5.41 (m, 1H, IsH-5), 6.97-7.06 (m, 2H, Ar_2H-3, Ar_2H-5), 7.14 (d, 2H, J = 7.51 Hz, Ar_1H-2/Ar_1H-6), 7.23 (t, 1H, J = 7.51 Hz, Ar_1H-4), 7.38 (t, 2H, J = 7.51 Hz, Ar_1H-3/Ar_1H-5), 7.42 (m, 1H Ar_2H-4), 7.88 (dd, 1H, J = 1.37 and 8.19 Hz, Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 55.48 (CH_3), 57.33 (IsC-2), 70.85 (IsC-6), 72.49 (IsC-1), 73.69 (IsC-5), 80.20 (IsC-4), 86.70 (IsC-3), 111.63 (Ar_2C-3), 115.98 (Ar_2C-1), 119.70 (Ar_2C-5), 121.06 (Ar_1C-2/Ar_1C-6), 125.08 (Ar_1C-4), 128.90 (Ar_1C-3/Ar_1C-5), 131.48 (Ar_2C-6), 133.60 (Ar_2C-4), 150.25 (Ar_1C-1), 153.43 (-C(O)OAr_1), 159.13 (Ar_2C-2), 164.80 (-C(O)Ar_2). HRMS (M + 23); $C_{21}H_{21}NO_7Na$ requires 422.1217; found 422.2633.

2-Deoxy-2-(benzyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **96**
(2-Benzyl reverse carbamate isosorbide)

Compound **96** was synthesised from 2-deoxy-2-azido-1,4:3,6-dianhydro-D-glucitol **89** (mol. wt. 171.15 g/mol, 5.84 mmol, 1 g) with benzyl chloroformate (mol. wt. 170.59 g/mol, d 1.195 g/ml, 6.43 mmol, 1.01 g, 0.85 ml) using the same method outlined for the preparation of compound **95**. The title compound was afforded as white crystalline needles (1.02 g, 62.6 %). M.pt 165°C, mol. wt. 279.29 g/mol. IR_{vmax} (KBr): 1089.8 (C-O-C), 1143.7 and 1253.3 (C(O)OR), 1543.9 (benzene), 1700.1 (C=O), 2924.7 (aliphatic CH), 3367.2 (OH) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 2.72 (s, 1H, OH), 3.62 (dd, 1H, J = 5.46 and 8.88 Hz, IsH-6), 3.82-4.01 (m, 3H, IsH-6', IsH-1, IsH-1'), 4.27 (m, 2H, IsH-2, IsH-5), 4.42 (d, 1H, J = 3.42 Hz, IsH-3), 4.53 (t, 1H, J = 4.78 Hz, IsH-4), 5.12 (s, 2H, CH_2), 5.51 (s, 1H, NH), 7.25-7.45 (m, 5H, ArH). **^{13}C-NMR ppm** ($CDCl_3$): 57.63 (IsC-2), 66.54 (CH_2), 71.74 (IsC-5), 72.99 (IsC-1), 73.14 (IsC-6), 81.09 (IsC-4), 86.30 (IsC-3), 127.81 (ArC-4), 127.85 (ArC-2/ArC-6), 128.13 (ArC-3/ArC-5), 135.67 (ArC-1), 155.25 (CO). $C_{14}H_{17}NO_5$ requires C, 60.21; H, 6.14; N, 5.02: found C, 60.56; H, 5.99; N, 4.78.

2-Deoxy-2-(benzyloxycarbonylamino-) 5-O-benzoyl-1,4:3,6-dianhydro-D-glucitol **108**
(2-Benzyl reverse carbamate 5-benzoyl isosorbide)

Compound **108** was synthesised from 2-deoxy-2-(benzyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **96** (mol. wt. 279.29 g/mol, 0.72 mmol, 200 mg) with benzoyl chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 0.79 mmol, 111 mg, 0.09 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane afforded the title compound as a white crystalline product 100 mg (36 %). M.pt 96°C, mol. wt. 383.39 g/mol. IR_{vmax} (KBr): 1090.8 (C-O-C), 1208.9 and 1284.0 (C(O)OR), 1521.9 (benzene), 1693.9 and 1730.7 (C=O), 2919.5 (aliphatic CH), 3320.0 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.81 (d, 1H, J = 9.41 Hz, IsH-1), 4.01 (m, 2H, IsH-1', IsH-6), 4.08 (dd, 1H, J = 3.39 and 10.17 Hz, IsH-6'), 4.34 (s, 1H, IsH-2), 4.49 (d, 1H, J = 3.76 Hz, IsH-3), 4.91 (t, 1H, J = 4.89 Hz, IsH-4), 4.95 (s, 1H, NH), 5.15 (s, 2H, CH_2) 5.42 (m, 1H, IsH-5), 7.32-7.42 (m, 5H, Ar_1H), 7.48 (d, 1H, J = 7.53 Hz, Ar_2H-3/Ar_2H-5),

7.61 (m, 1H, Ar_2H-4), 8.09 (d, 2H, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 57.47 (IsC-2), 66.89 (CH_2), 71.09 (IsC-6), 73.00 (IsC-1), 74.31 (IsC-5), 80.53 (IsC-4), 87.19 (IsC-3), 128.09 (Ar_1-H-4) 128.15 (Ar_1C-2/Ar_1C-6), 128.32 (Ar_1C-3/Ar_2C-5), 128.44 (Ar_1C-3/Ar_1C-5), 129.38 (Ar_2C-2/Ar_2C-6), 129.60 (Ar_2C-1), 133.13 (Ar_2C-4), 135.99 (Ar_1C-1), 155.38 (-C(O)OAr_1), 165.77 (-C(O)Ar_2). $C_{21}H_{21}NO_6$ requires C, 65.79; H, 5.52; N, 3.65: found C, 65.51; H, 5.62; N, 3.59.

2-Deoxy-2-(benzyloxycarbonylamino-) 5-O-(o-methyl-benzoyl)-1,4:3,6-dianhydro-D-glucitol **109** (2-benzyl reverse carbamate 5-methyl isosorbide)

Compound **109** was synthesised from 2-deoxy-2-(benzyloxycarbonylamino-)-1,4:3,6-dianhydro-D-glucitol **96** (mol. wt. 279.29 g/mol, 0.72 mmol, 200 mg) with *o*-toluoyl chloride (mol. wt. 154.6 g/mol, d 1.185 g/ml, 0.79 mmol, 122 mg, 0.10 ml) as reagent, using the method outlined for the preparation of esters. Purification by column chromatography yielded 137 mg (48 %) of the title compound as a clear oil, which crystallized upon standing. Mol. wt. 397.42 g/mol. IR_{vmax} (KBr): 1092.0 (C-O-C), 1143.9 and 1257.5 (C(O)OR), 1455.9 (CH_3), 1537.9 (benzene), 1722.4 (C=O), 2960.3 (aliphatic CH), 3320.2 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 2.60 (s, 3H, CH_3), 3.79 (d, 1H, J = 9.56 Hz, IsH-1), 3.87-4.03 (m, 3H, IsH-1', IsH-6, IsH-6'), 4.28 (m, 1H, IsH-2), 4.44 (d, 1H, J = 4.09 Hz, IsH-3), 4.87 (t, 1H, J = 5.14 Hz, IsH-4), 5.09 (s, 2H, CH_2), 5.31 (m, 1H, IsH-5), 5.51 (d, 1H, J = 7.51 Hz, NH), 7.19-7.46 (m, 8H, Ar_1H, Ar_2H-3/Ar_2H-4/Ar_2H-5), 7.93 (d, 1H, J = 8.19 Hz, Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 21.27 (CH_3), 57.24 (IsC-2), 66.41 (CH_2), 70.55 (IsC-6), 72.64 (IsC-1), 73.85 (IsC-5), 80.10 (IsC-4), 86.80 (IsC-3), 125.32 (Ar_2C-5), 127.74 (Ar_1C-2/Ar_1C-6, Ar_1C-4), 128.07 (Ar_1C-3/Ar_1C-5), 128.37 (Ar_2C-1), 130.21 (Ar_2C-3), 131.30 (Ar_2C-6), 131.82 (Ar_2C-4), 135.80 (Ar_1C-1), 140.08 (Ar_2C-2), 155.29 (-C(O)OAr_1), 166.25 (-C(O)Ar_2). $C_{22}H_{23}NO_6$ requires C, 66.49; H, 5.83; N, 3.52: found C, 66.35; H, 5.71; N, 3.33.

2-(Benzylaminocarbonyloxy-) 5-O-nitro-1,4:3,6-dianhydro-D-glucitol **111**

(2-Benzyl carbamate 5-*O*-nitro isosorbide)

IS-5-MN **62** (mol. wt. 191.14 g/mol, 52.36 mmol, 10 g) was dissolved in pyridine. 1.1 mol eq. of benzyl isocyanate (mol. wt. 133.15 g/mol, d 1.078 g/ml, 57.60 mmol, 7.67 g, 7.11 ml) was added. The mixture was heated to 105°C for two hours and the reaction was monitored by TLC (1:1 ethyl acetate: hexane) to observe the disappearance of the starting material. Potassium permanganate development solution was used to identify the reactant and product spots masked by pyridine when observed under UV light. The mixture was cooled upon completion of the reaction and methanol (20 ml) was added to remove excess isocyanate. The mixture was heated for a further 15 minutes at 105°C and cooled to room temperature. The pyridine was removed under vacuum and ice water was poured onto the crude product. The precipitate was filtered on a Buckner funnel and recrystallised from hot methanol to obtain the title compound as a white crystalline product with a 16.13 g (95.0 %). M.pt 93.5°C, mol. wt. 324.29 g/mol. IR_{vmax} (KBr): 1091.5 (C-O-C), 1138.9 and 1254.8 (C(O)OR), 1452.0 (CH_3), 1254.0 and 1630.3 (ONO_2), 1721.3 (C=O), 2926.5 (aliphatic CH), 3360.5 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.90 (dd, 1H, J = 5.52 and 11.04 Hz, IsH-6), 3.95-4.10 (m, 3H, IsH-1, IsH-1', IsH-6'), 4.36 (d, 2H, J = 6.02 Hz, CH_2), 4.51 (d, 1H, J = 5.02 Hz, IsH-3), 4.95 (t, 1H, J = 5.27, IsH-4), 5.21 (d, 1H, J = 3.01 Hz, IsH-2), 5.25 (m, IH, NH), 5.35 (m, 1H, IsH-5), 7.24-7.40 (m, 5H, Ar-H). **^{13}C-NMR ppm** ($CDCl_3$): 45.06 (CH_2), 69.15 (IsC-6), 73.70 (IsC-1), 77.77 (IsC-2), 81.30 (IsC-4), 81.39 (IsC-5), 86.69 (IsC-3), 127.50 (ArC-4), 127.61 (Ar-2/Ar-6), 128.68 (ArC-3/ArC-5), 138.42 (ArC-1), 155.54 (CO).

2-(Benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112**

(2-Benzyl carbamate isosorbide)

2-(Benzylaminocarbonyloxy-) 5-*O*-nitro-1,4:3,6-dianhydro-D-glucitol **111** (mol. wt. 324.29 g/mol, 46.25 mmol, 15 g) was dissolved in ethyl acetate: methanol 1:1 (100 ml) in a 250 ml round bottom flask. A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept

under an atmosphere of hydrogen gas and stirred for 24 hours. The progress of the reaction was monitored by TLC (1:1 ethyl acetate: hexane) to observe the disappearance of the starting material. TLC showed the formation of a single product. The solvent was evaporated under vacuum and the crude product was dissolved in DCM and filtered through silica. The filtrate was collected and evaporated under vacuum to yield 12.6 g of the target compound as a white crystalline product (98 %). M.pt. 76.0°C, mol. wt. 279.29 g/mol. 1083.9.5 (C-O-C) 1265.0 (C(O)OR), 1452.0 (CH_3), 1725.1 (C=O), 2877 (aliphatic CH), 3431.8 (OH) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 2.67 (d, 1H, J = 7.03 Hz, OH), 3.57 (dd, 1H, J = 5.53 and 8.54 Hz, IsH-6), 3.90 (dd, 1H, J = 6.03 and 9.54 Hz, IsH-6'), 4.00 (dd, 1H, J = 3.51 and 10.54 Hz, IsH-1), 4.10 (d, 1H, J = 10.45, IsH-1'), 4.31 (m, 1H, IsH-5), 4.38 (d, 2H, J = 6.03 Hz, CH_2), 4.51 (d, 1H, J = 4.01 Hz, IsH-3), 4.61 (t, 1H, J = 4.77 Hz, IsH-4), 5.17 (m, 1H, NH), 5.22 (d, 1H, J = 3.01 Hz, IsH-2), 7.23-7.40 (m, 5H, Ar-H). **^{13}C-NMR ppm** ($CDCl_3$): 44.70 (CH_2), 71.87 (IsC-5), 73.04 (IsC-6), 73.38 (IsC-1), 78.39 (IsC-2), 81.46 (IsC-4), 85.25 (IsC-3), 127.13 (ArC-4), 127.23 (Ar-2/Ar-6), 128.30 (ArC-3/ArC-5), 137.57 (ArC-1), 154.69 (CO).

2-(Benzylaminocarbonyloxy-) 5-O-acetyl-1,4:3,6-dianhydro-D-glucitol **114**
(2-Benzyl carbamate 5-acetyl isosorbide)

Compound **114** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.72 mmol, 200 mg) with acetic anhydride (mol. wt. 102.09, d 1.082, 0.79 mmol, 80 mg, 0.07 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane to afforded compound **114** as a white crystalline product (206 mg, 90 %). M.pt. 107.5°C, mol. wt. 321.33 g/mol. IR_{vmax} (KBr): 1092.2 (C-O-C), 1116.9, 1144.9, 1238.4, 1265.2, (C(O)OR), 1375.6 (CH_3), 1454.2 (aliphatic CH_2), 1540.6 (benzene), 1693.2, 1736.1 (C=O), 2880.1, 2944.8 (C-H stretching), and 3344.9 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 2.14 (s, 3H, CH_3), 3.80 (dd, 1H, J = 5.52 and 9.54 Hz, IsH-6), 3.93-4.07 (m, 3H, IsH-1, IsH-1', IsH-6'), 4.38 (d, 2H, J = 6.03 Hz, CH_2), 4.51 (d, 1H, J = 4.51 Hz, IsH-3), 4.81 (t, 1H, J = 5.02 Hz, IsH-4), 5.10-5.18 (m, 2H, IsH5, NH), 5.20 (d, 1H, J = 3.02 Hz, IsH-2), 7.27-7.40 (m, 5H, Ar-H). **^{13}C-NMR ppm** ($CDCl_3$): 20.26 (CH_3), 44.67

(CH_2), 69.71 (IsC-6), 73.24 (IsC-1), 73.57 (IsC-5), 78.05 (IsC-2), 80.22 (IsC-4), 85.54 (IsC-3), 127.10 (ArC-4), 127.21 (Ar-2/Ar-6), 128.29 (ArC-3/ArC-5), 137.59 (ArC-1), 154.76 (CO), 169.96 (-C(O)CH3). $C_{16}H_{19}NO_6$ requires C, 59.81; H, 5.96; N, 4.36: found C, 59.92; H, 5.96; N, 4.36.

2-(Benzylaminocarbonyloxy-) 5-O-propionyl-1,4:3,6-dianhydro-D-glucitol **115**
(2-Benzyl carbamate 5-propionyl isosorbide)

Compound **115** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.72 mmol, 200 mg) with propionic anhydride (mol. wt. 130.15 g/mol, d 1.01 g/ml, 0.79 mmol, 103 mg, 0.10 ml) as reagent, using the method outlined for the preparation of esters, Recrystallisation from hot ethyl acetate and hexane afforded compound **115** as a white crystalline product (202 mg, 84 %). M.pt. 84°C, mol.wt. 335.35 g/mol. IR_{vmax} (KBr): 1097.6 (C-O-C), 1141.6, 1176.3, 1236.9, 1268.9 (C(O)OR), 1374.6 (CH_3), 1432.1, 1456.4, 1497.2 (aliphatic CH_2), 1524.1 (benzene), 1697.7, 1746.1 (C=O), 2879.0, 2925.3, 2981.1 (C-H stretching), and 3376.4 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 1.18 (t, 3H, J = 7.53 Hz, CH_3), 2.42 (q, 2H, J = 7.53 and 15.05 Hz, $CO\underline{C}H_2CH_3$), 3.81 (dd, 1H, J = 5.02 and 9.53 Hz, IsH-6), 3.92-4.07 (m, 3H, IsH-1, IsH-1', IsH-6'), 4.38 (d, 2H, J = 6.03 Hz, CH_2), 4.51 (d, 1H, J = 4.52 Hz, IsH-3), 4.82 (t, 1H, J = 5.02 Hz, IsH-4), 5.07-5.18 (m, 2H, IsH5, NH), 5.20 (d, 1H, J = 3.01 Hz, IsH-2), 7.23-7.40 (m, 5H, Ar-H). **^{13}C-NMR ppm** ($CDCl_3$): 8.61 (CH_3), 26.82 ($CO\underline{C}H_2CH_3$) 44.66 (CH_2), 69.87 (IsC-6), 73.17 (IsC-1), 73.37 (IsC-5), 78.06 (IsC-2), 80.26 (IsC-4), 85.56 (IsC-3), 127.10 (ArC-4), 127.20 (Ar-2/Ar-6), 128.29 (ArC-3/ArC-5), 137.60 (ArC-1), 154.77 (CO), 173.45 ($\underline{C}OCH_2CH_3$). $C_{17}H_{21}NO_6$ requires C, 60.89; H, 6.31; N, 4.18: found C, 60.89; H, 6.29; N, 4.17.

2-(Benzylaminocarbonyloxy-) 5-O-valeryl-1,4:3,6-dianhydro-D-glucitol **116**
(2-Benzyl carbamate 5-valeryl isosorbide)

Compound **116** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.72 mmol, 200 mg) with valeric anhydride (mol.

wt. 186.25 g/mol, d 0.944 g/ml, 0.79 mmol, 147 mg, 0.16 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane afforded compound **116** as a white crystalline product (201 mg, 77 %). M.pt. 64°C, mol.wt. 335.35 g/mol. IR_{vmax} (KBr): 1087.7 (C-O-C), 1138.0, 1178.9, 1247.6, 1263.2 (C(O)OR), 1371.4 (CH_3), 1456.8, 1497.5 (aliphatic CH_2), 1540.2 (benzene), 1694.5, 1731.6 (C=O), 2860.7, 2926.5, 2965.5, 3032.5 (C-H stretching), and 3320.1 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 0.94 (t, 3H, J = 7.03 Hz, CH_3), 1.38 (m, 2H, $C(O)CH_2CH_2C\underline{H}_2CH_3$), 1.65 (m, 2H, $C(O)CH_2C\underline{H}_2CH_2CH_3$), 2.40 (q, 2H, J = 7.03 and 8.04 Hz, $C(O)C\underline{H}_2CH_2CH_2CH_3$), 3.81 (dd, 1H, J = 5.52 and 10.04 Hz, IsH-6), 3.90-4.07 (m, 3H, IsH-1, IsH-1', IsH-6'), 4.38 (d, 2H, J = 6.02 Hz, CH_2), 4.51 (d, 1H, J = 4.52 Hz, IsH-3), 4.82 (t, 1H, J = 5.02 Hz, IsH-4), 5.07-5.18 (m, 2H, IsH5, NH), 5.20 (d, 1H, J = 3.01 Hz, IsH-2), 7.23-7.40 (m, 5H, Ar-H). **^{13}C-NMR ppm** ($CDCl_3$): 13.28 (CH_3), 21.75 ($C(O)CH_2CH_2\underline{C}H_2CH_3$), 26.48 ($C(O)CH_2\underline{C}H_2CH_2CH_3$), 33.23 ($C(O)\underline{C}H_2CH_2CH_2CH_3$), 44.67 ($CH_2$), 69.91 (IsC-6), 73.16 (IsC-1), 73.30 (IsC-5), 78.07 (IsC-2), 80.26 (IsC-4), 85.57 (IsC-3), 127.10 (ArC-4), 127.20 (Ar-2/Ar-6), 128.29 (ArC-3/ArC-5), 137.59 (ArC-1), 154.77 (CO), 172.79 ($\underline{C}(O)CH_2CH_2CH_2CH_3$). $C_{19}H_{25}NO_6$ requires C, 62.80; H, 6.93; N, 3.85: found C, 62.87; H, 6.93; N, 3.78.

2-(Benzylaminocarbonyloxy-) 5-O-methanesulfonyl-1,4:3,6-dianhydro-D-glucitol **117**
(2-Benzyl carbamate 5-mesyl isosorbide)

Compound **117** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.72 mmol, 200 mg) with methanesulfonyl chloride (mol. wt. 114.55 g/mol, 0.79 mmol, 90 mg) as reagent. The reaction vessel was cooled to 0°C and followed the method outlined for the preparation of esters. Recrystallisation from hot methanol afforded compound **117** as a white crystalline product (202 mg, 84 %). M.pt. 84°C, mol.wt. 335.35 g/mol. IR_{vmax} (KBr): 1078.8 (C-O-C), 1135.2, 1242.5, (C(O)OR), 1176.8 (SO_2), 1343.0 (CH_3), 1463.9 (aliphatic CH_2), 1531.2 (benzene), 1690.7 (C=O), 2889.6, 2914.6, 2944.3, 2987.4, 3050.1 (C-H stretching), and 3353.0 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.15 (CH_3), 3.88 (dd, 1H, J = 6.53 and 10.04 Hz, IsH-6), 3.96-4.12 (m, 3H, IsH-1, IsH-1', IsH-6'), 4.38 (d, 2H, J =

6.02 Hz, CH_2), 4.56 (d, 1H, J = 4.02 Hz, IsH-3), 4.82 (t, 1H, J = 4.77 Hz, IsH-4), 5.06-5.18 (m, 2H, IsH5, NH), 5.22 (d, 1H, J = 3.02 Hz, IsH-2), 7.23-7.40 (m, 5H, Ar-H). **^{13}C-NMR ppm** ($CDCl_3$):38.25 (CH_3), 44.70 (CH_2), 69.69 (IsC-6), 73.52 (IsC-1), 77.86 (IsC-2), 78.14 (IsC-5), 80.00 (IsC-4), 85.64 (IsC-3), 127.12 (ArC-4), 127.26 (Ar-2/Ar-6), 128.32 (ArC-3/ArC-5), 137.52 (ArC-1), 154.62 (CO). $C_{15}H_{19}NO_7S$ requires C, 50.41; H, 5.36; N, 3.92: found C, 50.43; H, 5.33; N, 3.75.

2-(Benzylaminocarbonyloxy-) 5-O-trifluoromethanesulfonyl-1,4:3,6-dianhydro-D-glucitol **118**
(2-Benzyl carbamate 5-triflate isosorbide)

2-(Benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.90 mmol, 250 mg) was dissolved in chloroform (10 ml) and cooled to -20°C in an ice bath with acetone. 1.1 mol. eq. of triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 0.99 mmol, 108 mg, 0.15 ml) and 10 % DMAP (mol. wt. 122.17 g/mol, 0.21 mmol, 25 mg) were added. Trifluoromethanesulfonic anhydride (mol. wt. 282.13 g/mol, d 1.677 g/ml, 0.99 mmol, 278 mg, 0.17 ml) was added dropwise over 15 minutes. After stirring for one hour, the mixture was allowed to warm up to room temperature and was then diluted with chloroform. The mixture was washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml) saturated brine solution (20 ml) and dried over anhydrous sodium sulfate (1 g). The solution was filtered into a round bottom flask, and the organic solvent was evaporated under vacuum to give a yellow crystalline solid. Purification by column chromatography over silica gel using hexane and ethyl acetate (3:1) as eluent afforded the title compound as fine white needles (266 mg, 72 %). M.pt. 107°C, mol.wt. 411.35 g/mol. IR_{vmax} (KBr): 1087.0 (C-O-C), 1242.5, (C(O)OR), 1144.4 (SO_2), 1414.42 (CF_3), 1455.75 (aliphatic CH_2), 1529.6 (benzene), 1710.4 (C=O), 2899.6, 2932.3, (C-H stretching), and 3339.7 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.90 (dd, 1H, J = 5.02 and 11.54 Hz, IsH-6), 4.02-4.17 (m, 3H, IsH-1, IsH-1', IsH-6'), 4.39 (d, 2H, J = 6.02 Hz, CH_2), 4.52 (d, 1H, J = 4.52 Hz, IsH-3), 4.88 (t, 1H, J = 5.27 Hz, IsH-4), 5.09-5.17 (m, 1H, NH), 5.19-5.28 (m, 2H, IsH5, IsH-2), 7.23-7.40 (m, 5H, Ar-H). **^{13}C-NMR ppm** ($CDCl_3$): 44.70 (CH_2), 70.27 (IsC-6), 73.46 (IsC-1), 77.35 (IsC-2), 80.33 (IsC-5), 85.20 (IsC-4), 86.01 (IsC-3), 127.12 (ArC-4), 127.27 (ArC-2/ArC-6), 128.32 (ArC-3/ArC-5), 137.49 (ArC-1), 154.58 (CO).

$C_{15}H_{16}F_3NO_7S$ requires C, 43.80; H, 3.92; N, 3.41: found C, 43.82; H, 3.89; N, 3.19.

2-(Benzylaminocarbonyloxy-) 5-deoxy-L-xylohex-6-enitol **119**
(Alkene elimination product)

To a solution of 2-(Benzylaminocarbonyloxy-) 5-*O*-trifluoromethanesulfonyl-1,4:3,6-dianhydro-D-glucitol **118** (mol.wt. 411.35 g/mol, 0.36 mmol, 150 mg) in anhydrous toluene (10 ml) was added 1,5-diazabicyclo [4.3.0] non-5-ene (152.44 g/mol, d 1.019 g/ml, 0.40 mmol, 61 mg, 0.06 ml) and the mixture was stirred for 6 hours at room temperature. The organic solvent was removed under vacuum and the crude mixture was diluted with DCM and washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml) saturated brine solution (20 ml) and dried over anhydrous sodium sulfate (1 g). The solution was filtered into a round bottom flask, and the organic solvent was evaporated under vacuum to give a yellow crystalline solid. Purification by column chromatography over silica gel using hexane and ethyl acetate (3:1) as eluent, afforded the title compound as white crystalline solid (15 mg, 16 %). M.pt. 92°C, mol.wt. 261.27 g/mol. IR_{vmax} (KBr): 1055.8 (C-O-C), 1144.4, 1258.9 (C(O)OR) 1535.0 (benzene), 1611.0 (C=C), 1719.3 (C=O), 2859.2, 2924.4 (C-H stretching), and 3328.0 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.58 (dd, 1H, J = 2.51 and 10.56 Hz, IsH-1), 3.99 (d, 1H, J = 6.02 Hz, IsH-1'), 4.40 (d, 2H, J = 6.02 Hz, CH_2), 4.87 (d, 1H, J = 6.53 Hz, IsH-3), 5.06 (t, 1H, J = 2.51 Hz, IsH-4), 5.13 (m, 1H, NH), 5.20 (d, 1H, J = 2.01 and 6.03 Hz, IsH-2), 6.56 (d, 1H, J = 3.01 Hz, IsH-6), 7.28-7.40 (m, 5H, ArH).

2-(Benzylaminocarbonyloxy-) 5-(cyclopropylcarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **120**
(2-Benzyl carbamate 5-cyclopropyl isosorbide)

Compound **120** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.90 mmol, 250 mg) with cyclopropanccarbonyl chloride (mol. wt. 104.5 g/mol, d 1.152 g/ml, 0.99 mmol, 103 mg, 0.89 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot methanol afforded the target compound as a white crystalline product (265 mg, 85 %).

M.pt. 96°C, mol.wt. 347.36 g/mol. IR_{vmax} (KBr): 1094.4 (C-O-C), 1176.0, 1200.3, 1243.2, 1259.2 (C(O)OR), 1455.0 (aliphatic CH_2), 1533.7 (benzene), 1727.6 (C=O), 2876.9, 2931.6 (C-H stretching), and 3345.1 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 0.87-0.98 (m, 2H, cycloalkyl-H), 1.04 (m, 2H, cycloalkyl-H), 1.70 (m, 1H, cycloalkyl-H), 3.80 (dd, 1H, J = 5.52 and 9.54 Hz, IsH-6), 3.95 (dd, 1H, J = 6. 03 and 9.54 Hz, IsH-6'), 3.98-4.08 (m, 2H, IsH-1, IsH-1'), 4.37 (d, 2H, J = 6.02 Hz, CH_2), 4.52 (d, 1H, J = 4.51 Hz, IsH-3), 4.79 (t, 1H, J = 4.77 Hz, IsH-4), 5.14 (q, 1H, J = 5.52 and 11.54 Hz, IsH-5), 5.17-5.27 (m, 2H, IsH-2, NH), 7.22-7.40 (m, 5H, 5ArH). **^{13}C-NMR ppm** ($CDCl_3$): 8.41 (cycloalkyl CH_2), 8.53 (cycloalkyl CH_2), 12.19 (cycloalkyl CH), 44.64 (CH_2), 69.74 (IsC-6), 73.25 (IsC-1), 73.48 (IsC-5), 78.09 (IsC-2), 80.27 (IsC-4), 85.52 (IsC-3), 127.10 (ArC-4), 127.17 (ArC-2/ArC-6), 128.27 (ArC-3/ArC-5), 137.65 (ArC-1), 154.81 (-C(O)N-), 173.93 (-C(O)C_3H_5). $C_{18}H_{21}NO_6$ requires C, 62.24; H, 6.09; N, 4.03: found C, 62.20; H, 6.09; N, 3.97.

2-(Benzylaminocarbonyloxy-) 5-(cyclopentylcarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **121**
(2-Benzyl carbamate 5-cyclopentyl isosorbide)

Compound **121** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.90 mmol, 250 mg) with cyclopentanecarbonyl chloride (mol. wt. 132.5 g/mol, d 1.091 g/ml, 0.99 mmol, 131 mg, 0.14 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot methanol afforded the target compound as a white crystalline compound (276 mg, 82 %). M.pt. 94°C, mol.wt. 375.42 g/mol. IR_{vmax} (KBr): 1088.3 (C-O-C), 1184.8, 1247.1, 1263.7 (C(O)OR), 1454.9 (aliphatic CH_2), 1532.2 (benzene), 1693.5, 1731.8 (C=O), 2869.6, 2913.3, 2960.0, 3030.6 (C-H stretching), and 3314.6 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 1.53-2.00 (m, 8H, cycloalkyl-H), 2.83 (m, 1H, cycloalkyl), 3.73 (dd, 1H, J = 5.02 and 10.03 Hz, IsH-6), 3.89-4.06 (m, 3H, IsH-6', IsH-1, IsH-1'), 4.39 (d, 2H, J = 6.02 Hz, CH_2), 4.50 (d, 1H, J = 4.51 Hz, IsH-3), 4.81 (t, 1H, J = 5.02 Hz, IsH-4), 5.08-5.18 (m, 2H, IsH-5, NH), 5.20 (d, 1H, J = 3.01 Hz, IsH-2), 7.22-7.40 (m, 5H, 5Ar_1H) **^{13}C-NMR ppm** ($CDCl_3$): 25.35 (cycloalkyl CH_2), 25.37 (cycloalkyl CH_2), 29.43 (cycloalkyl CH_2), 29.73 (cycloalkyl CH_2), 43.04 (cycloalkyl CH), 44.65 (CH_2), 70.12 (IsC-6), 73.05

(IsC-1), 73.21 (IsC-5), 78.06 (IsC-2), 80.29 (IsC-4), 85.62 (IsC-3), 127.10 (ArC-4), 127.19 (ArC-2/ArC-6), 128.28 (ArC-3/ArC-5), 137.61 (ArC-1), 154.79 (-C(O)N-), 175.78 (-C(O)C_5H_9). $C_{20}H_{25}N0_6$ requires C, 63.99; H, 6.71; N, 3.73; found C, 63.98; H, 6.76; N, 3.64.

2-(benzylaminocarbonyloxy-) 5-O-toluenesulfonyl-1,4:3,6-dianhydro-D-glucitol **130**
(2-Benzyl carbamate 5-tosyl isosorbide)

Compound **130** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1.79 mmol, 500 mg) with *p*-toluenesulfonyl chloride (mol. wt. 190.5 g/mol, 1.58 mmol, 273 mg) as reagent using the method outlined for the preparation of esters. The procedure yielded the title compound (483 mg, 94 %) as a clear gum, which resisted recrystallisation. Mol. wt. 433.48 g/mol IR_{vmax} (NaCl): 1084.8 (C-O-C), 1177.0 (SO_2), 1190.7, 1241.6 (C(O)OR), 1365.2 (CH_3), 1455.4 (aliphatic CH_2), 1531.6 (benzene), 1724.3 (C=O), 2880.0, 2932.3, 3031.9 (C-H stretching), and 3344.9 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 2.46 (s, 3H, CH_3), 3.74 (dd, 1H, J = 6.53 and 9.54 Hz, IsH-6), 3.86 (dd, 1H, J = 6.52 and 10.04 Hz, IsH-6') 3.93-4.05 (m, 2H, IsH-1, IsH-1'), 4.36 (d, 2H, J = 6.03 Hz, CH_2), 4.48 (d, 1H, J = 4.02 Hz, IsH-3), 4.65 (t, 1H, J = 4.52 Hz, IsH-4), 4.92 (q, 1H, J = 6.53 and 12.05 Hz, IsH-5), 5.08 (s, 1H, NH), 5.15 (d, 1H, J = 2.42 Hz, IsH-2), 7.23-7.40 (m, 7H, 5Ar_1H, Ar_2H-2/ Ar_2H-6), 7.84 (d, 2H, J = 8.53 Hz, Ar_2H-3/ Ar_2H-5). **^{13}C-NMR ppm** ($CDCl_3$): 21.24 (CH_3) 44.57 (CH_2), 69.06 (IsC-6), 73.45 (IsC-1), 77.92 (IsC-2), 78.22 (IsC-5), 79.95 (IsC-4), 85.32 (IsC-3), 127.09 (Ar_1C-4), 127.13 (Ar_1C-2/Ar_1C-6), 127.48 (Ar_2C-2/Ar_2C-6), 128.24 (Ar_1C-3/Ar_1C-5), 129.51 (Ar_2C-3/Ar_2C-5), 132.64 (Ar_2C-1), 137.67 (Ar_1C-1), 144.83 (Ar_2C-4), 154.77 (CO). HRMS (M + 23); $C_{21}H_{23}NO_7SNa$ requires 456.1108; found, 456.1093.

2-(Benzylaminocarbonyloxy-) 5-deoxy-5-azido-1,4:3,6-dianhydro-L-iditol **131**
(2-Benzyl carbamate 5-azido isoiodide)

2-(Benzylaminocarbonyloxy-) 5-*O*-toluenesulfonyl-1,4:3,6-dianhydro-D-glucitol **130** (mol.wt. 433.12 g/mol, 150 mg, 0.35 mmol) was dissolved in 5 ml of DMSO. Sodium azide (mol. wt. 65.01 g/mol, 1.73 mmol, 113 mg) was added to the solution, which formed a slurry. The mixture was heated to 125°C. The reaction was monitored by TLC (1:1, ethyl acetate: hexane) to check for the disappearance of the starting material. The mixture was diluted with ethyl acetate (50 ml) was washed with three equal volumes of water and dried over anhydrous sodium sulfate (1 g). The solution was filtered into a round bottom flask, and the organic solvent was removed under vacuum, leaving a brown oil. Purification by column chromatography, using hexane and ethyl acetate (3:1) as eluent, yielded a crystalline solid, which was recrystallised from hot ethyl acetate and hexane to afford the target compound as a white crystalline product (75 mg, 74 %). M.pt. 58°C, mol.wt. 304.30 g/mol. IR_{vmax} (KBr): 1100.4 (C-O-C), 1244.4, (C(O)OR), 1454.8 (aliphatic CH_2), 1532.6 (benzene), 1723.1 (C=O), 28.76.1, 2908.3, (C-H stretching), and 3338.1 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.87-4.01 (m, 4H, IsH-1, IsH-1', IsH-6, IsH-6'), 4.07 (d, 1H, J = 3.51 Hz, IsH-3), 4.38 (d, 2H, J = 6.02 Hz, CH_2), 4.59-4,69 (m, 2H, IsH-4, IsH-5), 5.11 (s, 1H, NH), 5.20 (s, 1H, IsH-2), 7.24-7.40 (m, 5H, 5ArH). **^{13}C-NMR ppm** ($CDCl_3$): 44.72 (CH_2), 65.28 (IsC-5), 71.07 (IsC-6), 72.63 (IsC-1), 77.53 (IsC-2), 85.04 (IsC-4), 85.53 (IsC-3), 127.14 (ArC-4), 127.26 (ArC-2/ArC-6), 128.32 (ArC-3/ArC-5), 137.51 (ArC-1), 154.60 (CO). $C_{14}H_{16}N_4O_4$ requires C, 55.26; H, 5.30; N, 18.41: found C, 55.58; H, 5.10; N, 17.99.

2-(Benzylaminocarbonyloxy-) 5-O-(1-naphthoyl)-1,4:3,6-dianhydro-D-glucitol **122**
(2-Benzyl carbamate 5-(1-naphthoyl) isosorbide)

Compound **122** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1.79 mmol, 500 mg) with 1-naphthoylchloride (mol. wt. 190.63 g/mol, d 1.265 g/ml, 1.97 mmol, 376 mg, 0.30 ml) as reagent, using the method outlined for the preparation of esters. The procedure gave white solid, which was

washed with cold diethyl ether (1 ml) and recrystallised from hot methanol to give the target compound as a white crystalline product (674 mg, 87 %). M.pt. 126°C, mol.wt. 433.45 g/mol. IR_{vmax} (KBr): 1084.4 (C-O-C), 1133.4, 1196.8, 1240.4, 1282.64, (C(O)OR), 1469.4 (aliphatic CH_2), 1523.5 (benzene), 1688.98, 1717.1 (C=O), 2872.0, 2912.66, 2942.1, 2990.9, 3032.3, 3058.3 (C-H stretching), and 3314.7 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 4.01-4.16 (m, 4H, IsH-6, IsH-1), 4.38 (d, 2H, J = 6.03 Hz, CH_2), 4.61 (d, 1H, J = 4.52 Hz, IsH-3), 5.03 (t, 1H, J = 5.02 Hz, IsH-4), 5.11 (s, 1H, NH), 5.26 (s, 1H, IsH-2), 5.51 (q, 1H, J = 5.53 and 11.05 Hz, IsH-5), 7.23-7.40 (m, 5H, $5Ar_1H$), 7.50-7.60 (m, 2H, Ar_2H-3/Ar_2H-9), 7.65 (t, 1H, J = 7.28 Hz, Ar_2H-8), 7.92 (d, 1H, J = 8.03 Hz, Ar_2H-10), 8.07 (d, 1H, J = 8.03 Hz, Ar_2H-4), 8.25 (d, 1H, J = 7.72 Hz, Ar_2H-2), 8.96 (d, 1H, J = 8.53 Hz, Ar_2H-7). **^{13}C-NMR ppm** ($CDCl_3$): 44.69 (CH_2), 70.23 (IsC-6), 73.24 (IsC-1), 74.11 (IsC-5), 78.14 (IsC-2), 80.56 (IsC-4), 85.80 (IsC-3), 124.06 (Ar_2C-3), 125.31 (Ar_2C-9), 125.85 (Ar_2C-7), 125.90 (Ar_2C-1), 127.11 (Ar_1C-4), 127.21 (Ar_1C-2/Ar_1C-6), 127.47 (Ar_2C-8), 128.11 (Ar_2C-10), 128.30 (Ar_1C-3/Ar_1C-5), 130.01 (Ar_2C-2), 130.95 (Ar_2C-6), 133.30 (Ar_2C-4), 133.36 (Ar2C-5), 137.54 (Ar_1C-1), 154.77 (-C(O)N-), 166.33 (-C(O)Ar_2). $C_{25}H_{23}NO_6$ requires C, 69.27; H, 5.35; N, 3.23: found C, 68.96; H, 5.25; N, 2.94.

2-(Benzylaminocarbonyloxy-) 5-O-(2-naphthoyl)-1,4:3,6-dianhydro-D-glucitol **123**
(2-Benzyl carbamate 5-(2-naphthoyl) isosorbide)

Compound **123** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1.79 mmol, 500 mg) with 2-naphthoylchloride (mol. wt. 190.63 g/mol, 1.97 mmol, 376 mg) as reagent, using the method outlined for the preparation of esters. The procedure gave white solid, which was washed with cold diethyl ether (1 ml) and recrystallised from hot methanol to give the target compound as a white crystalline product (649 mg, 84 %). M.pt. 143°C, mol.wt. 433.45 g/mol. IR_{vmax} (KBr): 1082.4 (C-O-C), 1129.5, 1194.8, 1230.2, 1286.2, (C(O)OR), 1467.2 (aliphatic CH_2), 1545.2 (benzene), 1691.0, 1717.0 (C=O), 2870.0, 2911.4, 2938.0, 2989.0, 3010.9, 3058.2 (C-H stretching), and 3323.7 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.99-4.14 (m, 4H, IsH-6, IsH-1), 4.38 (d, 2H, J = 6.03 Hz, CH_2), 4.60 (d, 1H, J = 4.51 Hz, IsH-3), 5.01

(t, 1H, J = 5.02 Hz, IsH-4), 5.12 (s, 1H, NH), 5.27 (d, 1H, J = 2.51 Hz, IsH-2), 5.48 (q, 1H, J = 5.52 and 11.04, IsH-5), 7.23-7.40 (m, 5H, $5Ar_1H$), 7.54-7.68 (m, 2H, Ar_2H-8/Ar_2H-9), 7.92 (d, 2H, J = 8.54 Hz, Ar_2H-7/Ar_2H-10), 7.99 (d, 1H, J = 8.03 Hz, Ar_2H-4), 8.11 (dd, 1H, J = 1.50 and 8.53 Hz, Ar_2H-3), 8.66 (s, 1H, Ar_2H-1). **^{13}C-NMR ppm** ($CDCl_3$): 44.67 (CH_2), 70.30 (IsC-6), 73.27 (IsC-1), 74.18 (IsC-5), 78.18 (IsC-2), 80.64 (IsC-4), 85.80 (IsC-3), 124.79 (Ar_2C-3), 126.24 (Ar_2C-8), 126.30 (Ar_2C-2), 127.10 (Ar_1C-4), 127.20 (Ar_1C-2/ Ar_1C-6), 127.35 (Ar_2C-10), 127.84 (Ar_2C-9), 128.00 (Ar_2C-7), 128.29 (Ar_1C-3/ Ar_1C-5), 128.99 (Ar_2C-1), 130.93 (Ar_2C-6), 132.00 (Ar_2C-4), 135.22 (Ar_2C-5), 137.61 (Ar_1C-1), 154.87 (-C(O)N-), 165.64 (-C(O)Ar_2). $C_{25}H_{23}NO_6$ required C, 69.27; H, 5.35; N, 3.23: found C, 69.10; H, 5.31; N, 3.08.

*2-(Benzylaminocarbonyloxy-) 5-(*p*-phenyl-phenylcarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol* **124**
(2-Benzyl carbamate 5-bi-phenyl isosorbide)

Compound **124** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1.79 mmol, 500 mg) with biphenyl-4-carbonyl chloride (mol. wt. 216.67 g/mol, 1.97 mmol, 426 mg) as reagent, using the method outlined for the preparation of esters. The procedure gave white solid, which was washed with cold diethyl ether (1 ml) and recrystallised from hot methanol to give the target compound as a white crystalline product (743 mg, 90 %). M.pt. 109°C, mol.wt. 454.49 g/mol. IR_{vmax} (KBr): 1098.6 (C-O-C), 1125.4, 1267.8, 1276.1 (C(O)OR), 1455.0 (aliphatic CH_2), 1531.9 (benzene), 1691.2, 1716.8 (C=O), 2875.7, 2982.4, 3031.7 (C-H stretching), and 3347.2 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 4.00-4.10 (m, 4H, IsH-6, IsH-1), 4.38 (d, 2H, J = 6.02 Hz, CH_2), 4.58 (d, 1H, J = 4.52 Hz, IsH-3), 4.98 (t, 1H, J = 5.28, IsH-4), 5.15 (t, 1H, J = 5.78 Hz, NH), 5.26 (s, 1H, IsH-2), 5.43 (q, 1H, J = 5.52 and 10.02 Hz, IsH-5), 7.23-7.40 (m, 5H, $5Ar_1H$), 7.43 (m, 1H, Ar_2H-10), 7.50 (t, 2H, J = 7.53 Hz, Ar_2H-9/ Ar_2H-11), 7.64 (d, 2H, J = 8.03 Hz, Ar_2H-8/Ar_2H-12), 7.70 (d, 2H, J = 8.53 Hz, Ar_2H-3/Ar_2H-5), 8.16 (d, 2H, J = 8.53 Hz, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.67 (CH_2), 70.31 (IsC-6), 73.25 (IsC-1), 74.25 (IsC-5), 78.06 (IsC-2), 80.61 (IsC-4), 85.79 (IsC-3), 126.70 (Ar_2C-10), 126.85 (Ar_2C-3/Ar_2C-5), 127.10 (Ar_1C-4), 127.19 (Ar_1C-2/ Ar_1C-6), 127.73 (Ar_2C-1), 127.79 (Ar_2C-8/Ar_2C-12), 128.29 (Ar_1C-

3/Ar_1C-5), 128.52 (Ar_2C-9/Ar_2C-11), 129.86 (Ar_2C-2/Ar_2C-6), 137.62 (Ar_1C-1), 139.46 (Ar_2C-7), 145.57 (Ar_2C-4), 154.81 (-C(O)N-), 165.36 (-C(O)Ar_2). $C_{27}H_{25}NO_6$ requires C, 70.58; H, 5.48; N, 3.05: found C, 70.29; H, 5.46; N, 2.96.

2-(Benzylaminocarbonyloxy-) 5-(p-heptyloxyphenylcarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **126** (2-benzyl carbamate 5-*p*-heptyloxy-benzoyl isosorbide)

Compound **126** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.90 mmol, 250 mg) with *o*-heptoxybenzoyl chloride (mol. wt. 254.76g/mol, d 1.061 g/ml, 0.99 mmol, 251 mg, 0.24 ml) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethanol afforded the target compound as a white crystalline product (302 mg, 68 %). M.pt. 68°C, mol.wt. 497.58 g/mol. IR_{vmax} (KBr): 1100.2 (C-O-C), 1168.5 (-OCH_2-) 1126.5, 1255.3, 1281.3 (C(O)OR), 1455.8 (aliphatic CH_2), 1511.1, 1532.1 (benzene), 1716.7 (C=O), 2858.1, 2871.6, 2929.9, 3031.2, 3067.3 (C-H stretching), and 3345.3 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 0.92 (t, 3H, J = 6.78 Hz, CH_3), 1.25-1.53 (m, 8H, $OCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$), 1.82 (m, 2H, $OCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_3$), 3.94-4.07 (m, 6H, IsH-1, IsH-6, -O$C\underline{H}_2$-), 4.38 (d, 2H, J = 6.02 Hz, CH_2), 4.55 (d, 1H, J = 4.52 Hz, IsH-3), 4.93 (t, 1H, J = 5.02 Hz, IsH-4), 5.14 (t, 1H, J = 5.53, NH), 5.23 (s, 1H, IsH-2), 5.37 (q, 1H, J = 5.52 and 10.54 Hz, IsH-5), 6.93 (d, 2H, J = 9.03 Hz, Ar_2H-3/Ar_2H-5), 7.23-7.40 (m, 5H, 5Ar_1H), 8.01 (d, 2H, J = 9.04, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 13.65 (CH_3), 22.16 ($OCH_2CH_2CH_2CH_2CH_2\underline{C}H_2CH_3$), 25.40 ($OCH_2CH_2\underline{C}H_2CH_2CH_2CH_2CH_3$), 28.58 ($OCH_2CH_2CH_2\underline{C}H_2CH_2CH_2CH_3$), 28.64 ($OCH_2\underline{C}H_2CH_2CH_2CH_2CH_2CH_3$), 31.31 ($OCH_2CH_2CH_2CH_2\underline{C}H_2CH_2CH_3$), 44.65 ($CH_2$), 67.79 ($O\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_3$), 70.28 (IsC-6), 73.22 (IsC-1), 73.69 (IsC-5), 78.09 (IsC-2), 80.64 (IsC-4), 85.72 (IsC-3), 113.71 (Ar_2C-3/Ar_2C-3), 121.07 (Ar_2C-1), 127.10 (Ar_1C-4), 127.17 (Ar_1C-2/Ar_1C-6), 128.27 (Ar_1C-3/Ar_1C-5), 131.37 (Ar_2C-2/Ar_2C-6), 137.63 (Ar_1C-1), 154.83 (-C(O)N-), 162.82 (Ar_2C-4), 165.25 (-C(O)Ar_2). $C_{28}H_{35}NO_7$ requires C, 67.59; H, 7.09; N, 2.81: found C, 67.60; H, 7.09; N, 2.74.

2-(Benzylaminocarbonyloxy-) 5-O-cinnamoyl-1,4:3,6-dianhydro-D-glucitol **125**
(2-Benzyl carbamate 5-cinnamoyl isosorbide)

Compound **125** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 0.90 mmol, 250 mg) with cinnamoyl chloride (mol. wt. 166.60 g/mol, 0.99 mmol, 164 mg) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot ethyl acetate and hexane afforded the target compound as a white crystalline product (236 mg, 64 %) M.pt. 123°C, mol.wt. 409.15 g/mol. IR_{vmax} (KBr): 1094.4 (C-O-C), 1140.4, 1189.4, 1264.8, 1283.9, (C(O)OR), 1455.1 (aliphatic CH_2), 1528.6 (benzene), 1638.8 (-C=C-), 1695.6, 1709.1 (C=O), 2876.6, 2916.6, 3031.0, 3064.1 (C-H stretching), and 3365.6 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.87-3.94 (m, 1H, IsH-6), 3.98-4.10 (IsH-1, IsH-1', IsH-6'), 4.37 (d, 2H, J = 6.02 Hz, CH_2), 4.57 (d, 1H, J = 4.52 Hz, IsH-3), 4.88 (t, 1H, J = 4.77 Hz, IsH-4), 5.15 (t, 1H, J = 5.52, NH), 5.23 (s, 1H, IsH-2), 5.30 (q, 1H, J = 5.52 and 11.04 Hz, IsH-5), 6.52 (d, 1H, J = 16.06 Hz, -OC(O)C<u>H</u>-), 7.23-7.45 (m, 8H, $5Ar_1H$, Ar_2H-3, Ar_2H-4, Ar_2H-5), 7.56 (m, 2H, Ar_2H-2/Ar_2H-6), 7.76 (d, 1H, J = 16.07 Hz, -OC(O)CHC<u>H</u>-). **^{13}C-NMR ppm** ($CDCl_3$): 44.67 (CH_2), 69.84 (IsC-6), 73.33 (IsC-1), 73.68 (IsC-5), 78.04 (IsC-2), 80.45 (IsC-4), 85.62 (IsC-3), 116.60 (-OC(O)<u>C</u>HCHAr2), 127.10 (Ar_1C-4), 127.20 (Ar_1C-2/Ar_1C-6), 127.78 (Ar_2C-2/Ar_2C-6), 128.30 (Ar_1C-3/Ar_1C-5), 130.11 (Ar_2C-4), 133.72 (Ar_2C-1), 137.61 (Ar_1C-1), 145.45 (-C(O)CH<u>C</u>HAr_2), 154.80 (-<u>C</u>(O)N-), 165.85 (-<u>C</u>(O)CHCHAr_2). HRMS (M + 23); $C_{23}H_{23}NO_6Na$ requires 409.1423; found, 409.1440.

2-(Benzylaminocarbonyloxy-) 5-(coumarincarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **127**
(2-Benzyl carbamate 5-coumarin isosorbide)

2-(Benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1.79 mmol, 500 mg) was dissolved in chloroform (20 ml) and cooled to 0°C. Coumarin 3-carboxylic acid (mol. wt. 190.15 g/mol, 1.97 mmol, 375 mg), DCC (mol. wt. 206.33 g/mol, 1.97 mmol, 407 mg) and DMAP (mol. wt. 122.17 g/mol, 1.97 mmol, 219 mg) were added to the reaction vessel. The mixture was stirred overnight and the reaction vessel was allowed to return to room temperature. The reaction was monitored by TLC

(1:1 ethyl acetate: hexane) to observe the disappearance of the starting material. The organic solvent was evaporated under vacuum, yielding a crude crystalline compound. 20 ml of DCM was added to the reaction vessel and the unwanted urea precipitate was collected by filtration. The filtrate was washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml) saturated brine solution (20 ml) and dried over anhydrous sodium sulphate (1 g). The solution was filtered into a round bottom flask and the organic solvent was evaporated under vacuum to give a clear oil. Purification by column chromatography, using hexane and ethyl acetate (3:1) as eluent, yielded a white crystalline solid, which was recrystallised from hot ethyl acetate and hexane to afford the target compound as a white crystalline product (162 mg, 20 %). M.pt. 169°C, mol.wt. 451.43 g/mol. IR_{vmax} (KBr): 1085.5 (C-O-C), 1205.1, 1244.5 (C(O)OR), 1454.9 (aliphatic CH_2), 1535.0 (benzene), 1607.7 (-C=C-), 1691.3, 1763.8, 1777.7 (C=O), 2863.2, 2927.4 (C-H stretching), and 3334.7 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.98-4.17 (m, 4H, IsH-6, IsH-1), 4.38 (d, 2H, J = 5.54 Hz, CH_2), 4.57 (d, 1H, J = 4.51 Hz, IsH-3), 4.96 (t, 1H, J = 5.02 Hz, IsH-4), 5.10 (s, 1H, NH), 5.25 (d, 1H, J = 3.02 Hz, IsH-2), 5.40 (q, 1H, J = 5.52 and 11.04 Hz, IsH-5), 7.23-7.43 (m, 7H, Ar_1H, Ar_2H-6, Ar_2H-8), 7.62-7.73 (m, 2H, Ar_2H-7, Ar_2H-9), 8.58 (s, 1H, Ar_2H-3). **^{13}C-NMR ppm** ($CDCl_3$): 44.81 (CH_2), 71.18 (IsC-6), 73.51 (IsC-1), 75.79 (IsC-5), 78.62 (IsC-2), 81.51 (IsC-4), 86.97 (IsC-3), 116.81 (Ar_2C-6), 118.65 (Ar_2C-2), 125.29 (Ar_2C-8), 127.46 (Ar_1C-4), 127.79 (Ar_1C-2/Ar_1C-6), 128.86 (Ar_1C-3/Ar_1C-5), 130.61 (Ar_1C-1), 130.72 (Ar_2C-9), 134.97 (Ar_2C-7), 149.27 (Ar_2C-3), 155.85 (Ar_2C-5), 156.17 (-C(O)N-), 162.50 (Ar_2C-1), 165.31 (-C(O)Ar_2). $C_{24}H_{21}NO_8$ requires C, 63.85; H, 4.69; N, 3.10: found C, 63.59; H, 4.69; N, 2.98.

2-(Benzylaminocarbonyloxy-) 5-keto-1,4:3,6-dianhydro-D-glucitol **113**

(2-Benzyl carbamate 5-ketone isosorbide)

To a solution of 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1 g, 3.58 mmol) in 100 ml of DCM was added PCC (mol. wt. 215.02 g/mol, 13.95 mmol, 3 g) and silica gel (3 g). The reaction mixture was stirred for 30 minutes and then heated with stirring under reflux at a temperature of 65°C for 24 hours. The progress of the reaction was monitored by TLC (2:1 Ethyl Acetate: Hexane).

Vanillin development solution was used to distinguish the reactant and product spots. As the reactant and product spots gave similar Rf values, excess PCC (0.5 g) was added to the reaction vessel and the mixture was heated for a further eight hours to ensure that the reaction went to completion. The reaction mixture was cooled and filtered through dry silica gel and diethyl ether was used to elute the product through the silica gel yielding a clear oil which was crystallized from hot ethyl acetate and hexane giving 670 mg (68 %) of a white crystalline compound. M.pt. 107°C, Mol. wt. 277.27 g/mol. IR_{vmax} (KBr): 1078.0 (C-O-C), 1265.0 (C(O)OR), 1599 (benzene), 1725.0, 1774 (C=O), 2880, 2970 (C-H stretching), and 3326.4 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.97 (d, 1H, J = 17.57 Hz, IsH-1), 4.05-4.20 (m, 3H, IsH-1', IsH-6, IsH-6'), 4.30 (d, 1H, J = 4.02 Hz, IsH-4), 4.39 (d, 2H, J = 6.02 Hz, CH_2), 4.85 (d, 1H, J = 4.01 Hz, IsH-3), 5.19 (s, 1H, NH), 5.36 (d, 1H, J = 2.51 Hz, IsH-2), 7.22-7.40 (m, 5H, ArH). **^{13}C-NMR ppm** ($CDCl_3$): 44.73 (CH_2), 69.63 (IsC-1), 72.67 (IsC-6), 78.29 (IsC-2/IsC-4), 84.57 (IsC-3), 127.14 (ArC-4), 127.29 (ArC-2/ArC-6), 128.33 (ArC-3/ArC-5), 137.42 (ArC-1), 154.53 (CO), 209.28 (IsC-5). $C_{14}H_{15}NO_5$ requires C, 60.64; H, 5.45; N, 5.05: found 60.73; H, 5.43; N, 4.67.

2-(Benzylaminocarbonyloxy-) 5-ketoxime-1,4:3,6-dianhydro-D-glucitol **132**
(2-Benzyl carbamate 5-oxime isosorbide)

To a solution of 2-(benzylaminocarbonyloxy-) 5-keto-1,4:3,6-dianhydro-D-glucitol **113** (277.27 g/mol, 0.90 mmol, 250 mg) in methanol: water (2:1, 6ml) was added two mol. eq. of hydroxylamine hydrochloride (69.49 g/mol, 1.8032 mmol, 125.3 mg) and five mol. eq. of sodium acetate (82.03 g/mol, 4.5079 mmol, 369.8 mg). The reaction mixture was heated to 105°C for two hours. The reaction mixture was evaporated to dryness under vacuum and the residue was dissolved in chloroform. Any excess water was dried with sodium sulphate and the solution was filtered and evaporated. Purification by column chromatography using hexane and ethyl acetate (2:1) as eluent yielded 176 mg (67 %) of a clear oil. Mol. wt. 292.29 g/mol. IR_{vmax} (NaCl): 1086.5 (C-O-C), 1138.2, 1248.9 (C(O)OR), 1528.0 (benzene), 1697.2, 1720.1 (C=O), 2876.4, 2928.3, (C-H stretching), and 3332.5 (OH) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.90-4.10 (m, 2H, IsH-1, IsH-1'), 4.31 (d, 2H, J = 6.02 Hz, CH_2), 4.50 (d, 1H, J = 15.56 Hz, IsH-3), 4.54-4.70 (m, 2H, IsH-6, IsH-

6'), 4.96 (d, 1H, J = 3.01 Hz, IsH-4), 5.14 (s, 1H, IsH-2), 7.20-7.39 (m, 5H, 5ArH). **^{13}C-NMR ppm** ($CDCl_3$): 44.42 (CH_2), 69.13 (IsC-1), 72.21 (IsC-6), 78.53 (IsC-2), 80.32 (IsC-4), 86.50 (IsC-3), 127.17 (ArC-4), 127.28 (ArC-2/ArC-6), 128.48 (ArC-3/ArC-5), 139.14 (ArC-1), 156.58 (CO), 159.85 (ArC-5). HRMS (M + 23); $C_{14}H_{16}N_2O_5Na$ requires 315.0959: found, 315.0957.

2-(Benzylaminocarbonyloxy-) 5-(phenylcarbonyloxyimino-)-1,4:3,6-dianhydro-D-glucitol **133**
(2-Benzyl carbamate 5-oxime benzoate isosorbide)

2-(Benzylaminocarbonyloxy-) 5-ketoxime-1,4:3,6-dianhydro-D-glucitol **132** (292.29 g/mol, 0.34 mmol, 100 mg,) was dissolved in DCM (10 ml). Triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 0.38 mmol, 38 mg, 0.05 ml) and benzoyl chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 0.38 mmol, 53 mg, 0.04 ml) and DMAP (10 %, 5 mg) were added. The reaction mixture was stirred overnight. Chloroform (10 ml) was added to the reaction vessel and the mixture was washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml) saturated brine solution (20 ml) and dried with anhydrous sodium sulphate (1 g). The solution was filtered into a round bottom flask and was evaporated under vacuum to give a white crystalline solid, which was recrystallised from hot methanol to yield 106 mg (78 %) of a white crystalline product. M.pt. 91 °C, mol. wt. 396.39 g/mol. IR_{vmax} (KBr): 1089.2 (C-O-C), 1142.3, 1245.0 (C(O)OR), 1518.5 (benzene), 1710.3, 1725.9 (C=O), 2875.6, 2979.5 (C-H stretching), and 3325.5 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 4.07-4.25 (m, 2H, IsH-1, IsH-1'), 4.40 (d, 2H, J = 5.52 Hz, CH_2), 4.70 (m, 2H, IsH-6, IsH-6'), 4.87 (d, 1H, J = 16.57 Hz, IsH-3), 5.20 (m, 2H, IsH-4, NH), 5.35 (s, 1H, IsH-2), 7.23-7.43 (m, 5H, $5Ar_1H$), 7.51 (t, 2H, J = 7.53 Hz, Ar_2H-3/Ar_2H-5), 7.65 (t, 1H, J = 7.28 Hz, Ar_2H-4), 8.03 (d, 2H, J = 7.53 Hz, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.75 (CH_2), 69.47 (IsC-1), 72.72 (IsC-6), 77.60 (IsC-2), 79.74 (IsC-4), 86.30 (IsC-3), 127.16 (Ar_1C-4), 127.27 (Ar_1C-2/Ar_1C-6), 127.57 (Ar_2C-1), 128.25 (Ar_2C-3/Ar_2C-5), 128.33 (Ar_1C-3/Ar_1C-5), 129.27 (Ar_2C-2/Ar_2C-6), 133.37 (Ar_2C-4), 137.46 (Ar_1C-1), 154.53 (-C(O)N-), 162.61 (=NOC(O)Ar_2), 168.02 (IsC-5). $C_{21}H_{20}N_2O_6$ requires C, 63.63; H, 5.09; N, 7.07: found C, 63.69; H, 4.92; N, 6.77.

2-(Benzylaminocarbonyloxy-) 5-deoxy, 5-dehydro-((ethyloxycarbonyl)ene-)-1,4:3,6-dianhydro-D-glucitol **134** (Wittig product)

A solution of 2-(benzylaminocarbonyloxy-) 5-keto-1,4:3,6-dianhydro-D-glucitol **113** (mol. wt. 277.27 g/mol, 0.90 mmol, 250 mg) in DCM: THF (1:1, 10ml) was cooled to 0°C on ice in a dewer flask and kept under an atmosphere of nitrogen gas. Carbethoxymethylene triphenyl phosphorane (mol.wt. 348.88 g/mol, 0.99 mmol, 314 mg) was added to the solution and the mixture was stirred for six hours. The reaction mixture was evaporated to dryness under vacuum and the residue purified by column chromatography using hexane and ethyl acetate (2:1) as eluent yielded 142 mg of the title compounds as a white crystalline solid (45 %). M.pt. 121°C, mol. wt. 347.36 g/mol. IR_{vmax} (KBr): 1085.3 (C-O-C), 1140.6, 1248.6 (C(O)OR), 1370.7, 1390.6 (aliphatic CH), 1511.2 (benzene), 1688.8, 1721.4 (C=O), 2951.7, 2926.5, 3008.8 (C-H stretching), and 3324.8 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 1.30 (t, 3H, J = 7.31 Hz, CH_3), 4.00 (m, 2H, IsH-1), 4.20 (m, 2H, -$\underline{C}H_2CH_3$), 4.38 (d, 2H, J = 6.02 Hz, -NCH_2Ar), 4.55 (d, 1H, J = 4.10 Hz, IsH-3), 4.74 (dd, 1H, J = 2.92 and 17.54 Hz, IsH-4), 4.97 (m, 2H, IsH-6, IsH-6'), 5.15 (s, 1H, NH), 5.25 (s, 1H, IsH-2), 6.11 (s, 1H, -C=C$\underline{H}$-), 7.23-7.40 (m, 5H, 5ArH). **^{13}C-NMR ppm** ($CDCl_3$): 13.79 (CH_3), 44.69 (-NCH_2Ar), 60.16 (-CH_2CH_3), 70.92 (IsC-1), 71.77 (IsC-6), 78.24 (IsC-2), 83.28 (IsC-4), 84.74 (IsC-3), 116.12 (-C=$\underline{C}$H-), 127.12 (ArC-4), 127.22 (Ar-2/Ar-6), 128.29 (ArC-3/ArC-5), 137.59 (ArC-1), 154.71 (-N$\underline{C}$(O)O-), 158.13 (ArC-5), 165.30 (-C(O)CH_2CH_3). $C_{18}H_{21}NO_6$ requires C, 62.24; H, 6.09; N, 4.03: found C, 62.20; H, 6.08, N, 3.98.

7-exo-(Benzylaminocarbonyloxy-)-3-oxo-2,5,9-trioxabicyclo [4.3.0] nonane **137**
(Baeyer Villiger product)

2-(Benzylaminocarbonyloxy-) 5-keto-1,4:3,6-dianhydro-D-glucitol **113** (277.27 g/mol, 0.9016 mmol, 250 mg) was dissolved in DCM, stirred and cooled to 0°C. Chloroperbenzoic acid (172.57 g/mol, 500 mg) and sodium acetate (82.03 g/mol, 6.10 mmol, 500 mg) were added and the mixture was stirred overnight. The reaction was monitored by TLC (5:5:1) DCM: ethyl acetate: methanol) to observe the formation of the

desired product. The solvent was evaporated and the crude product was purified by flash chromatography (DCM: ethyl acetate, 9:1) to give 102 mg (39 %) of the title product as a clear oil. Mol. wt. 293.27 g/mol. IR_{vmax} (NaCl): 1082.0 (C-O-C), 1141.0, 1239.5 (C(O)OR), 1511.2 (benzene), 1719.5, 1725.1 (C=O), 2953.0 (C-H stretching), and 3328.3 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 4.03 (m, 2H, IsH-1, IsH-1'), 4.28-4.43 (m, 5H, IsH-6, IsH-6', IsH-3, CH_2), 5.24 (m, 2H, IsH-2, NH), 5.86 (d, 1H, J = 3.52 Hz, IsH-4), 7.22-7.42 (m, 5H, 5ArH). **^{13}C-NMR ppm** ($CDCl_3$): 44.76 (CH_2), 62.87 (IsC-1), 72.05 (IsC-6), 77.04 (IsC-2), 77.40 (IsC-3), 100.89 (IsC-4), 127.15 (ArC-4), 127.37 (Ar-2/Ar-6), 128.36 (ArC-3/ArC-5), 137.29 (ArC-1), 154.41 (CO), 165.29 (ArC-5). HRMS (M + 23); $C_{14}H_{15}NO_6Na$ requires 316.0798: found, 316.0881.

N-methyl-7-exo-(benzylaminocarbonyloxy-)-3-oxo-5,9-dioxa-2-oxabicyclo[4.3.0] nonane **139** (Beckmann product)

To a solution of 2-(benzylaminocarbonyloxy-) 5-keto-1,4:3,6-dianhydro-D-glucitol **113** (277.27 g/mol, 0.90 mmol, 250 mg) in anhydrous ethanol (6 ml) was added four mol. eq. of *N*-methylhydroxylamine hydrochloride (83.52 g/mol, 3.61 mmol, 301 mg) and six mol. eq. of sodium acetate (82.03 g/mol, 5.41 mmol, 444 mg). The reaction mixture was stirred for four hours. The reaction mixture then filtered and evaporated to dryness under vacuum. The crude intermediate product was purified by flash chromatography using a mobile phase of chloroform followed by chloroform: ethyl acetate: methanol (4.5:4.5:1) yielding a yellow viscous oil, which was diluted in pyridine (7 ml) and cooled to 0°C on an ice bath. The reaction mixture was stirred and kept under an atmosphere of nitrogen gas. *p*-toluenesulfonyl chloride (mol. wt. 190.5, 1.56 mmol, 300 mg) was added to the mixture and stirred for 6 hours. The solvent was evaporated and the crude product was purified by flash chromatography (DCM: ethyl acetate, 9:1) to give 97 mg (35 %) of the title product as a clear oil. Mol. wt. 306.31 g/mol. IR_{vmax} (NaCl): 1076.0 (C-O-C), 1170.9, 1271.6 (C(O)OR), 1521.5 (benzene), 1606.7 (C-N), 1718.7, 1721.4 (C=O), 2928.0, 3420.0 (C-H stretching), and 3321.5 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.06 (s, 3H, CH_3), 3.90 (d, 1H. J = 10.36 Hz, IsH-1) 4.05 (d, 1H, J = 16.06 Hz, IsH-6), 4.19 (d, 1H. J = 2.51 Hz, IsH-3) 4.25 (d, 1H. J = 16.57 Hz, IsH-6') 4.30-4.40 (m, 3H, IsH-1',

CH_2), 4.98 (d, 1H, J = 2.51 Hz, IsH-4), 5.17 (d, 1H, J = 4.02, IsH-2), 5.26-5.37 (s, 1H, NH), 7.23-7.40 (m, 5H, ArH). **^{13}C-NMR ppm** ($CDCl_3$): 32.01 (CH_3), 45.19 (CH_2), 66.24 (IsC-1), 71.17 (IsC-6), 77.28 (IsC-2), 77.43 (IsC-3), 87.47 (IsC-4), 127.14 (ArC-4), 127.31 (Ar-2/Ar-6), 128.33 (ArC-3/ArC-5), 137.43 (ArC-1), 154.45 (CO), 166.12 (ArC-5). HRMS (M + 23); $C_{15}H_{18}N_2O_6Na$ requires 329.1115: found, 329.1212.

*2-O-(*t*-(butyl)-dimethylsilyl-)-1,4:3,6-dianhydro-D-glucitol* **145**
(2-tBDMS isosorbide)

IS-5-MN **62** (mol. wt. 191.14 g/mol, 52.36 mmol, 10 g) was dissolved in DCM (150 ml). 1.1 mol eq. of imidazole (mol. wt. 68.08 g/mol, 57.60 mmol, 3.92 g), 1.1 mol eq. of *t*-(butyl)-dimethylsilyl chloride (mol. wt. 150.72 g/mol, 57.60 mmol, 5.94 g) and 10 % DMAP (mol. wt. 122.17 g/mol, 8.19 mmol, 1 g) were added and the mixture was stirred for two hours. The reaction was monitored by TLC (1:1, ethyl acetate: hexane) to observe the disappearance of the starting material. Potassium permanganate development solution was used to identify the reactant and product spots. The mixture was washed with 1M HCl (150 ml), 5 % $NaHCO_3$ (150 ml), saturated brine solution (150 ml) and dried over anhydrous sodium sulphate (10 g). The solution was filtered into a round bottom flask, and the organic solvent was evaporated under vacuum to give a clear oil. Purification by column chromatography, using hexane and ethyl acetate (3:1 and 1:1) as eluent, isolated the reaction product, which was a clear oil, which had a characteristic odour. The oil was diluted with ethyl acetate: methanol 1:1 (100 ml) in a 250 ml round bottom flask. A spatula tip-full of 10% palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas and stirred for 24 hours. TLC analysis showed the formation of a single product. The mixture was filtered through silica to remove the catalyst and the filtrate was evaporated under vacuum to give the title compound as a clear oil (13.25 g, 97 %). Mol. wt. 260.40 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 0.06 (d, 6H, J = 2.01 Hz, -OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.84 (m, 9H, -OSi(CH_3)$_2$C($C\underline{H}_3$)$_3$), 2.77 (s, 1H OH), 3.50 (dd, 1H, J = 6.02 and 9.03 Hz, IsH-6), 3.75-3.91 (m, 3H, IsH-1, IsH-1', IsH-6'), 4.18-4.35 (m, 3H, IsH-2, IsH-3, IsH-5), 4.61 (t, 1H, J = 4.77 Hz, IsH-4). **^{13}C-NMR ppm** ($CDCl_3$): -5.27 (-OSi($\underline{C}H_3$)$_2$C(CH_3)$_3$), 17.59 (-

$OSi(CH_3)_2\underline{C}(CH_3)_3$), 25.26 (-$OSi(\underline{C}H_3)_2C(CH_3)_3$), 72.26 (IsC-5), 73.31 (IsC-6), 76.17 (IsC-1), 77.30 (IsC-2), 81.53 (IsC-4), 88.50 (IsC-3).

2-(Benzylaminocarbonyloxy-) 5-O-benzyl-1,4:3,6-dianhydro-D-glucitol **140**
(2-Benzyl carbamate 5-benzyl isosorbide)

2-*O*-(*t*-(butyl)-dimethylsilyl-)-1,4:3,6-dianhydro-D-glucitol **145** (mol. wt. 384.88 g/mol, 1.15 mmol, 300 mg) and excess NaH (100 mg of 65 % NaH in an oil suspension) was placed in a clean and dry three neck round bottom flask and kept under an atmosphere of nitrogen gas. Anhydrous THF was added to the flask and the mixture was stirred for 30 minutes. Excess benzyl bromide (mol. wt. 171.03 g/mol, d 1.438 g/ml, 3.36 mmol, 575 mg, 0.40 ml) was added dropwise over 15 minutes and the reaction mixture was stirred for a further 24 hours. The mixture was diluted with water (25 ml), which was added slowly to exhaust the excess NaH, extracted with ethyl acetate (3 x 25ml) and dried over anhydrous sodium sulphate (2 g). The organic portions were collected and evaporated to dryness to give a clear oil. The remaining excess benzyl bromide was eliminated from the mixture by purification by column chromatography, which yielded a clear oil. The oil was diluted with THF (10 ml). Excess 0.1 M tetrabutylammonium fluoride (1.2 ml) was added to the solution and the mixture was stirred at room temperature for 15 minutes. The organic solvent was evaporated to dryness under vacuum giving a brown oil. DCM (10 ml) was added to the reaction vessel and the solution was stirred at room temperature. To the mixture was added excess triethylamine (mol. wt. 101g/mol, d 0.726 g/ml, 1.80 mmol, 182 mg, 0.25 ml), benzyl isocyanate (mol. wt. 133.15 g/mol, d 1.078 g/ml, 2.02 mmol, 270 mg, 0.25 ml) and DMAP (mol. wt. 122.17 g/mol, 0.25 mmol, 30 mg). The mixture was heated to 105°C for 2 hours. The mixture was cooled upon completion of the reaction and methanol (10 ml) was added to remove excess isocyanate. The mixture was heated for a further 15 minutes at 105°C and cooled to room temperature. The reaction mixture was evaporated to dryness giving a clear oil to which was added DCM (20 ml) and washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml), saturated brine solution (20 ml) and dried with anhydrous sodium sulphate (2 g). The solution was filtered into a round bottom flask and the organic solvent was evaporated to dryness under vacuum giving a

white crystalline compound. Purification by column chromatography using hexane and ethyl acetate (3:1 and 1:1) as eluent, afforded the title compound as a white crystalline product (109 mg, 26 %). M.pt. 107°C, Mol. wt. 369.41 g/mol. IR_{vmax} (KBr): 1100.9 (C-O-C Is), 1135.5 (C-O-C Ether), 1242.2 (C(O)OR), 1454.7 (aliphatic CH_2), 1528.0 (benzene), 1697.2 (C=O), 2876.4, 2928.3, (C-H stretching), and 3349.1 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.65 (t, 1H, J = 8.28 Hz, IsH-6), 3.90 (dd, 1H, J = 6.53 and 8.54 Hz, IsH-6'), 4.04-4.15 (m, 3H, IsH-5, IsH-1, IsH-1'), 4.39 (d, 2H, J = 6.02 Hz, CH_2), 4.53 (d, 1H, J = 4.02 Hz, IsH-3), 4.59 (d, 1h, J = 12.04 Hz, OCH_2Ar_1), 4.68 (t, 1H, J = 4.27 Hz, IsH-4), 4.78 (d, 1H, J = 12.04 Hz, OCH_2Ar_2), 5.08 (s, 1H, NH), 5.19 (d, 1H, J = 3.01 Hz, IsH-2), 7.23-7.43 (m, 10H, $5Ar_1H$, $5Ar_2H$). **^{13}C-NMR ppm** ($CDCl_3$): 45.06 (CH_2), 69.82 (IsC-6), 72.05 (OCH_2Ar_2), 73.55 (IsC-1), 78.58 (IsC-2), 78.68 (IsC-5), 80.06 (IsC-4), 85.54 (IsC-3), 127.12 (Ar_1C-4), 127.20 (Ar_1C-2/Ar_1C-6), 127.52 (Ar_2C-2/Ar_2C-6/Ar_2C-4), 128.06 (Ar_2C-3/Ar_2C-5), 128.29 (Ar_1C-3/Ar_1C-5), 137.19 (Ar_2C-1), 137.64 (Ar_1C-1), 154.79 (CO). $C_{21}H_{23}NO_5$ requires C, 68.28; H, 6.28; N, 3.79: found C, 68.22; H, 6.27; N, 3.69.

2-(Benzylaminocarbonyloxy-) 5-(phenylpropyloxy-)-1,4:3,6-dianhydro-D-glucitol **148**
(2-Benzyl carbamate 5-propyl phenyl isosorbide)

Compound **148** was prepared by using the same procedure as for 2-(Benzylaminocarbonyloxy-) 5-*O*-benzyl-1,4:3,6-dianhydro-D-glucitol **140** above. Excess propyl phenyl bromide (mol. wt. 199.09 g/mol, 3.01 mmol 600 mg) was used as the reagent for the reaction. The procedure afforded the title compound as a white crystalline compound (88 mg, 19 %). M.pt. 97°C, mol. wt. 397.46 g/mol. IR_{vmax} (KBr): 1106.7 (C-O-C Is), 1151.1 (C-O-C Ether), 1208.7, 1266.9 (C(O)OR), 1454.6 (aliphatic CH_2), 1542.2 (benzene), 1691.2 (C=O), 2873.7, 2939.1, 3026.6, 3065.0 (C-H stretching), and 3328.5 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 1.97 (m, 2H, $OCH_2C\underline{H}_2CH_2Ar_2$), 2.73 (t, 2H, J = 7.78 Hz, $OCH_2CH_2C\underline{H}_2Ar_2$), 3.48 (m, 1H, $OC\underline{H}_2CH_2CH_2Ar_2$), 3.63 (t, 1H, J = 7.78 Hz, IsH-6), 3.71 (m, 1H, $OCH_2CH_2CH_2Ar_2$'), 3.92-4.15 (m, 4H, IsH-6', IsH-5, IsH-1, IsH-1'), 4.38 (d, 2H, J = 6.02 Hz, CH_2), 4.54 (d, 1H, J = 4.02 Hz, IsH-3), 4.64 (t, 1H, J = 4.02 Hz, IsH-4), 5.11 (s, 1H, NH), 5.19 (d, 1H, J = 3.51 Hz, IsH-2), 7.17-7.40 (m, 10H,

5Ar_1H, 5Ar_2H). **^{13}C-NMR ppm** ($CDCl_3$): 31.21 ($OCH_2CH_2\underline{C}H_2Ar_2$), 32.11 ($OCH_2\underline{C}H_2CH_2Ar_2$), 45.06 ($CH_2$), 69.94 ($O\underline{C}H_2CH_2CH_2Ar_2$), 70.06 (IsC-6), 73.94 (IsC-1), 78.96 (IsC-2), 80.33 (IsC-5), 80.41 (IsC-4), 85.95 (IsC-3), 125.77 (Ar_2C-4), 127.51 (Ar_1C-4), 127.59 (Ar_1C-2/Ar_1C-6), 128.29 (Ar_1C-3/Ar_1C-5), 128.41 (Ar_2C-2/Ar_2C-6), 128.68 (Ar_2C-3/Ar_2C-5), 138.05 (Ar_1C-1), 141.70 (Ar_2C-1), 155.21 (CO). $C_{23}H_{27}NO_5$ requires C, 69.50; H, 6.85; N, 3.52: C, 69.46; H, 6.88; N, 3.39.

2-O-Benzyl 5-O-nitro-1,4:3,6-dianhydro-D-glucitol **150**

(2-Benzyl 5-nitro isosorbide)

Compound **150** was synthesised from IS-5-MN **62** (191.14 g/mol, 2.62 mmol, 500 mg) and excess NaH (500 mg of 65 % NaH in an oil suspension) was placed in a clean and dry three neck round bottom flask and kept under an atmosphere of nitrogen gas. Anhydrous THF was added to the flask and the mixture was stirred for 30 minutes. Excess benzyl bromide (mol. wt. 171.03 g/mol, d 1.438 g/ml, 3.36 mmol, 575 mg, 0.40 ml) was added dropwise over 15 minutes and the reaction mixture was stirred for a further 24 hours. The mixture was diluted with water (25 ml), which was added slowly to exhaust the excess NaH, extracted with ethyl acetate (3 x 25ml) and dried over anhydrous sodium sulphate (2 g). The organic portions were collected and evaporated to dryness to give a clear oil. Purification by column chromatography gave the title compound as a white crystalline product (447 mg, 61 %). M.pt. 121°C, mol. wt. 281.26 g/mol. IR_{vmax} (KBr): 1106.7 (C-O-C Is), 1150.2 (C-O-C Ether), 1281.5 and 1643.6 (ONO_2), 1440.5 (aliphatic CH_2), 1534.0 (benzene) and 2932.4, 2945.3, (C-H stretching) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.90 (m, 2H, IsH-1), 4.01 (dd, 1H, J = 2.73 and 10.92 Hz, IsH-6), 4.12 (m, 2H, IsH-2, IsH-6'), 4.54 (d, 1H, J = 4.78 Hz, IsH-3), 4.59 (s, 1H, CH_2), 5.00 (t, 1H, J = 5.47 Hz, IsH-4), 5.35 (m, 1H, IsH-5), 7.30-7.40 (m, 5H, ArH). **^{13}C-NMR ppm** ($CDCl_3$): 68.62 (CH_2), 70.93 (IsC-6), 72.79 (IsC-1), 80.89 (IsC-5), 81.04 (IsC-2), 82.03 (IsC-4), 86.44 (IsC-3), 127.28 (ArC-2/ArC-6), 127.52 (ArC-4), 128.10 (ArC-3/ArC-5), 136.92 (ArC-1). HRMS (M + 23): $C_{13}H_{15}NO_6Na$ requires 304.0798: found C, 304.0861.

2-(Benzylaminocarbonyloxy-) 5-O-nicotinoyl-1,4:3,6-dianhydro-D-glucitol **128**

(2-Benzyl carbamate 5-nicotinoyl isosorbide)

To a solution of 2-*O*-(*t*-(butyl)-dimethylsilyl-)-1,4:3,6-dianhydro-D-glucitol **145** (mol. wt. 260.40 g/mol, 1.15 mmol, 300 mg) in dry THF (50 ml) was added 1.25 mol. eq. of anhydrous nicotinoyl chloride hydrochloride (mol. wt. 178.05 g/mol, 1.44 mmol, 256 mg). The mixture was kept under an atmosphere of nitrogen and stirred at room temperature for 15 minutes. To the mixture was added four mol. eq. of triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 4.61 mmol, 465 mg, 0.64 ml) and stirring was continued for a further 24 hours. The solvent was evaporated under vacuum and to give a white residue, which was dissolved in a saturated aqueous solution of Na_2CO_3 (25 ml) and extracted with DCM (3 x 25 ml). The collected organic portions were dried over anhydrous sodium sulphate (5 g) and evaporated to dryness to give a clear oil. The oil was diluted with THF (10 ml) and 1.1 mol. eq. of tetrabutylammonium fluoride (mol. wt. 261.47g/mol, 0.75 mmol, 197 mg, 0.75 ml of a 1M solution) was added and the mixture was stirred at room temperature for 15 minutes. The solution was evaporated to dryness under vacuum giving a brown oil. DCM (10 ml) was added to the reaction vessel and the solution was stirred at room temperature. To the mixture was added triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 0.75 mmol, 76 mg, 0.11 ml), benzyl isocyanate (mol. wt. 133.15 g/mol, d 1.078 g/ml, 0.75 mmol, 100 mg, 0.09 ml) and DMAP (mol. wt. 122.17 g/mol, 0.25 mmol, 30 mg). The mixture was heated to 105°C for two hours. The mixture was cooled upon completion of the reaction and methanol (10ml) was added to remove excess isocyanate. The mixture was heated for a further 15 minutes at 105°C and cooled to room temperature. All organic solvent was removed under vacuum giving a clear oil to which was added DCM (20 ml) and washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml), saturated brine solution (20 ml) and dried over anhydrous sodium sulphate (2 g). The solution was filtered into a round bottom flask, which was evaporated under vacuum to give a white crystalline compound. Purification by column chromatography using hexane and ethyl acetate (3:1 and 1:1) as eluent, afforded compound **128** as a white crystalline compound (188 mg, 42 %). M.pt. 136°C, mol. wt. 384.38 g/mol. $IR_{\nu max}$ (KBr): 1092.8 (C-O-C), 1134.9, 1247.8, 1283.4 (C(O)OR), 1455.4 (aliphatic CH_2), 1533.7 (benzene), 1592.3 (C=N), 1726.7 (C=O), 2875.6, 2927.5, 3063.4 (C-H stretching), and 3342.3 (2°

amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.93-4.07 (m, 4H, IsH-1, IsH-6), 4.37 (d, 2H, J = 6.03 Hz, CH_2), 4.55 (d, 1H, J = 4.52 Hz, IsH-3), 4.94 (t, 1H, J = 5.02 Hz, IsH-4), 5.22 (d, 1H, J = 2.01 Hz, IsH-2), 5.39 (m, 2H, IsH-5, NH), 7.20-7.37 (m, 5H, $5Ar_1H$), 7.38-7.48 (s, 1H, Ar_2H -3), 8.32 (d, 1H, J = 7.53 Hz, Ar_2H-2) 8.80 (s, 1H, Ar_2H-4), 9.35 (s, 1H, Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.64 (CH_2), 70.17 (IsC-6), 73.18 (IsC-1), 74.50 (IsC-5), 77.85 (IsC-2), 80.44 (IsC-4), 85.82 (IsC-3), 123.12 (Ar_2C-3), 126.78 (Ar_2C-1), 127.10 (Ar_1C-4), 127.15 (Ar_1C-2/Ar_1C-6), 128.25 (Ar_1C-3/Ar_1C-5), 136.74 (Ar_2C-2), 137.65 (Ar_1C-1), 150.47 (Ar_2C-6), 153.23 (Ar_2C-4), 154.81 (-C(O)N-), 165.36 (-C(O)Ar_2). $C_{20}H_{20}N_2O_6$ requires C, 62.49; H, 5.24; N, 7.29; found C, 62.42; H, 5.22; N, 6.96.

2-(Benzylaminocarbonyloxy-) 5-O-isonicotinoyl-1,4:3,6-dianhydro-D-glucitol **129**
(2-Benzyl carbamate 5-isonicotinoyl isosorbide)

Compound **129** was prepared by using the same procedure as for 2-(benzylaminocarbonyloxy-) 5-*O*-nicotinoyl-1,4:3,6-dianhydro-D-glucitol **128** above. Anhydrous isonicotinoyl chloride hydrochloride (mol. wt. 178.05 g/mol, 1.44 mmol, 256 mg) was used as reagent. The procedure afforded the title compound as a clear gum, which resisted recrystallisation (162 mg, 37 %). M.pt. 146°C, mol. wt. 384.38 g/mol. IR_{vmax} (NaCl): 1092.8 (C-O-C), 1134.9, 1247.8, 1283.4 (C(O)OR), 1455.4 (aliphatic CH_2), 1533.7 (benzene), 1592.3 (C=N), 1726.7 (C=O), 2875.6, 2927.5, 3063.4 (C-H stretching), and 3342.3 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.94-4.09 (m, 4H, IsH-1, IsH-1', IsH-6, IsH-6'), 4.35-4.43 (d, 2H, J = 6.02 Hz, CH_2), 4.54-4.60 (d, 1H, J = 4.52 Hz, IsH-3), 4.93-5.00 (t, 1H, J = 5.02 Hz, IsH-4), 5.10-5.20 (s, 1H, NH), 5.19-5.25 (d, 1H, J = 2.51 Hz, IsH-2), 5.38-5.47 (q, 1H, J = 5.02 and 10.54 Hz, IsH-5), 7.23-7.40 (m, 5H, $5Ar_1H$), 7.85-7.93 (d, 2H, J = 6.02 Hz, Ar_2H-2/Ar_2H-6), 8.78-8.88 (d, 2H, J = 5.52 Hz, Ar_2H-3/Ar_2H-5). **^{13}C-NMR ppm** ($CDCl_3$): 45.10 (CH_2), 70.58 (IsC-6), 73.64 (IsC-1), 75.22 (IsC-5), 78.27 (IsC-2), 80.83 (IsC-4), 86.24 (IsC-3), 122.86 (Ar_2C-2/Ar_2C-6), 127.52 (Ar_1C-4), 127.64 (Ar_1C-2/Ar_1C-6), 128.72 (ArC-3/ArC-5), 136.62 (Ar_2C-1), 137.96 (Ar_1C-1), 150.69 (Ar_2C-5/Ar_2C-3), 158.05 (-C(O)N-), 164.48 (-C(O)Ar_2). HRMS(M + 23); $C_{21}H_{23}NO_7SNa$ requires 407.1233; found, 407.1219.

2,5-O-(Di-toluenesulfonyl)-1,4:3,6-dianhydro-D-mannitol **19**

(Di-tosyl isomannide)

2,5-*O*-(Di-toluenesulfonyl)-1,4:3,6-dianhydro-D-mannitol **19** was synthesised from isomannide (146.14 g/mol, 68.43 mmol, 10 g) with *p*-toluenesulfonyl chloride (mol. wt. 190.5 g/mol, 171.07 mmol, 32.6 g) as reagent. The reaction vessel was cooled to 0°C and followed the method outlined for the preparation of esters. Recrystallisation from ethanol yielded compound **19** as a white crystalline product (28 g, 90 %). Mol. wt. 454.52 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 2.45 (s, 6H, $(CH_3)_2$), 3.71 (dd, 2H, J = 8.03 and 9.53 Hz, IsH-1, IsH-6), 3.93 (dd, 2H, J = 6.52 and 9.54 Hz, IsH-1', IsH-6'), 4.46 (m, 2H, IsH-3, IsH-4), 4.84 (m, 2H, IsH-2, IsH-5), 7.34 (d, 4H, J = 8.54, Ar_1H-3/Ar_1H-5, Ar_2H-3/Ar_2H-5), 7.79 (d, 4H, J = 8.54, Ar_1H-2/Ar_1H-6, Ar_2H-2/Ar_2H-6), **^{13}C-NMR ppm** ($CDCl_3$): 21.25 $(CH_3)_2$, 69.63 (IsC-1, IsC-6), 77.42 (IsC-2, IsC-5), 79.48 (IsC-3, IsC-4), 127.51 (Ar_1C-2/Ar_1C-6, Ar_2C-2/Ar_2C-6), 129.49 (Ar_1C-3/Ar_1C-5, Ar_2C-3/Ar_2C-5) 132.50 (Ar_1C-1, Ar_2C-1), 144.89 (Ar_1C-4, Ar_2C-4).

2,5-O-(Di-acetyl)-1,4:3,6-dianhydro-L-iditol **20**

(Di-acetyl isoiodide)

To a solution of 2,5-*O*-(di-toluenesulfonyl)-1,4:3,6-dianhydro-D-mannitol **19** (mol.wt. 454.52 g/mol, 44 mmol, 20 g) in of DMSO (50 ml) was added excess sodium acetate (mol. wt. 62.03 g/mol, 183 mmol, 15 g). The mixture was heated to 125°C and stirred for 48 hours. The reaction was monitored by TLC (1:1 ethyl acetate: hexane) to monitor the disappearance of the starting material. The mixture was diluted with water (200 ml) was washed with three equal volumes of ethyl acetate and dried over anhydrous sodium sulphate (10 g). The organic fractions were collected and evaporated to dryness to give a brown oil. Purification by column chromatography, using hexane and ethyl acetate (3:1) as eluent, afforded the title compound a clear oil, which crystallized upon standing (4.7 g, 47 %) of a white crystalline product. Mol. wt. 230.21 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 2.05-2.11 (s, 6H, $(CH_3)_2$), 3.83-4.00 (m, 4H, IsH-1, IsH-6), 4.64 (s, 2H, IsH-3, IsH-4), 5.20 (d, 2H, J = 2.51 Hz, IsH-2, IsH-5). **^{13}C-NMR ppm** ($CDCl_3$): 20.45 $(CH_3)_2$), 72.10 (IsC-1,

IsC-6), 77.13 (IsC-2, IsC-5), 84.88 (IsC-3, IsC-4), 169.47 ($(CO)_2$).

1,4:3,6-Dianhydro-L-iditol **3**
(Isoiodide)

2,5-*O*-(Di-acetyl) 1,4:3,6-dianhydro-L-iditol **20** (mol.wt. 230.21 g/mol, 17.37 mmol, 4 g) was stirred in methanol: 5 % NaOH (1:1, 100 ml) for one hour. The solution was naturalized with HCl and evaporated to dryness on a high vacuum rotary evaporator. The crude product was dissolved in chloroform. Sodium sulphate (5 g) was added to absorb any remaining water. The solution was filtered and evaporated under vacuum to give a clear gum, which resisted crystallization. The procedure gave a yield of 2 g (80 %). **^{1}H-NMR** δ ($CDCl_3$): 2.80-3.20 (s, 2H, OH), 3.65-3.78 (m, 4H, IsH-1, IsH-6), 4.16 (m, 2H, IsH-2, IsH-5). 4.45 (s, 2H, IsH-3, IsH-4). **^{13}C-NMR ppm** ($CDCl_3$): 73.80 (IsC-1, IsC-6), 75.17 (IsC-2, IsC-5), 87.08 (IsC-3, IsC-4).

5-O-Toluenesulfonyl-1,4:3,6-dianhydro-L-iditol **157**
(5-Tosyl isoiodide)

Compound **157** was synthesised from isoiodide **3** (146.14 g/mol, 6.84 mmol, 1 g) with *p*-toluenesulfonyl chloride (mol. wt. 190.5 g/mol, 6.84 mmol, 1.3 g) as reagent in acetone (20 ml). The reaction vessel was cooled to 0°C and followed the method outlined for the preparation of esters. Recrystallisation from ethanol yielded the title compound as a white crystalline product (1.27 g, 62 %). Mol. wt. 300.33 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 2.48 (s, 3H, CH_3), 3.76-3.90 (m, 4H, IsH-6, IsH-1, IsH-1'), 3.98 (d, 1H, J = 11.04 Hz, IsH-6'), 4.34 (s, 1H, IsH-2), 4.56 (d, 1H, J = 4.31 Hz, IsH-3), 4.65 (d, 1H, J = 3.51 Hz, IsH-4), 4.90 (m, 1H, J = 3.52 Hz, IsH-5), 7.39 (d, 2H, J = 8.03, ArH-3/ArH-5), 7.82 (d, 2H, J = 8.03, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 21.69 (CH_3), 72.03 (IsC-6), 74.72 (IsC-1), 75.71 (IsC-2), 83.08 (IsC-5), 84.81 (IsC-4), 87.57 (IsC-3), 127.84 (ArC-2/ArC-6), 130.09 (ArC-3/ArC-5), 133.10 (ArC-1), 145.37 (ArC-4).

5-Deoxy-5-azido-1,4:3,6-dianhydro-D-glucitol **155**

(5-Azido isosorbide)

5-*O*-(Toluenesulfonyl)-1,4:3,6-dianhydro-L-iditol **157** (mol. wt. 300.3 g/mol, 4.16 mmol, 1.25 g) was dissolved a minimum volume of DMSO (7.5 ml). Excess sodium azide (mol. wt. 65.01 g/mol, 20.8 mmol, 1.36 g) was added to form a slurry. The mixture was heated to 125°C and stirred for 24 hours. The mixture was diluted with ethyl acetate (50 ml) was washed with three equal volumes of water (50 ml x 3) and dried over anhydrous sodium sulphate (5 g). The solution was filtered into a round bottom flask, and the organic solvent was evaporated under vacuum to give a brown oil. The crude product was purified by flash chromatography using hexane and ethyl acetate (3:1) as eluent giving a white solid, which was recrystallised from hot ethyl acetate and hexane to afford the title compound as a white crystalline product (514 mg, 72 %). Mol. wt. 171.15 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 3.13 (d, 1H, J = 4.52 Hz, OH), 3.64 (m, 1H, IsH-5), 3.85-4.00 (m, 4H, IsH-1, IsH-6), 4.32 (s, 1H, IsH-2), 4.42 (d, 1H, J = 4.02 Hz, IsH-3), 4.82 (d, 1H, J = 4.52 Hz, IsH-4). **^{13}C-NMR ppm** ($CDCl_3$): 61.80 (IsC-5), 69.25 (IsC-6), 75.43 (IsC-1), 75.86 (IsC-2), 82.04 (IsC-4), 88.21 (IsC-3).

2-(Benzylaminocarbonyloxy-) 5-deoxy-5-azido-1,4:3,6-dianhydro-D-glucitol **154**

(2-Benzyl carbamate 5-benzylamide isosorbide)

To a solution of 5-deoxy-5-azido -1,4:3,6-dianhydro-D-glucitol **155** (Mol. wt. 171.15 g/mol, 2.92 mmol, 500 mg), in DCM was added triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 3.21 mmol, 325 mg, 0.45 ml), benzyl isocyanate (mol. wt. 133.15 g/mol, d 1.078g/ml, 3.21 mmol, 428 mg, 0.40 ml) and 10 % DMAP (mol.wt. 122.17, 0.41 mmol, 50 mg). The mixture was heated to 105°C for two hours and the reaction was monitored by TLC (1:1 ethyl acetate: methanol) to observe the starting material. The mixture was cooled upon completion of the reaction and methanol (10 ml) was added to remove excess isocyanate. The mixture was heated for a further 15 minutes at 105°C and cooled to room temperature. All organic solvent was removed under vacuum giving a clear oil to which was purified by column chromatography using hexane and ethyl acetate (3:1 and

1:1) as eluent to isolate the primary product as a white crystalline solid. The white crystalline solid was dissolved in ethyl acetate: methanol (1:1, 15 ml) in a 50 ml round bottom flask. A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas and stirred for 24 hours. The palladium catalyst was removed by filtration and the filtrate was collected and evaporated under vacuum to give a clear oil. This oil was diluted with DCM (10 ml) and excess triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 3.59 mmol, 363 mg, 0.5 ml), benzoyl chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 3.59 mmol, 505 mg, 0.42 ml) and DMAP (mol.wt. 122.17, 0.41 mmol, 50 mg) were added to the reaction vessel. The reaction mixture was stirred for four hours. DCM (10 ml) was added to the reaction vessel and the mixture was washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml) saturated brine solution (20 ml) and dried over anhydrous sodium sulphate (1 g). Purification by column chromatography, using hexane and ethyl acetate (2:1) as eluent afforded compound **154** as a white crystalline (219 mg, 20 %). M.pt. 164°C, mol. wt. 382.41 g/mol. IR_{vmax} (KBr): 1102.0 (C-O-C), 1136.2, 1274.2 (C(O)OR), 1530.0 (benzene), 1698.6, 1720.1 (C=O), 2933.5, 3032.2 (C-H stretching), and 3326.5, 3350.6 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.45 (t, 1H, J = 8.79 Hz, IsH-6), 4.02-4.11 (m, 2H, IsH-1, IsH-1'), 4.32 (t, 1H, J = 8.03 Hz, IsH-6'), 4.39 (d, 2H, J = 6.02 Hz, CH_2), 4.55-4.77 (m, 3H, IsH-3, IsH-4, IsH-5), 5.15 (s, 1H, NH), 5.24 (s, 1H, IsH-2), 6.74 (d, 1H, J = 7.02 Hz, NH-benzylamide), 7.23-7.40 (m, 5H, $5Ar_1H$), 7.47 (m, 2H, Ar_2H-3/Ar_2H-5), 7.53 (m, 1H, Ar_2H-4), 7.81 (m, 2H, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.73 (CH_2), 52.83 (IsC-5), 70.77 (IsC-6), 73.44 (IsC-1), 78.55 (IsC-2), 81.36 (IsC-4), 85.37 (IsC-2), 126.59 (Ar_2C-2/Ar_2C-6), 127.14 (Ar_1C-4), 127.26 (Ar_1C-2/Ar_1C-6), 128.19 (Ar_2C-3/Ar_2C-5), 128.32 (Ar_1C-3/Ar_1C-5), 131.36 (Ar_2C-4), 133.35 (Ar_2C-1), 137.53 (Ar_1C-1), 154.65 (-$Ar_1CH_2NC(O)O$-), 166.33 (-$NC(O)Ar_2$). HRMS (M + 23); $C_{21}H_{22}N_2O_5Na$ requires 405.1427; found, 405.1367.

2-benzyloxy-benzoic acid **165**

Salicylic acid (mol. wt. 138.12 g/mol, 72.4 mmol, 10 g) and potassium carbonate (mol. wt. 157.17g/mol, 159 mmol, 25 g) were dissolved in acetone (300 ml) in a two-neck

round bottom flask. The mixture was kept under an atmosphere of nitrogen and heated under reflux for 45 minutes. The mixture was then cooled and 2.5 mol. eq. of benzyl bromide (mol. wt. 171.03g/mol, d 1.438 g/ml, 148 mmol, 15 g, 22 ml) was added. The mixture was again heated under reflux and was stirred for 24 hours. The reaction was monitored by TLC (3:1 hexane: ethyl acetate) to observe the disappearance of the acid. The solution was cooled and filtered and the solution was evaporated to dryness giving a yellow oil. The oil was diluted with methanol: 5 % NaOH (1:1, 150ml) and heated under reflux for two hours. The methanol was then evaporated from the solution under vacuum and then remaining aqueous solution was acidified with HCl and washed with ethyl acetate (3 x 40ml). The aqueous layer was evaporated to dryness and the residue was washed with DCM (40 ml) to extract any remaining product in order to maximize the yield. The combined organic solutions were dried over anhydrous sodium sulfate (10 g) and evaporated under vacuum to give compound **165** as a white crystalline product (12 g, 73 %). This compound, which was used to synthesize compounds **162** and **181**, was not characterized. 3-Benzyloxybenzoic acid **166** was synthesised by the same process from 3-hydroxyl benzoic acid giving a yield of 87 %

2-(Benzylaminocarbonyloxy-) 5-O-salicyloyl-1,4:3,6-dianhydro-D-glucitol **162**
(2-Benzyl carbamate 5-salicylate isosorbide)

2-benzyloxy-benzoic acid **165** (mol.wt. 228.24 g/mol, 2.19 mmol, 500 mg) was dissolved in chloroform (20 ml) in a 100 ml round bottom flask and cooled to -20°C in a Dewer flask. Two mol. eq. of compound of 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 4.38 mmol 1.2 g,), 1.1 mol. eq. of DCC (mol. wt. 206.33g/mol, 2.41 mmol, 497 mg) and DMAP (mol. wt. 122.17g/mol, 2.41 mmol, 295 mg) were added. The mixture was stirred overnight whilst the reaction vessel was allowed to return to room temperature. The reaction was monitored by TLC (2:1 hexane: ethyl acetate). The organic solvent was evaporated under vacuum, yielding a crude crystalline compound. DCM (10 ml) was added to the reaction vessel and the unwanted urea precipitate was collected by filtration. The filtrate was washed with 1M HCl (20 ml), 5 % $NaHCO_3$ (20 ml) saturated brine solution (20 ml) and dried over anhydrous sodium

sulfate (1 g). The solution was filtered into a round bottom flask and was evaporated under vacuum to give a white crystalline solid. The solid was dissolved in ethyl acetate: methanol (1:1, 20 ml). A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas and stirred for 24 hours. TLC showed the formation of a single product. The palladium catalyst by filtration and the solvent was evaporated under vacuum The crude product was dissolved in DCM and purified by flash chromatography using hexane and ethyl acetate (1:1) as eluent giving of the title compound as a white crystalline product (486 mg, 56 %). M.pt. 120°C, mol. wt. 399.35 g/mol. IR_{vmax} (KBr): 1080.9 (C-O-C), 1136.8, 1216.1, 1245.1, 1257.1 (C(O)OR), 1455.8 (aliphatic CH_2), 1539.3 (benzene), 1689.5, 1698.9 (C=O), 2873.0, 2920.4, 2990.5, 3048.9 (C-H stretching), and 3325.7 (2° amine) 3410.5 (OH) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.94-4.10 (m, 4H, IsH-1, IsH-6), 4.34-4.42 (d, 2H, J = 6.02 Hz, CH_2), 4.56 (d, 1H, J = 5.02 Hz, IsH-3), 4.96 (t, 1H, J = 5.27 Hz, IsH-4), 5.12 (t, 1H, J = 5.27 Hz, NH), 5.22 (d, 1H, J = 3.01 Hz, IsH-2), 5.42 (q, 1H, J = 5.52 and 10.04 Hz, IsH-5), 6.89-6.96 (m, 1H, Ar_2H-5), 7.01 (dd, 1H, J = 1.01 and 8.54 Hz, Ar_2H-3), 7.23-7.40 (m, 5H, Ar_1H), 7.51 (m, 1H, Ar_2H-4), 7.89 (dd, 1H, J = 1.5 and 8.03 Hz, Ar_2H-6), 10.60 (s, 1H, OH). **^{13}C-NMR ppm** ($CDCl_3$): 44.67 (CH_2), 70.27 (IsC-6), 73.24 (IsC-1), 74.36 (IsC-5), 77.87 (IsC-2), 80.57 (IsC-4), 85.83 (IsC-3), 111.45 (Ar_2C-1), 117.26 (Ar_2C-3), 118.89 (Ar_2C-5), 127.10 (Ar_1C-4), 127.21 (Ar_1C-2/Ar_1C-6), 128.29 (Ar_1C-3/Ar_1C-5), 129.50 (Ar_2C-6), 135.63 (Ar_2C-4), 137.58 (Ar_2C-1), 154.73 (-C(O)N-), 161.29 (Ar_2C-2), 168.90 (-C(O)Ar_2). $C_{21}H_{21}NO_7$ requires C, 63.15; H, 5.30; N, 3.51: found C, 62.96; H, 5.29; N, 3.41.

*2-(Benzylaminocarbonyloxy-) 5-O-(*m*-hydroxybenzoyl)-1,4:3,6-dianhydro-D-glucitol* **171**
(2-Benzyl carbamate 5-(*m*-hydroxybenzoyl) isosorbide)

Compound **171** was synthesised from 3-benzyloxy-benzoic acid **166** (mol.wt. 228.24 g/mol, 2.19 mmol, 500 mg) and two mol. eq. of 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 4.38 mmol 1.2 g,) using the same method as described for the synthesis of compound **162**. The procedure afforded compound **171** as a clear gum, which resisted recrystallisation (270 mg, 31 %). Mol.wt. 399.35 g/mol. IR_{vmax} (NaCl): 1095.1 (C-O-C), 1227.3, 1288.5 (C(O)OR), 1453.9 (aliphatic CH_2), 1528.9 (benzene), 1701.9, 1716.5 (C=O), 2876.3, 2931.7 (C-H stretching), and 3342.4 (2° amine, OH) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.93-4.05 (m, 4H, IsH-1, IsH-6), 4.36 (d, 2H, J = 6.02 Hz, CH_2), 4.54 (d, 1H, J = 4.52 Hz, IsH-3), 4.92 (t, 1H, J = 5.02 Hz, IsH-4), 5.21 (s, 1H, IsH-2), 5.32 (q, 1H, J = 5.52 and 10.54 Hz, IsH-5), 5.53 (t, 1H, J = 6.03 Hz, NH), 7.05 (dd, J = 2.01 and 8.53 Hz, 1H, Ar_2H-4), 7.14 (s, 1H, Ar_2H-5), 7.20-7.37 (m, 5H, $5Ar_1H$), 7.55 (s, 1H, Ar_2H-2), 7.59 (d, J = 7.53 Hz, 1H, Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.64 (CH_2), 70.32 (IsC-6), 73.15 (IsC-1), 74.07 (IsC-5), 77.93 (IsC-2), 80.61 (IsC-4), 85.64 (IsC-3), 115.87 (Ar_2C-2), 120.40 (Ar_2C-4), 121.15 (Ar_2C-6), 127.11 (Ar_1C-4), 127.17 (Ar_1C-2/Ar_1C-6), 128.25 (Ar_1C-3/Ar_1C-5), 129.31 (Ar_2C-5), 130.02 (Ar_2C-1), 137.52 (Ar_2C-1), 155.07 (-C(O)N-), 156.06 (Ar_2C-3), 165.62 (-C(O)Ar_2). HRMS (M + 23); $C_{21}H_{21}NO_7Na$ requires 422.1216; found, 422.1215

*2-(Benzylaminocarbonyloxy-) 5-O-(*p*-hydroxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **172**
(2-Benzyl carbamate 5-(*p*-hydroxybenzoyl) isosorbide)

Compound **172** was synthesised from 4-benzyloxy-benzoic acid (mol.wt. 228.24 g/mol, 2.19 mmol, 500 mg) and of 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 4.38 mmol 1.2 g) using the same method as described for the synthesis of compound **162**. The procedure afforded compound **172** as a clear gum, which resisted recrystallisation (242 mg, 28 %). Mol. wt. 399.35 g/mol. IR_{vmax} (NaCl): 1096.2 (C-O-C), 1165.2, 1270.9 (C(O)OR), 1454.5 (aliphatic CH_2), 1515.0 (benzene), 1701.8 (C=O), 2877.6, 2929.9 (C-H stretching), and 3332.4 (2° amine, OH) cm^{-1}. **^{1}H-**

NMR δ ($CDCl_3$): 3.94-4.15 (m, 4H, IsH-1, IsH-6), 4.33-4.41 (d, 2H, J = 5.52 Hz, CH_2), 4.54 (d, 1H, J = 4.52 Hz, IsH-3), 4.94 (t, 1H, J = 5.02 Hz, IsH-4), 5.21 (s, 1H, IsH-2), 5.34 (q, 1H, J = 5.02 and 10.04 Hz, IsH-5), 5.42 (t, 1H, J = 5.78 Hz, NH), 6.85 (d, 2H, J = 9.04, Ar_2H-3/ Ar_2H-5), 7.20-7.38 (m, 5H, $5Ar_1H$), 7.93 (d, 2H, J = 8.53, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.65 (CH_2), 70.41 (IsC-6), 73.16 (IsC-1), 73.68 (IsC-5), 78.09 (IsC-2), 80.73 (IsC-4), 85.65 (IsC-3), 114.97 (Ar_2C-3/Ar_2C-5), 120.49 (Ar_2C-1), 127.08 (Ar_1C-4), 127.22 (Ar_1C-2/Ar_1C-6), 128.29 (Ar_1C-3/Ar_1C-5), 131.65 (Ar_2C-2/Ar_2C-6), 137.42 (Ar_2C-1), 155.11 (-C(O)N-), 160.84 (Ar_2C-4), 165.56 (-C(O)Ar_2). HRMS (M + 23); $C_{21}H_{21}NO_7Na$ requires 422.1216; found, 422.1207.

2-(Benzylaminocarbonyloxy-) 5-O-(o-nitro-benzoyl)-1,4:3,6-dianhydro-D-glucitol **173**
(2-Benzyl carbamate 5-(*o*-nitro-benzoyl)-isosorbide)

Compound **173** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1.79 mmol, 500 mg) with *o*-nitrobenzoyl chloride (mol. wt. 185.50 g/mol, d 1.404 g/ml, 1.97 mmol, 365 mg, 0.26 ml) as reagent, using the method outlined for the preparation of esters. The procedure yielded the title compound as a clear oil, which resisted recrystallisation (681 mg, 86 %). Mol. wt. 428.39 g/mol. IR_{vmax} (NaCl): 1094.3 (C-O-C), 1134.3, 1254.7, 1286.1 (C(O)OR), 1350.7 (C-NO_2), 1455.3 (aliphatic CH_2), 1534.6 (benzene), 1729.3 (C=O), 2877.5, 2929.1, 3065.6 (C-H stretching), and 3338.9 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.91 (m, 2H, IsH-6, IsH-6'), 4.06 (m, 2H, IsH-1, IsH-1'), 4.38 (d, 2H, J = 6.03 Hz, CH_2), 4.56 (d, 1H, J = 4.52 Hz, IsH-3), 4.93 (t, 1H, J = 5.02 Hz, IsH-4), 5.18 (m, 2H, IsH-2, NH), 5.40 (q, 1H, J = 5.52 and 11.04 Hz, IsH-5), 7.23-7.40 (m, 5H, $5Ar_1H$), 7.62-7.82 (m, 3H, Ar_2H-3/Ar_2H-4/Ar_2H-5), 7.98 (d, 1H, J = 7.53 Hz, Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.66 (CH_2), 69.60 (IsC-6), 73.22 (IsC-1), 75.24 (IsC-5), 77.94 (IsC-2), 80.28 (IsC-4), 85.66 (IsC-3), 123.60 (Ar_2C-3), 126.73 (Ar_2C-1), 127.11 (Ar_1C-4), 127.20 (Ar_1C-2/Ar_1C-6), 128.29 (Ar_1C-3/Ar_1C-5), 129.48 (Ar_2C-5), 131.50 (Ar_2C-6), 132.71 (Ar_2C-4), 137.61 (Ar_1C-1), 154.74 (-C(O)N-), 156.54 (Ar_2C-2), 164.48 (-C(O)Ar_2). HRMS (M + 23); $C_{21}H_{20}N_2O_8Na$ requires 451.1117: found, 451.1132.

2-(Benzylaminocarbonyloxy-) 5-O-(o-nitroso-benzoyl)-1,4:3,6-dianhydro-D-glucitol **176** (2-benzyl carbamate 5-(*o*-nitroso-benzoyl) isosorbide) and *2-(Benzylaminocarbonyloxy-) 5-O-(o-amino-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **178** (2-benzyl carbamate 5-(*o*-amino-benzoyl) isosorbide)

Compounds **176** and **178** were synthesised as two products of the same reaction. 2-(Benzylaminocarbonyloxy-) 5-*O*-(*o*-nitro-benzoyl)-1,4:3,6-dianhydro-D-glucitol **173** (mol. wt. 428.12 g/mol, 0.67 mmol, 300 mg) was dissolved in ethyl acetate: methanol (1:1, 15 ml) in a 50 ml round bottom flask. A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas and stirred for 24 hours. The progress of the reaction was monitored by TLC (1:1 ethyl acetate: hexane) to observe the disappearance of the starting material. TLC showed the formation of two products. The palladium catalyst was removed by filtration and the filtrate was collected and evaporated under vacuum. Purification by column chromatography, using ethyl acetate and hexane (3:1, 2:1, 1:1) as eluent, separated the compounds **176** and **178** as clear oils.

2-(Benzylaminocarbonyloxy-) 5-*O*-(*o*-nitroso-benzoyl)-1,4:3,6-dianhydro-D-glucitol **176** was obtained with a yield of 70 mg (25 %). Rf = 0.78 (m.p. ethyl acetate: hexane 1:1), mol. wt. 412.39 g/mol. IR_{vmax} (NaCl): 1095.4 (C-O-C), 1131.8, 1175.0, 1239.7, 1258.5 (C(O)OR), 1455.5 (aliphatic CH_2), 1519.9 (benzene), 1579.9 (N=O), 1687.9, 1726.9 (C=O), 2873.3, 2932.5, 3030.7, 3063.9 (C-H stretching), and 3380.6 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.96-4.08 (m, 4H, IsH-1, IsH-6), 4.39 (d, 2H, J = 6.02 Hz, CH_2), 4.56 (d, 1H, J = 4.51 Hz, IsH-3), 4.94 (t, 1H, J = 5.27 Hz, IsH-4), 5.08 (s, 1H, NH) 5.24 (s, 1H, IsH-2), 5.33 (q, 1H, J = 5.52 and 10.54 Hz, IsH-5), 6.52 (t, 1H, J = 7.53 Hz Ar_2H-3), 6.69 (d, 1H, J = 8.53 Hz, Ar_2H-5), 7.23-7.45 (m, 5H, $5Ar_1H$), 7.62 (s, 1H, Ar_2H-4), 7.94 (dd, 1H, J = 2.01 and 8.03 Hz, Ar_2H-2). HRMS (M + 23): $C_{21}H_{20}N_2O_7Na$ requires 435.1169; found, 435.1270.

2-(Benzylaminocarbonyloxy-) 5-*O*-(*o*-amino-benzoyl)-1,4:3,6-dianhydro-D-glucitol **178** was obtained with a yield of 121 mg (45 %). Rf = 0.71 (m.p. ethyl acetate: hexane 1:1),

mol. wt. 398.41 g/mol. IR_{vmax} (NaCl): 1096.8 (C-O-C), 1161.9, 1244.1 (C(O)OR), 1455.6 (aliphatic CH_2), 1528.2 (benzene), 1589.6 (N-H bending), 1694.1, 1720.9 (C=O), 2876.6, 2931.3, 3031.5, 3062.4 (C-H stretching), and 3367.4 (2° amine) 3475.5 (N-H stretching) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.97 (m, 4H, IsH-1, IsH-6), 4.35 (d, 2H, J = 6.02 Hz, CH_2), 4.53 (d, 1H, J = 4.52 Hz, IsH-3), 4.89 (t, 1H, J = 5.02 Hz, IsH-4), 5.20 (s, 1H, IsH-2), 5.31 (q, 1H, J = 5.53 and 10.55 Hz, IsH-5), 5.43 (t, 1H, J = 5.77 Hz, NH), 5.75 (s, 2H, NH_2), 6.65 (m, 2H, Ar_2H-3/Ar_2H-5), 7.19-7.37 (m, 6H, Ar_1H, Ar_2H-4), 7.89 (dd, 1H, J = 8.03 Hz, Ar_2H-2). **^{13}C-NMR ppm** ($CDCl_3$): 44.59 (CH_2), 70.32 (IsC-6), 73.12 (IsC-1), 73.44 (IsC-5), 78.02 (IsC-2), 80.65 (IsC-4), 85.69 (IsC-3), 109.48 (Ar_2C-1), 115.84 (Ar_2C-3), 116.32 (Ar_2C-5), 127.09(Ar_1C-4), 127.11 (Ar_1C-2/Ar_1C-6), 128.24 (Ar_1C-3/Ar_1C-5), 130.85 (Ar_2C-6), 134.02 (Ar_2C-4), 137.78 (Ar_1C-1), 150.31 (Ar_2C-2), 154.92 (-C(O)N-), 166.85 (-C(O)Ar_2). HRMS (M + 23): $C_{21}H_{22}N_2O_6Na$ requires 421.1393; found, 421.1376.

*2-(Benzylaminocarbonyloxy-) 5-O-(*m*-nitro-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **174**
(2-Benzyl carbamate 5-(*m*-nitro-benzoyl) isosorbide)

Compound **174** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1.79 mmol, 500 mg) with *m*-nitrobenzoyl chloride (mol. wt. 185.50 g/mol, 1.97 mmol, 365 mg) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot methanol to afforded the title compounds as a white crystalline product (718 mg, 90 %). M.pt. 126°C, mol.wt. 428.39 g/mol. IR_{vmax} (KBr): 1092.3 (C-O-C), 1134.7, 1257.9, 1276.1 (C(O)OR), 1358.3 (C-NO_2), 1465.3 (aliphatic CH_2), 1537.8 (benzene), 1727.2 (C=O), 2889.9, 2954.7 3063.9 (C-H stretching), and 3337.9 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.96-4.11 (m, 4H, IsH-1, IsH-6), 4.39 (d, 2H, J = 6.02 Hz, CH_2), 4.58 (d, 1H, J = 5.02 Hz, IsH-3), 4.98 (t, 1H, J = 5.02 Hz, IsH-4), 5.13 (t, 1H, J = 5.52, NH), 5.25 (d, 1H, J = 2.51, IsH-2), 5.43 (q, 1H, J = 5.52 and 10.54 Hz, IsH-5), 7.23-7.40 (m, 5H, Ar_1H), 7.69 (t, 1H, J = 8.04, Ar_2H-5), 8.40 (m, 1H, Ar_2H-4), 8.47 (m, 1H, Ar_2H-6), 8.90 (s, 1H, Ar_2H-2). **^{13}C-NMR ppm** ($CDCl_3$): 44.68 (CH_2), 70.09 (IsC-6), 73.24 (IsC-1), 74.96 (IsC-5), 77.88 (IsC-2), 80.40 (IsC-4), 85.85 (IsC-3), 124.28 (Ar2C-2), 127.10 (Ar1C-4), 127.22 (Ar1C-2/Ar1C-6), 127.30

(Ar_2C-4), 128.30 (Ar_1C-3/Ar_1C-5), 129.34 (Ar_2C-6), 130.80 (Ar_2C-1), 134.93 (Ar_2C-5), 137.56 (Ar_1C-1), 147.88 (Ar_2C-3), 154.71 (-OC(O)N-), 163.42 (-OC(O)Ar_2). HRMS (M + 23): $C_{21}H_{22}N_2O_6$ requires 451.1117; found, 451.1104

*2-(Benzylaminocarbonyloxy-) 5-O-(*m*-amino-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **179**
(2-Benzyl carbamate 5-(*m*-amino-benzoyl) isosorbide)

2-(Benzylaminocarbonyloxy-) 5-*O*-*m*-nitro-benzoyl-1,4:3,6-dianhydro-D-glucitol **174** (mol. wt. 428.12 g/mol, 0.67 mmol, 300 mg) was dissolved in ethyl acetate: methanol (1:1, 15 ml) in a 50 ml round bottom flask. A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas and stirred for 24 hours. The progress of the reaction was monitored by TLC (1:1 ethyl acetate: hexane) to observe the disappearance of the starting material. The palladium catalyst was removed by filtration and the filtrate was collected and evaporated under vacuum. Purification by column chromatography, using ethyl acetate and hexane (3:1, 2:1, 1:1) as eluent, afforded the title compound as a clear oil (135 mg, 50 %). Mol. wt. 398.41 g/mol. IR_{vmax} (NaCl): 1092.7 (C-O-C), 1143.9, 1237.9 (-C(O)OR), 1454.6 (aliphatic CH_2), 1529.2 (benzene), 1587.9 (N-H bending), 1696.3, 1721.2 (C=O), 2878.8, 2934.6, 3033.3, 3058.9 (C-H stretching), and 3367.0 (2° amine) 3476.9 (N-H stretching) cm^{-1} . **^{1}H-NMR δ** ($CDCl_3$): 3.50 (s, 2H, NH_2), 3.93-4.07 (m, 4H, IsH-1, IsH-6), 4.38 (d, 2H, J = 6.02 Hz, CH_2), 4.53 (d, 1H, J = 4.52 Hz, IsH-3), 4.92 (t, 1H, J = 5.02 Hz, IsH-4), 5.17-5.30 (m, 2H, IsH-2, NH), 5.36 (q, 1H, J = 5.52 and 10.54 Hz, IsH-5), 6.89 (d, 1H, J = 8.03, Ar_2H-4), 7.20-7.40 (m, 7H, Ar_1H, Ar_2H-2/Ar_2H-5), 7.46 (d, 1H, J = 7.53 Hz, Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.64 (CH_2), 70.25 (IsC-6), 73.21 (IsC-1), 73.94 (IsC-5), 78.02 (IsC-2), 80.57 (IsC-4), 85.72 (IsC-3), 115.43 (Ar_2C-2), 119.38 (Ar_2C-4), 119.49 (Ar_2C-6), 127.09(Ar_1C-4), 127.16 (Ar_1C-2/Ar_1C-6), 128.27 (Ar_1C-3/Ar_1C-5), 128.91 (Ar_2C-5), 129.94 (Ar_2C-1), 137.65 (Ar_1C-1), 143.58 (Ar_2C-3), 154.85 (-C(O)N-), 166.63 (-C(O)Ar_2). HRMS (M + 23) $C_{21}H_{22}N_2O_6Na$ requires 421.1393; found, 421.1376.

*2-(Benzylaminocarbonyloxy-) 5-O-(*p*-nitro-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **175**
(2-Benzyl carbamate 5-(*p*-nitro-benzoyl) isosorbide)

Compound **175** was synthesised from 2-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **112** (mol. wt. 279.29 g/mol, 1.79 mmol, 500 mg) with *p*-nitrobenzoyl chloride (mol. wt. 185.50 g/mol, 1.97 mmol, 365 mg) as reagent, using the method outlined for the preparation of esters. Recrystallisation from hot methanol to afforded the title compound as a white crystalline product (737 mg, 93 %). M.pt. 120°C, mol. wt. 428.12 g/mol. IR_{vmax} (KBr): 1086.6 (C-O-C), 1127.2, 1248.5, 1269.0 (C(O)OR), 1345.4 (C-NO_2), 1456.5 (aliphatic CH_2), 1531.3 (benzene), 1694.9, 1732.9 (C=O), 2871.9, 2993.3, 3034.2, 3060.1 (C-H stretching), and 3324.2 (2° amine) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 4.01 (m, 4H, IsH-1, IsH-6), 4.38 (d, 2H, J = 6.02 Hz, CH_2), 4.57 (d, 1H, J = 4.52 Hz, IsH-3), 4.97 (t, 1H, J = 5.02 Hz, IsH-4), 5.12 (t, 1H, J = 5.52, NH), 5.25 (d, 1H, J = 3.01 Hz, IsH-2), 5.42 (q, 1H, J = 5.02 and 10.04 Hz, IsH-5), 7.23-7.40 (m, 5H, $5Ar_1H$), 8.25 (d, 2H, J = 9.04 Hz, Ar_2H-3/Ar_2H-5), 8.32 (d, 2H, J = 9.04 Hz, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.68 (CH_2), 70.13 (IsC-6), 73.23 (IsC-1), 74.94 (IsC-5), 77.84 (IsC-2), 80.44 (IsC-4), 85.83 (IsC-3), 123.20 (Ar_2C-3/Ar_2C-5), 127.10 (Ar_1C-4), 127.23 (Ar_1C-2/Ar_1C-6), 128.30 (Ar_1C-3/Ar_1C-5), 130.44 (Ar_2C-2/Ar_2C-6), 134.39 (Ar_2C-1), 137.54 (Ar_1C-1), 150.28 (Ar_2C-4), 154.70 (-C(O)N-), 163.61 (-C(O)Ar_2). $C_{21}H_{20}N_2O_8$ requires C, 58.88; H, 4.71; N, 6.54: found C, 58.65; H, 4.71; N, 6.36.

*2-(Benzylaminocarbonyloxy-) 5-O-(*p*-amino-benzoyl)-1,4:3,6-dianhydro-D-glucitol* **180**
(2-Benzyl carbamate 5-(*p*-amino-benzoyl) isosorbide)

2-(Benzylaminocarbonyloxy-) 5-*O*-*p*-nitrobenzoyl-1,4:3,6-dianhydro-D-glucitol **175** (mol.wt. 428.12 g/mol, 0.67 mmol, 300 mg) was dissolved in ethyl acetate: methanol 1:1 (15 ml) in a 50 ml round bottom flask. A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas and stirred for 24 hours. The progress of the reaction was monitored by TLC (1:1 ethyl acetate: hexane) to observe the disappearance of the starting material. The palladium catalyst was removed by filtration and the filtrate

was collected and evaporated under vacuum. Purification by column chromatography, using ethyl acetate and hexane (3:1, 2:1, 1:1) as eluent, afforded the title compound as a clear oil, which was crystallized from hot methanol (161 mg, 60 %). M.pt. 197°C, mol.wt. 398.41 g/mol. IR_{vmax} (KBr): 1088.4 (C-O-C), 1130.6, 1174.1, 1249.6, 1277.1 (C(O)OR), 1462.8 (aliphatic CH_2), 1537.9 (benzene), 1599.7 (N-H bending), 1683.3, 1698.5 (C=O), 2867.0, 2917.4, 2984.7, 3031.6 (C-H stretching), and 3313.3 (2° amine) 3375.8 (N-H stretching) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 3.91-4.03 (m, 4H, IsH-1, IsH-6), 4.36 (d, 2H, J = 6.02 Hz, CH_2), 4.52 (d, 1H, J = 4.52 Hz, IsH-3), 4.89 (t, 1H, J = 5.02 Hz, IsH-4), 5.20 (m, 1H, IsH-2), 5.29-5.40 (m, 2H, NH, IsH-5), 6.63 (d, 1H, J = 8.54, Ar_2H-3/Ar_2H-5), 7.23-7.38 (m, 5H, 5Ar_1H), 7.87 (d, 1H, J = 8.53 Hz, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 43.75 (CH_2), 70.56 (IsC-6), 72.67 (IsC-1), 73.43 (IsC-5), 77.64 (IsC-2), 80.76 (IsC-4), 85.85 (IsC-3), 111.65 (Ar_2C-3/Ar_2C-5), 116.30 (Ar_2C-1), 126.82 (Ar_1C-4), 127.00 (Ar_1C-2/Ar_1C-6), 128.29 (Ar_1C-3/Ar_1C-5), 131.01 (Ar_2C-3/Ar_2C-5), 139.54 (Ar_1C-1), 151.72 (Ar_2C-4), 155.51 (-C(O)N-), 165.01 (-C(O)Ar_2). $C_{21}H_{22}N_2O_6$ requires C, 63.31; H, 5.57; N, 7.03: found C, 62.91; H, 5.39; 6.57.

5-O-(o-Benzyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **181**

(5-*o*-Benzyloxy-benzoyl isosorbide)

2-Benzyloxy-benzoic acid (mol.wt. 228.24 g/mol, 6.57 mmol, 1.5 g) was dissolved in DCM (5 ml) in a 100 ml round bottom flask and cooled to 0°C a Dewer flask containing ice. 3 mol. eq. of 2-*O*-(*t*-(butyl)-dimethylsilyl-)-1,4:3,6-dianhydro-D-glucitol **145** (mol. wt. 260.40 g/mol, 19.72 mmol 5.13 g) and DMAP (mol. wt. 122.17 g/mol, 7.22 mmol, 883 mg) were added and the mixture was stirred for 30 minutes. DCC (mol. wt. 206.33 g/mol, 7.22 mmol, 1.5 g) was added to the mixture over the course of an hour. The mixture was stirred overnight whilst the reaction vessel was allowed to return to room temperature. The reaction was monitored by TLC (95:5 DCM: ethyl acetate). The mixture was filtered to remove the urea precipitate and the filtrate was evaporated under vacuum giving a brown oil. The crude oil was dissolved in DCM (1 ml) and purified by flash chromatography (DCM: ethyl acetate 95:5) to eliminate any excess starting materials. A clear oil, which was obtained as the primary product, was diluted with THF

(10 ml). Excess 0.1M tetrabutylammonium fluoride (8 ml) was added to the solution and the mixture was stirred at room temperature for 15 minutes. The organic solvent was evaporated to dryness under vacuum giving a brown oil. Purification by column chromatography, using ethyl acetate and hexane (3:1) as eluent, yielded compound **181** as a clear oil (2.1 g, 90 %). Mol.wt. 356.37 g/mol. **^{1}H-NMR δ** ($CDCl_3$): 3.79-3.89 (m, 3H, IsH-1' IsH-6, IsH-6'), 3.96 (q, 1H, J = 6.03 and 10.04, IsH-1'), 4.26 (s, 1H, IsH-2), 4.43 (d, 1H, J = 4.52 Hz, IsH-3), 4.96 (t, 1H, J = 5.02 Hz, IsH-4), 5.18 (s, 2H, CH_2), 5.34 (q, 1H, J = 5.52 and 11.04 Hz, IsH-5), 6.97-7.05 (m, 2H, Ar_1H-3/ Ar_1H-5), 7.27-7.53 (m, 6H, Ar_2H, Ar_1H-4), 7.89 (dd, 1H, J = 1.51 and 7.53, Ar_1H-6).

2-(Dimethylaminocarbonyloxy-) 5-O-(o-benzyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **184**
(2-Dimethyl carbamate 5-*O*-(*o*-benzyloxy-benzoyl) isosorbide)

5-*O*-(*o*-Benzyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **181** (mol.wt. 356.37 g/mol, 0.5612 mmol, 200 mg) was dissolved in anhydrous pyridine (4 ml). Excess di-methyl carbamoyl chloride (107.54 g/mol d 1.168 g/ml, 2.24 mmol, 241 mg, 0.21 ml) was added to the mixture and was heated to 120°C for 24 hours and kept under an atmosphere of nitrogen. The mixture was evaporated to dryness under high vacuum to remove pyridine and the residue was diluted with DCM (20 ml) and washed with water (3 x 20 ml). The organic layer was dried over anhydrous sodium sulphate (2 g) and evaporated to dryness. Purification by column chromatography, using ethyl acetate and hexane (3:1, 2:1, 1:1) as eluent, yielded the target compound as a clear oil (186 mg, 78 %). Mol.wt. 427.45 g/mol. **^{1}H-NMR δ** ($CDCl_3$): 3.88 (q, 1H, J = 6.03 and 10.04, IsH-6), 3.93-4.05 (m, 3H, IsH-6', IsH-1, IsH-1'), 4.57 (d, 1H, J = 4.52 Hz, IsH-3), 4.97 (t, 1H, J = 5.02 Hz, IsH-4), 5.11 (s, 1H, IsH-2), 5.19 (s, 2H, CH_2), 5.37 (q, 1H, J = 6.03 and 11.55 Hz, IsH-5), 6.97-7.07 (m, 2H, Ar_1H-3/Ar_1H-5), 7.27-7.55 (m, 6H, 5Ar_2H, Ar_1H-4), 7.91 (dd, 1H, J = 1.50 and 8.03, Ar_1H-6). **^{13}C-NMR ppm** ($CDCl_3$): 35.47 (CH_3), 35.98 (CH_3), 69.75 (CH_2), 70.08 (IsC-6), 73.24 (IsC-1), 74.01 (IsC-5), 78.48 (IsC-2), 80.30 (IsC-4), 85.75 (IsC-3), 113.17 (Ar_1C-3), 119.32 (Ar_1C-5), 120.08 (Ar_1C-1), 126.72 (Ar_2C-2/Ar_2C-6), 127.45 (Ar_2C-4), 128.09 (Ar_2C-3/Ar_2C-5), 131.80 (Ar_1C-6), 133.40 (Ar_1C-4), 136.13 (Ar_2C-1), 154.88 (-C(O)N-), 157.93 (Ar_1C-2), 165.27 (-C(O)Ar-).

2-(Diethylaminocarbonyloxy-) 5-O-(o-benzyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **185**
(2-Diethyl carbamate 5-*O*-(*o*-benzyloxy-benzoyl) isosorbide)

Compound **185** was synthesised from 5-*O*-(*o*-benzyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **181** (mol.wt. 356.37 g/mol, 0.56 mmol, 200 mg) and diethylcarbamoyl chloride (135.59 g/mol d 1.070 g/ml, 2.25 mmol, 304 mg, 0.21 ml) using the same method as described for the synthesis of compound **184**. The procedure yielded the target compound as a clear oil (170 mg, 66 %). Mol.wt. 455.50 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 1.12 (q, 6H, J = 7.03 and 16.06 Hz, $(CH_3)_2$), 3.15-3.40 (m, 4H, $(CH_2)_2$), 3.87 (q, 1H, J = 5.52 and 9.54 Hz, IsH-6), 4.00 (m, 3H, IsH-6', IsH-1, IsH-1'), 4.57 (d, 1H, J = 4.52 Hz, IsH-3), 4.94 (t, 1H, J = 5.02 Hz, IsH-4), 5.15 (s, 1H, IsH-2), 5.20 (s, 2H, CH_2), 5.38 (q, 1H, J = 6.02 and 11.54 Hz, IsH-5), 6.98-7.07 (m, 2H, Ar_1H-3/Ar_1H-5), 7.27-7.54 (m, 6H, Ar_2H, Ar_1H-4), 7.91 (dd, 1H, J = 1.51 and 7.53, Ar_1H-6). **^{13}C-NMR ppm** ($CDCl_3$): 13.00 (CH_3), 13.63 (CH_3), 40.94 (-NCH_2), 41.51 (-NCH_2), 69.73 (OCH_2), 70.10 (IsC-6), 73.28 (IsC-1), 74.06 (IsC-5), 78.10 (IsC-2), 80.34 (IsC-4), 85.74 (IsC-3), 113.16 (Ar_1C-3), 119.31 (Ar_1C-5), 120.08 (Ar_1C-1), 126.72 (Ar_2C-2/Ar_2C-6), 127.45 (Ar_2C-4), 128.09 (Ar_2C-3/Ar_2C-5), 131.83 (Ar_1C-6), 133.40 (Ar_1C-4), 136.13 (Ar_2C-1), 154.16 (-C(O)N), 157.95 (Ar_1C-2), 165.29 (-C(O)Ar-).

2-(Morpholinocarbonyloxy-) 5-O-(o-benzyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **186**
(2-Morpholinocarbamate 5-*O*-(*o*-benzyloxy-benzoyl) isosorbide)

Compound **186** was synthesised from 5-*O*-(*o*-benzyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **181** (mol.wt. 356.37 g/mol, 0.56 mmol, 200 mg) and 4-morpholinecarbonyl chloride (mol. wt. 149.58 g/mol, d 1.282 g/ml, 2.25 mmol, 336 mg, 0.26 ml) using the same method as described for the synthesis of compound **184**. The procedure yielded the target compound as a white crystalline product (151 mg, 57 %). Mol. wt. 469.48 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 3.38-3.55 (s, 4H, Morph H), 3.58-75 (s, 4H, Morph H), 3.89 (dd, 1H, J = 5.52 and 10.17, IsH-6), 3.93-4.05 (m, 3H, IsH-6', IsH-1, IsH-1'), 4.57 (d, 1H, J = 4.52 Hz, IsH-3), 4.97 (t, 1H, J = 5.02 Hz, IsH-4), 5.15 (d, 1H, J = 1.51 Hz, IsH-2), 5.20 (s, 2H, CH_2), 5.37 (q, 1H, J = 5.52 and 11.55 Hz, IsH-5), 6.98-7.07 (m, 2H, Ar_1H-

3/Ar_1H-5), 7.30-7.55 (m, 6H, 5Ar_2H, Ar_1H-4), 7.91 (dd, 1H, J = 2.01 and 8.03, Ar_1H-6). **^{13}C-NMR ppm** ($CDCl_3$): 43.80 (Morph C), 65.96 (Morph C), 69.85 (OCH_2), 70.10 (IsC-6), 73.06 (IsC-1), 73.93 (IsC-5), 78.72 (IsC-2), 80.31 (IsC-4), 85.63 (IsC-3), 113.17 (Ar_1C-3), 119.26 (Ar_1C-5), 120.09 (Ar_1C-1), 126.73 (Ar_2C-2/Ar_2C-6), 127.47 (Ar_2C-4), 128.10 (Ar_2C-3/Ar_2C-5), 131.82 (Ar_1C-6), 133.44 (Ar_1C-4), 136.12 (Ar_2C-1), 153.70 (-C(O)N-), 157.96 (Ar_1C-2), 165.26 (-C(O)Ar-).

2-(Ethylaminocarbonyloxy-) 5-O-(o-benzyloxy benzoyl)-1,4:3,6-dianhydro-D-glucitol **183**
(2-Ethyl carbamate 5-*O*-(*o*-benzyloxy-benzoyl) isosorbide)

5-*O*-(*o*-Benzyloxy-benzoyl)-1,4:3,6-dianhydro-D-glucitol **181** (mol.wt. 356.37 g/mol, 0.56 mmol, 200 mg) was dissolved in 4 ml of anhydrous pyridine (4 ml). Excess ethyl isocyanate (71.08 g/mol, d 0.8980 g/ml, 2.25 mmol, 160 mg, 0.18 ml) was added and the mixture was heated to 120°C for 24 hours and kept under an atmosphere of nitrogen. The mixture was evaporated to dryness under high vacuum to remove pyridine and the residue was diluted with DCM (20 ml) and washed with water (3 x 20 ml). The organic layer was dried over anhydrous sodium sulphate (2 g) and evaporated to dryness. Purification by column chromatography, using ethyl acetate and hexane (3:1, 1:1, 1:3) as eluent, yielded the target compound a white crystalline solid (153.8 mg, 64.1 %). Mol. wt. 427.45 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 1.14 (t, 3H, J = 7.28 Hz, CH_3), 3.21 (m, 2H, CH_2), 3.87 (dd, 1H, J = 5.52 and 10.04 Hz, IsH-6), 3.91-4.03 (m, 3H, IsH-6', IsH-1, IsH-1'), 4.53 (d, 1H, J = 4.51 Hz, IsH-3), 4.83 (s, 1H, NH), 4.93 (t, 1H, J = 5.02 Hz, IsH-4), 5.12 (s, 1H, IsH-2), 5.19 (s, 2H, CH_2), 5.37 (q, 1H, J = 5.52 and 11.54 Hz, IsH-5), 6.98-7.07 (m, 2H, Ar_1H-3/Ar_1H-5), 7.27-7.54 (m, 6H, Ar_2H, Ar_1H-4), 7.90 (dd, 1H, J = 2.01 and 8.03 Hz, Ar_1H-6). **^{13}C-NMR ppm** ($CDCl_3$): 14.70 (CH_3), 35.43 (-NCH_2), 69.90 (-OCH_2), 70.09 (IsC-6), 73.11 (IsC-1), 73.90 (IsC-5), 77.82 (IsC-2), 80.33 (IsC-4), 85.74 (IsC-3), 113.18 (Ar_1C-3), 119.32 (Ar_1C-5), 120.09 (Ar_1C-1), 126.72 (Ar_2C-2/Ar_2C-6), 127.47 (Ar_2C-4), 128.11 (Ar_2C-3/Ar_2C-5), 131.80 (Ar_1C-6), 133.41 (Ar_1C-4), 136.12 (Ar_2C-1), 154.67 (-C(O)N), 157.94 (Ar1C-2), 165.19 (-C(O)Ar-).

2-(Dimethylaminocarbonyloxy-) 5-O-salicyloyl-1,4:3,6-dianhydro-D-glucitol **188**
(2-Dimethylcarbamate 5-salicyloyl-isosorbide)

Compound **184** (mol.wt. 427.45 g/mol, 0.35 mmol, 150 mg) was dissolved in ethyl acetate: methanol (1:1, 10 ml) in a 50 ml round bottom flask. A spatula tip-full of 10 % palladium on activated carbon was added to the solution. Air was expelled from the flask and the mixture was kept under an atmosphere of hydrogen gas and stirred for 24 hours. The progress of the reaction was monitored by TLC (1:1 ethyl acetate: hexane) to observe the disappearance of the starting material. TLC showed the formation of a single product. The palladium catalyst was removed by filtration and the filtrate evaporated under vacuum. Purification by column chromatography, using ethyl acetate and hexane (3:1, 2:1, 1:1) as eluent, afforded compound **188** as a clear oil (74 mg, 62 %). Mol.wt. 337.32 g/mol. IR_{vmax} (NaCl): 1087.6 (C-O-C), 1159.5, 1188.1, 1250.9 (C(O)OR), 1300.3 (N-C bending), 1399.2 (CH_3), 1583.3 (benzene), 1680.7, 1708.3 (C=O), 2875.7, 2927.6 (C-H stretching), and 3203.5 (2° amine), 3410.5 (OH) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 2.90 (d, 6H, J = 15.06 $(CH_3)_2$), 3.95-4.08 (m, 4H, IsH-1, IsH-6), 4.57 (d, 1H, J = 4.52 Hz, IsH-3), 4.99 (t, 1H, J = 5.02 Hz, IsH-4), 5.19 (s, 1H, IsH-2), 5.41 (q, 1H, J = 5.52 and 10.04 Hz, IsH-5), 6.91 (m, 1H, ArH-5), 7.00 (d, 1H, J = 8.53 Hz, ArH-3), 7.48 (m, 1H, ArH-4), 7.88 (dd, 1H, J = 1.51 and 7.53 Hz, ArH-6), 10.59 (s, 1H, OH). **^{13}C-NMR ppm** ($CDCl_3$): 35.46 (CH_3), 35.99 (CH_3), 70.14 (IsC-6), 73.38 (IsC-1), 74.47 (IsC-5), 78.15 (IsC-2), 80.53 (IsC-4), 85.93 (IsC-3), 111.44 (ArC-1), 117.23 (ArC-3), 118.87 (ArC-5), 129.52 (ArC-6), 135.61 (ArC-4), 154.81 (-C(O)N-), 161.28 (ArC-2), 168.96 (-OC(O)Ar). HRMS (M + 23): $C_{16}H_{19}NO_7Na$ requires 360.1054: found 360.1059.

2-(Diethylaminocarbonyloxy-) 5-O-salicyloyl-1,4:3,6-dianhydro-D-glucitol **189**
(2-Diethylcarbamate 5-salicyloyl-isosorbide)

Compound **189** was synthesised 2-(diethylaminocarbonyloxy-) 5-*O*-*o*-benzyloxy benzoyl-1,4:3,6-dianhydro-D-glucitol **185** (mol. wt. 455.50 g/mol, 0.33 mmol, 150 mg) using the same method as described for the synthesis of compound **188**. The procedure afforded compound **189** as a clear oil (83 mg, 69 %). Mol. wt. 365.38 g/mol. IR_{vmax} (NaCl): 1088.7 (C-O-C), 1160.2, 1173.1, 1216.6, 1251.3 (C(O)OR), 1300.7 (N-C bending), 1354.8, 1379.8 (CH_3), 1485.2 (CH_2), 1583.3 (benzene), 1698.9, 1720.5 (C=O), 2876.3, 2934.6, 2976.7 (C-H stretching), and 3213.6 (2° amine), 3415.5 (OH) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 1.03-1.20 (m, 6H, $(CH_3)_2$), 3.14-3.38 (m, 4H, $(CH_2)_2$), 3.95-4.10 (m, 4H, IsH-1, IsH-6), 4.58 (d, 1H, J = 4.52 Hz, IsH-3), 4.99 (t, 1H, J = 5.02 Hz, IsH-4), 5.23 (d, 1H, J = 2.0 Hz, IsH-2), 5.42 (q, 1H, J = 5.02 and 10.04 Hz, IsH-5), 6.92 (m, 1H, ArH-5), 7.01 (d, 1H, J = 8.53 Hz, ArH-3), 7.49 (m, 1H, ArH-4), 7.89 (dd, 1H, J = 1.51 and 8.03 Hz, ArH-6), 10.60 (s, 1H, OH). **^{13}C-NMR ppm** ($CDCl_3$): 13.62 (CH_3), 12.97 (CH_3), 40.93 (CH_2), 41.54 (CH_2), 70.14 (IsC-6), 73.41 (IsC-1), 74.51 (IsC-5), 77.78 (IsC-2), 80.57 (IsC-4), 85.92 (IsC-3), 111.44 (ArC-1), 117.24 (ArC-3), 118.87 (ArC-5), 129.53 (ArC-6), 135.62 (ArC-4), 154.09 (-C(O)N-), 161.29 (ArC-2), 168.98 (-C(O)Ar). HRMS (M + 23): $C_{16}H_{19}NO_7Na$ requires 388.1373: found 388.1361.

2-(Morpholinocarbonyloxy-) 5-O-salicyloyl-1,4:3,6-dianhydro-D-glucitol **190**
(2-Morpholinocarbamate 5-salicyloyl-isosorbide)

Compound **190** was synthesised from 2-(morpholinocarbonyloxy-) 5-*O*-*o*-benzyloxy benzoyl-1,4:3,6-dianhydro-D-glucitol **186** (mol. wt. 469.48 g/mol, 0.32 mmol, 150 mg) using the same method as described for the synthesis of compound **188**. The procedure afforded compound **190** as a clear oil which crystallized upon standing to form a white crystalline solid (56 mg, 47 %). M.pt 158°C, mol. wt. 379.36 g/mol. IR_{vmax} (KBr): 1107.9 (C-O-C), 1134.7, 1159.6, 1216.0, 1243.5 (C(O)OR), 1294.1 (N-C bending), 1435.4, 1485.9 (CH_2), 1583.3 (benzene), 1690.3, 1705.0 (C=O), 2859.2, 2919.2, 2977.4 (C-H stretching), and 3203.0 (2° amine), 3447.1 (OH) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.38-3.55 (s,

4H, Morph H), 3.56-3.75 (s, 4H, Morph H), 3.95-4.11 (m, 4H, IsH-1, IsH-6), 4.59 (d, 1H, J = 4.51 Hz, IsH-3), 5.00 (t, 1H, J = 5.27 Hz, IsH-4), 5.24 (s, 1H, IsH-2), 5.42 (q, 1H, J = 5.52 and 10.04 Hz, IsH-5), 6.92 (m, 1H, ArH-5), 7.02 (dd, 1H, J = 1.0 and 8.53 Hz, ArH-3), 7.50 (m, 1H, ArH-4), 7.88 (dd, 1H, J = 2.01 and 8.03 Hz, ArH-6), 10.60 (s, 1H, OH). **^{13}C-NMR ppm** ($CDCl_3$): 43.83 (Morph C), 65.95 (Morph C), 70.23 (IsC-6), 73.22 (IsC-1), 74.40 (IsC-5), 78.40 (IsC-2), 80.54 (IsC-4), 85.80 (IsC-3), 111.40 (ArC-1), 117.28 (ArC-3), 118.89 (ArC-5), 129.49 (ArC-6), 135.65 (ArC-4), 153.62 (-C(O)N-), 161.31 (ArC-2), 168.94 (-C(O)Ar). $C_{18}H_{21}NO_8$ requires C, 56.99; H, 5.58; N, 3.69: found C, 56.68; H, 5.63; N, 3.69.

2-(Ethylaminocarbonyloxy-) 5-O-salicyloyl-1,4:3,6-dianhydro-D-glucitol **187**
(2-Ethylcarbamate 5-salicyloyl-isosorbide)

Compound **187** was synthesised from 2-(ethylaminocarbonyloxy-) 5-*O*-*o*-benzyloxy-benzoyl-1,4:3,6-dianhydro-D-glucitol **183** (mol.wt. 427.45 g/mol, 0.33 mmol, 150 mg) using the same method as described for the synthesis of compound **188**. The procedure afforded compound **187** as a white crystalline product (69 mg, 59 %). M.pt 118°C, mol. wt. 337.32 g/mol. IR_{vmax} (KBr): 764.3 (ortho-substitution), 1085.8 (C-O-C), 1157.7, 1187.4, 1234.5, 1253.3 (C(O)OR), 1304.9 (N-C bending), 1354.8, 1356.9 (CH_3), 1482.2 (CH_2), 1512.1 (benzene), 1688.6, 1720.0 (C=O), 2867.5, 2922.8, 2970.7 (C-H stretching), and 3216.0 (2° amine), 3431.1 (OH) cm^{-1}. **^{1}H-NMR δ** ($CDCl_3$): 1.14 (t, 3H, J = 7.28 Hz, CH_3), 3.19 (m, 2H, CH_2), 3.92-4.10 (m, 4H, IsH-1, IsH-6), 4.55 (d, 1H, J = 4.52 Hz, IsH-3), 4.84 (s, 1H, NH), 4.97 (t, 1H, J = 5.27 Hz, IsH-4), 5.21 (s, 1H, IsH-2), 5.41 (q, 1H, J = 5.02 and 9.54 Hz, IsH-5), 6.91 (m, 1H, ArH-5), 6.99 (dd, 1H, J = 1.0 and 8.53 Hz, ArH-3), 7.48 (m, 1H, ArH-4), 7.87 (dd, 1H, J = 1.51 and 8.03 Hz, ArH-6), 10.58 (s, 1H, OH). **^{13}C-NMR ppm** ($CDCl_3$): 14.68 (CH_3), 35.46 (CH_2), 70.23 (IsC-6), 73.24 (IsC-1), 74.40 (IsC-5), 77.51 (IsC-2), 80.54 (IsC-4), 85.87 (IsC-3), 111.45 (ArC-1), 117.23 (ArC-3), 118.89 (ArC-5), 129.51 (ArC-6), 135.62 (ArC-4), 154.59 (-C(O)N-), 161.27 (ArC-2), 168.90 (-C(O)Ar). $C_{16}H_{19}NO_7$ requires C, 56.97; H, 5.68; N, 4.15: found C, 56.77; H, 5.69; N, 4.02.

5-O-o-Benzoyl-1,4:3,6-dianhydro-L-iditol **196**

(5-Benzoyl isoiodide)

Compound **196** was synthesised from isoiodide **3** (mol. wt. 146.14 g/mol, 3.42 mmol, 500 mg) with benzoyl chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 3.76 mmol, 529 mg, 0.44 ml) as reagent, using the method outlined for the preparation of esters. Re-crystallization from hot ethyl acetate and hexane to gave 398 mg (47 %) of a white crystalline product. Mol. wt. 250.25 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 3.20-3.40 (s, 1H, OH), 3.86-3.96 (m, 2H, IsH-1, IsH-1'), 3.98-4.07 (m, 2H, IsH-6, IsH-6'), 4.35-4.43 (s, 1H, IsH-2), 4.64-4.70 (d, 1H, J = 4.01 Hz, IsH-3), 4.81-4.87 (d, 1H, J = 3.52 Hz, IsH-4), 5.38-5.45 (s, 1H, IsH-5), 7.38-7.47 (t, 2H, J = 7.78 Hz, ArH-3/ArH-5), 7.53-7.60 (t, 1H, J = 7.53 Hz, ArH-4), 7.97-8.07 (m, 2H, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 71.99 (IsC-6), 74.26 (IsC-1), 75.38 (IsC-2), 77.75 (IsC-5), 84.63 (IsC-4), 87.39 (IsC-3), 128.03 (ArC-3/ArC-5), 128.90 (ArC-1), 129.29 (Ar-2/Ar-6), 133.02 (ArC-4), 165.24 (CO).

2-(Benzylaminocarbonyloxy-) 5-O-benzoyl-1,4:3,6-dianhydro-L-iditol **193**

(2-Benzylcarbamate 5-benzoyl isoiodide)

5-*O*-*o*-Benzoyl-1,4:3,6-dianhydro-D-glucitol **196** (mol. wt. 250.25 g/mol, 1.40 mmol, 350 mg) was dissolved in DCM. Triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 1.54 mmol, 155 mg, 0.21 ml) and benzyl isocyanate (mol. wt. 133.15 g/mol, d 1.078 g/ml, 1.54 mmol, 205 mg, 0.19 ml) were added and the mixture was heated to 105°C for two hours. The reaction was monitored by TLC (1:1 ethyl Acetate: hexane) to observe the disappearance of the starting material. The mixture was cooled upon completion of the reaction and methanol (20ml) was added to remove excess isocyanate. The mixture was heated for a further 15 minutes at 105°C and cooled to room temperature. All organic solvent was removed under vacuum giving a clear oil to which was added DCM (50 ml) and washed with 1M HCl (50 ml), 5 % sodium bicarbonate (50 ml), saturated brine solution (50 ml) and dried with anhydrous sodium sulphate (2 g). Purification by column chromatography using hexane and ethyl acetate (3:1 and 1:1) as eluent, afforded 377 mg (70 %) of a white crystalline compound. M.pt. 85°C, mol. wt. 383.39 g/mol. IR_{vmax}

(KBr): 1096.2 (C-O-C), 1140.3, 1258.7, 1276.9 (C(O)OR), 1546.0 (benzene), 1693.9, 1726.1 (C=O), 2934.9, 2973.9, 3032.7 (C-H stretching), and 3341.2 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.98-4.04 (s, 2H, IsH-6, IsH-6'), 4.05-4.10 (m, 2H, J = 2.0 Hz, IsH-1, IsH-1'), 4.35-4.43 (d, 2H, J = 6.02 Hz, CH_2), 4.73-4.80 (s, 2H, IsH-3, IsH-4), 5.15-5.20 (s, 1H, NH), 5.23-5.28 (s, 1H, IsH-2), 5.43-5.48 (s, 1H, IsH-5), 7.23-7.40 (m, 5H, $5Ar_1H$), 7.43-7.51 (t, 2H, J = 7.53 Hz, Ar_2H-3/Ar_2H-5), 7.57-7.64 (t, 1H, J = 7.53 Hz, Ar_2H-4), 8.02-8.08 (d, 2H, J = 7.03 Hz, Ar_2H-2/Ar_2H-6). $C_{21}H_{21}NO_6$ requires C, 65.79; H, 5.52; N, 3.65: found C, 65.56; H, 5.63; N, 3.54.

5-O-Benzoyl-1,4:3,6-dianhydro-D-mannitol **195**

(5-Benzoyl isomannide)

Compound **195** was synthesised from isomannide **2** (mol. wt. 146.14 g/mol, 3.42 mmol, 500 mg) with benzoyl chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 3.76 mmol, 529. mg, 0.44 ml) as reagent, using the method outlined for the preparation of esters. Re-crystallization from hot ethyl acetate and hexane to gave 438 mg (51 %) of a white crystalline product. Mol. wt. 250.25 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 3.00 (s, 1H, OH), 3.58 (dd, 1H, J = 7.53 and 8.53 Hz, IsH-6), 3.92 (dd, 1H, J = 6.52 and 9.03 Hz, IsH-6'), 4.03 (dd, 1H, J = 6.03 and 9.54 Hz, IsH-1), 4.15 (dd, 1H, J = 6.53 and 10.04 Hz, IsH-1'), 4.29 (q, 1H, J = 6.03 and 13.04 Hz, IsH-2), 4.47 (t, 1H, J = 5.02 Hz, IsH-3), 4.81 (t, 1H, J = 5.27 Hz, IsH-4), 5.37 (q, 1H, J = 6.03 and 12.05 Hz, IsH-5), 7.42 (m, 2H, ArH-3/ArH-5), 7.54 (m, 1H, ArH-4), 8.06 (m, 2H, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 70.69 (IsC-6), 71.81 (IsC-2), 73.03 (IsC-1), 74.17 (IsC-5), 80.29 (IsC-4), 81.21 (IsC-3), 128.01 (ArC-3/ArC-5), 128.93 (ArC-1), 129.33 (Ar-2/Ar-6), 132.88 (ArC-4), 165.48 (CO).

2-(Benzylaminocarbonyloxy-) 5-O-benzoyl-1,4:3,6-dianhydro-D-mannitol **192**

(2-Benzylcarbamate 5-benzoate isomannide)

5-*O*-Benzoyl-1,4:3,6-dianhydro-D-mannitol **195** (mol. wt. 250.25 g/mol, 1.60 mmol, 400 mg) was dissolved in DCM. Triethylamine (mol. wt. 101 g/mol, d 0.726 g/ml, 1.76 mmol, 178 mg, 0.25 ml) and benzyl isocyanate (mol. wt. 133.15 g/mol, d 1.078 g/ml,

1.76 mmol, 234 mg, 0.22 ml) were added. The mixture was heated to 105°C for two hours and the reaction was monitored by TLC (1:1 ethyl Acetate: hexane) to observe the disappearance of the starting material. The mixture was cooled upon completion of the reaction and methanol (20 ml) was added to remove excess isocyanate. The mixture was heated for a further 15 minutes at 105°C and cooled to room temperature. All organic solvent was removed under vacuum giving a clear oil to which was added DCM (50 ml) and washed with 1M HCl (50 ml), 5 % $NaHCO_3$ (50 ml), saturated brine solution (50 ml) and dried with anhydrous sodium sulfate (2 g). Purification by column chromatography using hexane and ethyl acetate (3:1 and 1:1) as eluent, afforded 401 mg (66 %) of a white crystalline compound. M.pt. 138°C, mol. wt. 383.39 g/mol. IR_{vmax} (KBr): 1096.1 (C-O-C), 1146.9, 1269.9 (C(O)OR), 1536.0 (benzene), 1699.2, 1724.0 (C=O), 2874.9, 2986.8, 3035.7 (C-H stretching), and 3318.2 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.81 (t, 1H, J = 8.53 Hz, IsH-1), 4.08 (m, 2H, IsH-6, IsH-1'), 4.16 (dd, 1H, J = 6.03 and 9.54 Hz, IsH-6'), 4.42 (m, 2H, CH_2), 4.72 (t, 1H, J = 5.02 Hz, IsH-3), 4.88 (t, 1H, J = 5.27 Hz, IsH-4), 5.15 (q, 1H, J = 7.53 and 13.05 Hz, IsH-5), 5.22 (s, 1H, NH), 5.39 (q, 1H, J = 5.52 and 11.54 Hz, IsH-2), 7.29-7.40 (m, 5H, $5Ar_1H$), 7.47 (t, 2H, J = 7.78 Hz, Ar_2H-3/Ar_2H-5), 7.60 (t, 1H, J = 7.53 Hz, Ar_2H-4), 8.10 (d, 2H, J = 7.03 Hz, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.77 (CH_2), 69.67 (IsC-6), 70.72 (IsC-1), 73.53 (IsC-2), 74.00 (IsC-5), 80.35 (IsC-4/IsC-3), 127.08 (Ar_1C-4), 127.17 (Ar_1C-2/Ar_1C-6), 127.99 (Ar_2C-3/Ar_2C-5), 128.26 (Ar_1C-3/Ar_1C-5), 129.36 (Ar_2C-2/Ar_2C-6), 129.94 (Ar_2C-1), 130.98 (Ar_2C-4), 132.85 (Ar_2C-1), 155.06 (-O<u>C</u>(O)N-), 165.51 (-OC(O)Ar_2). $C_{21}H_{21}NO_6$ requires C, 65.79; H, 5.52; N, 3.65: found C, 65.66; H, 5.66; N, 3.38.

2-(Benzylaminocarbonyloxy-) 5-O-nitro-1,4:3,6-dianhydro-D-glucitol **197**
(2-Benzoyl 5-nitro isosorbide)

Compound **197** was synthesised from IS-5-MN **62** (mol. wt. 191.14 g/mol, 2.62 mmol, 500 mg) with benzoyl chloride (mol. wt. 140.57 g/mol, d 1.211 g/ml, 2.88 mmol, 405 mg, 0.33 ml) as reagent, using the method outlined for the preparation of esters. Re-crystallization from hot ethyl acetate and hexane to gave 707 mg (92 %) of a white crystalline product. Mol. wt. 295.29 g/mol. **^{1}H-NMR** δ ($CDCl_3$): 3.91-3.99 (dd, 1H, J =

5.52 and 11.04 Hz, IsH-6), 4.03-4.23 (m, 3H, IsH-6', IsH-1, IsH-1'), 4.65 (d, 1H, J = 5.02 Hz, IsH-3), 5.09 (t, 1H, J = 5.27 Hz, IsH-4), 5.40 (m, 1H, IsH-5), 5.49 (d, 1H, J = 3.02 Hz, IsH-2), 7.46 (t, 2H, J = 7.78 Hz, ArH-3/ArH-5), 7.60 (m, 1H, ArH-4), 8.04 (m, 2H, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 68.86 (IsC-6), 73.14 (IsC-1), 77.37 (IsC-2), 80.92 (IsC-5), 81.18 (IsC-4), 86.27 (IsC-3), 128.05 (ArC-3/ArC-5), 128.83 (ArC-1), 129.29 (Ar-2/Ar-6), 133.07 (ArC-4), 165.07 (CO).

2-(Benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **198**

(2-Benzoyl isosorbide)

2-(Benzylaminocarbonyloxy-) 5-*O*-nitro-1,4:3,6-dianhydro-D-glucitol **197** (mol.wt. 295.29 g/mol, 2.37 mmol, 700 mg) was dissolved in ethyl acetate: methanol 1:1 (15 ml). A spatula tip-full of 10 % palladium on activated carbon was added to the solution and the mixture was kept under an atmosphere of hydrogen gas and stirred for 24 hours. TLC showed the formation of a single product. The solvent was evaporated under vacuum and the crude product was dissolved in DCM and filtered through silica. The filtrate was collected and evaporated under vacuum to yield 561 mg (95 %) of a white crystalline product. Mol. wt. 250.25 g/mol. **^{1}H-NMR δ** ($CDCl_3$): 2.79 (d, 1H, J = 6.02 Hz, OH), 3.52 (dd, 1H, J = 6.03 and 9.54 Hz, IsH-6), 3.84 (dd, 1H, J = 6.03 and 9.54 Hz, IsH-6'), 4.05 (m, 2H, IsH-1, IsH-1'), 4.26 (m, 1H, IsH-5), 4.55 (d, 1H, J = 4.51 Hz, IsH-3), 4.63 (t, 1H, J = 5.02 Hz, IsH-4), 5.38 (d, 1H, J = 3.51 Hz, IsH-2), 7.36 (t, 2H, J = 7.78 Hz, ArH-3/ArH-5), 7.49 (m, 1H, ArH-4), 7.95 (m, 2H, ArH-2/ArH-6). **^{13}C-NMR ppm** ($CDCl_3$): 71.96 (IsC-5), 72.94 (IsC-1), 73.18 (IsC-6), 78.41 (IsC-2), 81.65 (IsC-4), 85.23 (IsC-3), 128.02 (ArC-3/ArC-5), 128.93 (ArC-1), 129.29 (Ar-2/Ar-6), 133.01 (ArC-4), 165.09 (CO).

2-O-Benzoyl 5-(benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **194**

(2-Benzoyl 5-benzyl carbamate isosorbide)

2-(Benzylaminocarbonyloxy-)-1,4:3,6-dianhydro-D-glucitol **198** (mol. wt. 250.25 g/mol, 1.60 mmol, 400 mg) was dissolved in DCM. Triethylamine (mol. wt. 101 g/mol, d 0.726

g/ml, 1.76 mmol, 178 mg, 0.25 ml) and benzyl isocyanate (mol. wt. 133.15 g/mol, d 1.078 g/ml, 1.76 mmol, 234 mg, 0.22 ml) were added. The mixture was heated to 105°C for two hours and the reaction was monitored by TLC (1:1 ethyl acetate: hexane) to observe the disappearance of the starting material. The mixture was cooled upon completion of the reaction and methanol (20ml) was added to remove excess isocyanate. The mixture was heated for a further 15 minutes at 105°C and cooled to room temperature. All organic solvent was removed under vacuum giving a clear oil to which was added DCM (50 ml) and washed with 1M HCl (50 ml), 5 % $NaHCO_3$ (50 ml), saturated brine solution (50 ml) and dried with anhydrous sodium sulfate (2 g). Purification by column chromatography using hexane and ethyl acetate (3:1 and 1:1) as eluent, afforded 454 mg (74 %) of a white crystalline compound. M.pt. 125°C, mol. wt. 383.39 g/mol. IR_{vmax} (KBr): 1097.2 (C-O-C), 1148.7, 1266.0 (C(O)OR), 1536.7 (benzene), 1699.1, 1725.9 (C=O), 2925.5, 2983.1, 3032.8 (C-H stretching), and 3325.3 (2° amine) cm^{-1}. **^{1}H-NMR** δ ($CDCl_3$): 3.82 (dd, 1H, J = 6.52 and 9.03 Hz, IsH-6), 4.05 (dd, 1H, J = 6.54 and 9.54 Hz, IsH-6'), 4.16 (s, 2H, IsH-1, IsH-1'), 4.42 (d, 2H, J = 6.02 Hz, CH_2), 4.67 (d, 1H, J = 4.52 Hz, IsH-3), 4.96 (t, 1H, J = 4.27 Hz, IsH-4), 5.23 (m, 2H, NH, IsH-5), 5.48 (s, 1H, IsH-2), 7.27-7.40 (m, 5H, $5Ar_1H$), 7.48 (t, 2H, J = 7.78 Hz, Ar_2H-3/Ar_2H-5), 7.61 (t, 1H, J = 7.27 Hz, Ar_2H-4), 8.05 (d, 2H, J = 7.53 Hz, Ar_2H-2/Ar_2H-6). **^{13}C-NMR ppm** ($CDCl_3$): 44.75 (CH_2), 69.73 (IsC-6), 73.22 (IsC-1), 73.99 (IsC-5), 78.12 (IsC-2), 80.78 (IsC-4), 85.44 (IsC-3), 127.07 (Ar_1C-4), 127.16 (Ar_1C-2/Ar_1C-6), 128.02 (Ar_2C-3/Ar_2C-5), 128.26 (Ar_1C-3/Ar_1C-5), 128.97 (Ar_2C-1), 129.31 (Ar_2C-2/Ar_2C-6), 132.99 (Ar_2C-4), 137.64 (Ar_2C-1), 155.15 (-O<u>C</u>(O)N-), 165.15 (-OC(O)Ar_2). $C_{21}H_{21}NO_6$ requires C, 65.79; H, 5.52; N, 3.65: found C, 65.90; H, 5.52; N, 3.57.

6.4 ENZYME ACTIVITY AND INHIBITION ASSAY

6.4.1 SAMPLE PREPARATION

Phosphate Buffer (0.1M., pH 8.0)

Solution A: 13.8 g NaH_2PO_4 diluted to 1 L with distilled H_2O

Solution B: 14.2 g Na_2HPO_4 diluted to 1 L with distilled H_2O

Solution A (53 ml) was added to Solution B (947 ml)

5, 5'-dithiobis(2-nitrobenzoic) acid (DTNB)

DTNB (19.8 mg) was dissolved in 10 ml of phosphate buffer pH 8.0 in amber glassware. [25 µl of this solution in 250 µl of test solution will give a concentration of 0.5 mM]

Substrates

Butyrylthiocholine Iodide (BTCI): BTCI (15.9 mg) was dissolved in 10 ml of phosphate buffer pH 8.0. [25 µl of this solution in 250 µl of test solution will give a concentration of 0.5 mM] [25 µl of this solution in 250 µl of test solution will give a concentration of 0.5 mM]

Acetylthiocholine Iodide (ATCI): ATCI (14.5 mg) was dissolved in 10 ml of phosphate buffer pH 8.0. [25 µl of this solution in 250 µl of test solution will give a concentration of 0.5 mM]

Enzyme solutions

Plasma: Human blood samples were collected in venipuncture into Li-Heparin Sarstedt Monovette tubes (9 ml). Plasma was obtained by centrifugation of the blood samples at 10,000 rpm for 5 minutes. Plasma was stored at 2 to 6 °C. A plasma solution for the activity/inhibition assay was prepared by diluting 1 ml of plasma to 20 ml with phosphate buffer pH 8.0.

AChE: 10 µl of electric eel AChE was diluted to 10 ml with phosphate buffer pH 8.0.

Inhibitor solutions:

1M solutions of each inhibitor were prepared in 10 ml of acetonitrile: distilled water (1:1). [25 µl of a 1M inhibitor solution in 250 µl of test solution (see below) gave an inhibitor concentration of 100 mM]

6.4.2 DETERMINATION OF CHOLINESTERASE ACTIVITY AND INHIBITION

HuBuChE activity was measured in replicate samples using a 96-well plate reader. The total volume of test solution in each well was 250 µl. This consisted of 25 µl of plasma solution, 175 µl of phosphate buffer pH 8.0, 25 µl of DTNB solution [0.5 mM] and 25 µl of acetonitrile: distilled water (1:1). The 96-well plate was incubated for 30 minute before 25 µl of BTCI solution [0.5 mM] was added and the reaction was measured at 412 nm over five minutes using an Anthos bt2 plate reader. For the determination of AChE activity, 25 µl of AChE solution and 25 µl of ATCI solution were used instead of the plasma solution and BTCI solution. For determination of the inhibition of enzymes, 25 µl of an inhibitor solution was added to the test solution instead of the acetonitrile: water (1:1) solution.

References

[1] Williams, F.M. *Clinical Pharmacokinetics*, 1985, (10), 392-403.

[2] Walker, C.H., Mackness, M.I. *Biochemical Pharmacology*, 1983, (32), 3265-3269.

[3] Soreq, H., Seidman, S. *Nature Reviews: Neuroscience*, 2001, (2), 294-302.

[4] Gilmer, J.F., Moriarty, L.M., McCafferty, D.F., Clancy, J.M. *European Journal of Pharmaceutical Sciences*, 2002, (16), 297-304.

[5] Silk, E., King, J., Whittaker, M. *Annals of Clinical Biochemistry*, 1979, (16), 57-75.

[6] Giacobini, E. In *Butyrylcholinesterase: its Function and Inhibitors*, 2003, 1-21, Martin Duntz, London.

[7] Atack, J.R., Perry, E.K., Bonham, J.R., Candy, J.M. Perry, R.H. *Journal of Neurochemistry*, 1986, (47), 263-277.

[8] Dale, H.H. *Journal of Pharmaceutical and Experimental Therapeutics*, 1914, (6), 147-190.

[9] Massoulie, J., Pezzementi, L., Krejci, E., Vallette, F.M. *Progress in Neurobiology*, 1993, (41), 31-91.

[10] Silver, A. *The Biology of Cholinesterases,* 1974, 426-447, Elsevier/Agricultural Research Council, New York.

[11] Stedman, E., Easson, L.H. *Biochemistry Journal*, 2932, (26), 2056-2066.

[12] Alles, G.A., Hawes, R.C. *Journal of Biological Chemistry*, 1940, (133), 375-390.

[13] Mendel, B., Rudney, H. *Science*, 1943, (98), 201-202.

[14] Chatonet, A. *Biochemistry Journal*, 1989, (260), 625-634.

[15] Silverman, R.B. *The organic chemistry of enzyme-catalysed reactions*, 2000, 60-62, Academic Press, London.

[16] Mesulam, M.M. *Neuroscience*, 2002, (110), 627-639.

[17] Li, B. *Journal of Neurochemistry*, 2000, (75), 1320-1331.

[18] Geula, C., Mesulam, M.M. In *Alzheimer's disease*, 1994, 263-291, Raven Press Ltd., New York.

[19] Mesulam, M.M. In *Cholinesterases and cholinesterase inhibitors*, 2000, 121-136, Martin Duntz, London.

[20] Giacobini, E., Holmstedt, B. *Acta Physiology Scandinavia*, 1958, (42), 12-27.

[21] Darvesh, S., Hopkins, D. A. *Nature Reviews: Neuroscience,* 2003, (2), 131-8.

[22] Salmon, A. Y., Goren, Z. *Clinical and Experimental Pharmacology & Physiology*, 1999, (26), 596-600.

[23] Stewart, D. J., Inaba, T. *Life Sciences*, 1977, (20), 1557-63.

[24] Valentino, R. J., Lockridge, O. *Biochemical Pharmacology,* 1981, (30), 1643-9.

[25] Johnson, G., Moore, S. W. *Molecular Immunology,* 2000, (37), 707-19.

[26] Sparks, S. E., Quistad, G. B. *Journal of Biochemical and Molecular Toxicology* 1999, (13), 113-8.

[27] Whittaker, V. P. *Progress in Brain Research*, 1993, (98), 155-9.

[28] Van Dyke, C., Barash, P. G. *Science*, 1976, (191), 859-61.

[29] Jatlow, P., Barash, P. G. *Anesthesia and Analgesia*, 1979, (58), 235-8.

[30] Jbilo, O., Bartels, C. F. *Toxicon : Official Journal of the International Society on Toxinology*, 1994, (32), 1445-57.

[31] Loewenstein-Lichtenstein, Y., Glick, D. *Molecular Pharmacology*, 1996, (50), 1423-31.

[32] Lehmann, H., Ryan, E. *Lancet*, 1956, (271), 124.

[33] Kalow, W., Staron, N. *Canadian Journal of Biochemistry and Physiology*, 35 (12), 1305-20.

[34] La Du, B. N., Bartels, C. F. *Clinical Biochemistry*, 1990, (23), 423-31.

[35] La Du, B. N., Bartels, C. F. *Cellular and Molecular Neurobiology*, 1991, (11), 79-89.

[36] Nassar, B. A., Darvesh, S. *Clinical Biochemistry*, 2002, (35), 205-9.

[37] Xie, W., Altamirano, C. V. *Molecular Pharmacology*, 1999, (55), 83-91.

[38] Whittaker, M. Britten, J. J. *Human Heredity*, 1987, (37), 54-8.

[39] Jensen, F. S., Bartels, C. F. *Pharmacogenetics*, 1992, (2), 234-40.

[40] Sakamoto, N., Hidaka, K. *Clinica Chimica Acta*, 1998, (274), 159-66.

[41] Areekul, S. *Journal of the Medical Association of Thailand*, 1981, (64), 363-6.

[42] Primo-Parmo, S. L., Bartels, C. F. *American Journal of Human Genetics*, 1996, (58), 52-64.

[43] Rubinstein, H. M., A. A. Dietz. *Journal of Clinical Investigation,* 1970, (49) 479-86.

[44] Giacobini, E. *Pharmacological Research*, 2004, (50), 433-440.

[45] Small, D. H., Michaelson, S. *Neurochemistry International*, 1996, (28), 453-83.

[46] Harel, M., Sussman, J. L. *Proceedings of the National Academy of Sciences of the USA*, 1992, (89), 10827-31.

[47] Nachon, F. In *Butyrylcholinesterase: its Function and Inhibitors*, 2003, 39-54, Martin Duntz, London

[48] Sussman, J. L., Harel, M. *Science*, 1991, (253), 872-9.

[49] Nicolet, Y., Lockridge, O. *Journal of Biological Chemistry*, 2003, (278), 41141-7.

[50] Nachon, F., Nicolet, Y. *European Journal of Biochemistry*, 2002, (269), 630-7.

[51] Massoulie, J., Anselmet, A. *Chemical and Biological Interactions*, 1999, (119-120), 29-42.

[52] Vellom, D. C., Radic, Z. *Biochemistry*, 1993, (32), 12-7.

[53] Sutherland, D., McClellan, J. S. *The Journal of Experimental Zoology*, 1997, (277), 213-29.

[54] Masson, P., Xie, W. *Biochimica et Biophysica Acta*, 1999, (1433), 281-93.

[55] Massoulie, J. and Bon, S. *Annual Review of Neuroscience*, 1982, (5), 57-106.

[56] Greig, N. H., Utsuki, T. *Current Medical Research and Opinion*, 2001, (17), 159-65.

[57] Mesulam, M., Guillozet, A. *Neurobiology of Disease*, 2002, (9), 88-93.

[58] Giacobini, E. *Neurobiological Aging*, 1986, (7), 392-396.

[59] Giacobini, E. *Abstracts - Society for Neuroscience*, 1996, (22), 203.

[60] Shafferman, A., Velan, B. *The EMBO journal,* 1992, (11), 3561-3568.

[61] Busker, R. W., Zijlstra, J. J. *Neurochemical Research*, 1994, (19), 713-9.

[62] Greig, N. H. *Alzheimer's insights*, 2001, (7), 1-4.

[63] Duysen, E. G. *Brain Research, developmental brain research*, 2002, (137), 43-54.

[64] Lockridge, O., In *Butyrylcholinesterase: its Function and Inhibitors*, 2003, 21-28, Martin Duntz, London.

[65] Xie, W., Stribley, J. A. *The Journal of Pharmacology and Experimental Therapeutics*, 2000, (293), 896-902.

[66] Palmer, A. M. *Trends in Pharmacological Sciences*, 2002, (23), 426-33.

[67] Gauthier, S., Emre, M. *Current Medical Research and Opinion*, 2003, (19), 707-14

[68] Davies, P. *Brain Research, developmental brain research*, 1979, (171), 319-27.

[69] Perry, E. K., Tomlinson, B. E. *British Medical Journal*, 1978, (2), 1457-9.

[70] Geula, C., M. Mesulam. *Brain Research, developmental brain research*, 1989, (498), 185-9.

[71] Perry, E. K., Perry, R. H. *Neuropathology and Applied Neurobiology*, 1978, (4), 273-7.

[72] Arendt, T., Bruckner, M. K. *Neurochemistry International*, 1992, (21), 381-96.

[73] Wright, C. I., Geula, C. *Annals of Neurology*, 1993, (34), 373-84.

[74] Selkoe, D. J. *Scientific American*, 1991 (265), 68-71.

[75] Guillozet, A. L., Smiley, J. F. *Annals of Neurology*, 1997, (42), 909-18.

[76] Moran, M. A., Mufson, E. J. *Acta Neuropathologica*, 1994, (87), 284-92.

[77] Geula, C., Mesulam, M. M. *Alzheimer Disease and Associated Disorders*, 1995 (9 Suppl 2), 23-8.

[78] Wright, C. I., Guela, C. *Proceedings of the National Academy of Sciences of the USA*, 1993, (90), 683-6.

[79] Mesulam, M. M., Geula. C. *Annals of Neurology*, 1994, (36), 722-7.

[80] Atack, J. R., Perry, E. K. *Journal of Neurochemistry*, 1987, (48), 1687-92.

[81] Arendt, T., M. K. Bruckner. *Neurochemistry International*, 1992, (21), 381-96.

[82] Lehmann, D. J., Johnston, C. *Human Molecular Genetics*, 1997, (6), 1933-6.

[83] McIlroy, S. P., Crawford, V. L. *Journal of Medical Genetics*, 2000 (37), 182-5.

[84] Wiebusch, H., Poirier, J. *Human Genetics*, 1999, (104), 158-63.

[85] Alvarez-Arcaya, A., Combarros, O. *Acta Neurologica Scandinavica*, 2000, (102), 350-3.

[86] Singleton, A. B., Smith, G. *Human Molecular Genetics*, 1998, (7), 937-9.

[87] Lockridge, O. *Journal of Neurochemistry*, 1982 (39), 106-10.

[88] Darvesh, S., Kumar, R. *Cellular and Molecular Neurobiology*, 2001, (21), 285-96.

[89] Layer, P. G. *Proceedings of the National Academy of Sciences of the USA*, 1983, (80), 6413-7.

[90] Layer, P. G. *Cellular and Molecular Neurobiology*, 1991, (11), 7-33.

[91] Layer, P. G., Sporns, O. *Proceedings of the National Academy of Sciences of the USA*, 1987, (84), 284-8.

[92] Olson, P. F., L. I. Fessler, O. *The EMBO Journal*, 1990, (9), 1219-27.

[93] Arendt, T., Bigl, V. *Lancet*, 1984, (1), 173.

[94] Razon, N., Soreq, H. *Experimental Neurology,* 1984, (84), 681-95.

[95] Drews, U. *Progress in Histochemistry and Cytochemistry,* 1975, (7), 1-52.

[96]Zakut, H., Even, L. *Cancer*, 1988 (61), 727-37.

[97] Small, D. H., Michaelson, S. *Neurochemistry International*, 1996, (28), 453-83.

[98] Small, D. H., Moir, R. D. *Biochemistry*, 1991, (30), 10795-9.

[99] Katona, G., Berglund, G. I. *Journal of Molecular Biology*, 2002, (315), 1209-18.

[100] Giacobini, E. *Neuroscience Research*, 2003, (28), 515-522.

[101] Scarpini, E., P. Scheltens. *Lancet Neurology*, 2003 (2), 539-47.

[102] Asthana, S., Greig, N. H. *Clinical Pharmacology and Therapeutics*, 1995, (58), 299-309.

[103] Sim, A. *Hospital Medicine*, 1999, (60), 731-5.

[104] Darvesh, S., Grantham, D. L. *The Journal of Comparative Neurology*, 1998, (393), 374-90.

[105] Holmstedt, B. In *Cholinesterases and cholinesterase inhibitors*, 2000, 1-9, Martin Duntz, London.

[106] Liston, D. R., Nielsen, J. A. *European Journal of Pharmacology*, 2004, (486), 9-17.

[107] Yang, Z. P., Dettbarn, W. D. *Biochemical Pharmacology*, 1998, (55), 1419-26.

[108] Yu, Q. *Journal of Medical Chemistry*, 1997, (40), 2895-2901.

[109] Yu, Q. *Journal of Medical Chemistry*, 1998, (41) 2371-2379.

[110] Yu, Q. *Journal of Medical Chemistry*, 2001 (44), 4062-4072.

[111] Gilmer, J. F., L. M. Moriarty. *European Journal of Pharmaceutical Sciences*, 2001, (14), 221-7.

[112] Thiem, J., Luders, H. *Polymer Bulletin,* 1984, (11), 365-369.

[113] Chatti, S., Bortolussi, M., Loupy, A. *Tetrahedron Letters*, 2000, (41), 3367-3370.

[114] Jacquet, F., Audinos, R., Delmas, M., Gaset, A. *Biomass,* 1985, (6), 193-209.

[115] Thiem, J., Luders, H. *Starch,* 1984, (36), 170-176.

[116] Thiem, J., Luders, H. *Makromolekular Chemie*, 1986, (187), 2775-2785.

[117] Thiem, J., Bachmann, F. *Makromolekular Chemie,* 1991, (192), 2163-2182.

[118] Cope, A.C., Shen, T. *Journal of the American Chemical Society,* 1956, (78), 3177-3182.

[119] Buck, K.W., Duxbury, J.M., Foster, A.B., Perry, A.R., Webber, J.M. *Carbohydrate Research*, 1966, (2), 122-131.

[120] Kurochkina, G., Bratsch, G.S., Soboleva, N.O., Vasyanina, L.K., Grachev, M.K., Nifant'ev, E.E. *Russian Journal of General Chemistry*, 2004, (74), 10, 1616-1619.;

[121] McInnes, L. *Canadian Journal of Chemistry*, 1960, (1), 38, 136-138

[122] Vogler, M., Koert, U., Dorsch, D., Gleitz, J., Raddatz, P. *Synlett*, 2003, (11), 1683 1687.

[123] Stoss, P., Merrath, P., Schluter, G. *Synthesis*, 1987, 174-177.

[124] Gizur, T., Harsanyi, K. *Synthetic Communications*, 1990, (20), 15, 2365-2371.

[125] Chalecki, Z., Guibe-Jampel, E. *Synthetic Communications,* 1997, (27), 22, 3847-3852.

[126] Tamion, R., Abenhaim, D., Loupy, A., Munnier, L., Marsais, F., Queguiner, G. *Carbohydrate Research*, 1994, (261), 255-266.

[127] Szeja, W. *Journal of the Chemical Society, Chemical Communications*, 1981, 215-216.

[128] Cekovic, Z., Tokic, Z. *Synthesis*, 1989, 610-612.

[129] Jackson, M., Hayward, L.D. *Canadian Journal of Chemistry*, 1960, (38), 496-502.

[130] Wiggins, L.F., Wood, J.C. *Journal of the Chemical Society*, 1951, 1180-1184.

[131] Tamion, R., Marsais, F., Ribereau, P., Queguiner, G., Aberhaim, D., Loupy, A., Munnier, L. *Tetrahedron: Asymmetry*, 1993, (4), 8, 1879-1890.

[132] Arya, V.P. *Indian Journal of Chemistry*, 1978, (16B), 153-155.

[133] Archibald, T.G., Baum, K. *Synthetic Communications*, 1989, (19), 1493-1498.

[134] Pickering, L., Nair, V. *Nucleosides and Nucleotides*, 1996, (15), 1751-1769.

[135] De Coster, G., Vandyck, K., Van der Eycken, E., Van der Eycken, J., Elserviers, M., Roper, H. *Tetrahedron: Asymmetry*, 2002, (13), 1673-1679.

[136] Jauquet, F., Grando, C., Rigal, L., Gaset, A. *Applied Catalysis*, 1985, (18), 157-172.

[137] Limberg, G., Thiem, J. *Synthesis*, 1994, 317-321.

[138] Liu, F.W., Yan, L., Zhang, J.Y., Liu, H.M. *Carbohydrate Research*, 2006, (341), 332-338.

[139] Chatti, S., Bortolussi, M., Bogdal, D., Blais, J.C., Loupy, A. *European Polymer Journal*, 2006, (42), 410-424.

[140] Bachmann, F., Reimer, J., Ruppenstein, M., Thiem, J. *Macromolecular Rapid Communications*, 1998, (19), 21-26.

[141] Caouther, A.A., Loupy, A., Bortolussi, M., Blais, J.C., Dubreucq, L., Meddour, A. *Journal of Polymer Science*, 2005, (43), 6480-6491.

[142] Bachmann, F., Thiem, J. *Journal of Polymer Science*, 1992, (30), 2059-2062.

[143] Okada, M., Tsunoda, K., Tachikawa, K., Aoi, K. *Journal of Applied Polymer Science*, 2000, (77), 338-346.

[144] Huang, L.L., Xu, M.H., Lin, G.Q. *Journal of Organic Chemistry*, 2005, (70), 529-532.

[145] Xu, M.H., Lin, G.Q., Wang, W., Xia, L.J. *Journal of Organic Chemistry*, 2001, (66), 3953-3962

[146] Loupy, A., Monteux, D.A. *Tetrahedron*, 2002, (58), 1541-1549.

[147] Kumar, S., Ramachandran, U. *Tetrahedron*, 2005, (61), 4141-4148.

[148] Sano, S., Teranishi, R., Nakano, F., Shiro, M., Nagao, Y. *Heterocycles*, 2003, (59), 793-801.

[149] Cekovic, Z., Tokic-Vujosevic, Z., Stepanovc-Petrovic, R.M., Bokonjic, D., Dobric, S. *Pharmazie*, 2002, (57), 507-508.

[150] Hayashi, H., Ueno, H., Suzuki, F. *Bioorganic and Medicinal Chemistry Letters*, 1992, (2), 1187-1192.

[151] Cekovic, Z., Tokic-Vujosevic, Z. *Synthesis,* 2001, (13), 2028-2034.

[152] Cekovic, Z., Tokic-Vujosevic, Z. *Heterocyclic Communications,* 2001, (7), 43-48.

[153] Muri, E.M.F., Gomes, M., Albuquerque, M.G., da Cunha, E.F.F., de Alencastro, R.B. *Amino Acids*, 2005, (28), 413-419.

[154] Bencsik, J.R., Kercher, T. *Organic Letters*, 2003, (5), 2727-2730.

[155] Dietrich, E.; Lubell, W.D. *Journal of Organic Chemistry,* 2003, (68), 6988-6996.

[156] Chao, Q., Zhang, J., Pickering, L., Jahnke, T.S., Nair, V. *Tetrahedron,* 1998, (54), 3113-3124.

[157] Hrebabecky, H., Dockal, J., Holy, A. *Collection of Czechoslovak Chemical Contributors,* 1994, (59), 1408-1419.

[158] Matsuura, D., Nojiri, T., Suzuki, Y., Takabe, K., Yoda, H. *Synlett*, 2005, (2), 287-288.

[159] Liu, H.M., Liu, F.W., Zou, D.P., Dia, G.F. *Bioorganic and Medicinal Letters*, 2005, (15), 1821-1824.

[160] Girard, C. Greck, C. *Tetrahedron Letters*, 1996, (37), 7967.

[161] Pitts, M.R., Harrison, J.R., Moody, C. *The Journal of the Chemical Society; Perkins Transmissions 1,* 2001, 955.

[162] Rahman, A., Fraser-Reid, B. *Journal of the American Chemical Society*, 1985, (107), 5576-5578.

[163] Booker-Milburn, K.I., Dunkin, I.R. *The Journal of the Chemical Society; Perkins Transmissions 1*, 1997, 3261.

[164] Sherman, A. R, Ed: L. Paquette. *Encyclopedia of Reagents for Organic Synthesis*, 2004, J. Wiley & Sons

[165] Kurochkina, G., Bratsch, G.S., Soboleva, N.O., Vasyanina, L.K, Grachev, M.K., Nifant'ev, E.E. *Russian Journal of General Chemistry*, 2004, (74), 10, 1616-1619.

[166] Adamczyk, M., Chen, Y., Moore J.A., Mattingly, P.G. *Bioorganic & Medicinal Chemistry Letters,* 1998, (8), 1281-1284.

[167] Kolakowski, R. V., N. Shangguan, N. *Journal of the American Chemical Society*, 2006, (128), 5695-702.

[168] Barlett, K. N., Kolakowski, R. V. *Organic Letters*, 2006, (8), 823-6.

[169] Hudlicky, M. *Reductions in Organic Chemistry,* 1984, Chichester publishing, New York.

[170] Saito, S. *Tetrahedron Letters*, 1989, (30), 837-838

[171] Chong, P.Y., Janicki, S.Z., Petillo, P.A. *Journal of Organic Chemistry*, 1998, (63), 8515-8521.

[172] Ireland, R., Norbeck, D.W. *Journal of the American Chemical Society*, 1985, (107), 3279-3285..

[173] Gawrorski, J.K., Reddy, S.M., Walbarsky, H.M. *Journal of the American Chemical Society*, 1987, (109), 69-26-6730.

[174] Silveira, M.H., Okamura, W.H. *Journal of Organic Chemistry*, 1985, (50), 2390-2392.

[175] Hopton, F.J., Thomas, G.H.S. *Canadian Journal of Chemistry*, 1969, (47) 2395.

[176] Ellman, G.L., Courtney, K.D. *Biochemical Pharmacology*, 1961, (7), 88-95.

[177] Kamban, J.R., Horton, B., Parris, W.C. *Anaesthesia and Analgesia*, 1989, (68), 486-488.

[178] Venkataraman, B. V., Rani M. A. *Indian Journal of Physiology and Pharmacology*, 1994, (38), 211-3.

[179] Abdel-Aal, Y. A. I., Lampert, E.P. *Cellular and Molecular Life Sciences*, 1993, (49), 571-575.

[180] Radic, Z., Pickering, N. A. *Biochemistry*, 1993, (32), 12074-84.

[181] Costagli, C., Galli, A. *Biochemical Pharmacology*, 1998, (55), 1733-7.

[182] Sandler, M. (Ed.). *Enzyme Inhibitors as Drugs*, 1980, Macmillan, London.

[183] Gnatt, A., Loewenstein, Y. *Journal of Neurochemistry*, 1994, (62), 749-55.

[184] Ogura, H., Kosasa, T. *Methods and Findings in Experimental and Clinical Pharmacology*, 2000, (22), 609-13.

[185] Ibach, B., Haen, E. *Current Pharmaceutical Design*, 2004, (10), 231-251.

[186] Lane, R. M., Kivipelto, M. *Clinical Neuropharmacology*, 2004 (27), 141-9.

[187] Reale, M., Iarlori, C. *Neuropharmacology*, 2006 (50), 606-13.

[188] Almkvist, O., Darreh-Shori, T. *European Journal of Neurology*, 2004, (11), 253-61.

[189] Grossberg, G. T. *Current Medical Research and Opinion*, 2005 (21), 1631-9.

[190] Gaynor, J.M., Dillon, G.P., Reidy, S., Gilmer, J.F. *Chemico-Biological Interactions*, 2005, (157-158), 380-381.

[191] Hirschmann, R., Ducry, L. *Journal of Organic Chemistry*, 2000, (65), 8307-8316.

[192] Robins, M.J., Miles, R.W. *Journal of Organic Chemistry*, 2001, (66), 8204-8210.

[193] Uchiyama, M., Hirai, M. *Tetrahedron Letters,* 2001, (42), 4653-4656.

[194] Wang, X., Yang, X., Wu, Y. *Reaction Kinetics,* 1998, (64), 309-316.

[195] Dinesh, V., Patel, F. *Journal of the American Chemical Society*, 1986, (108), 4603-4614.

[196] Mereyala, H.B., *Tetrahedron: Assymetry*, 2004, (15), 3457-3460.

[197] Acar, M.H., Gulkanat, A. *Polymer*, 2000, (41), 6709-6713.

[198] Ito, M., Okui, H., Nakagawa, H. *Bioscience, Biotechnology, and Biochemistry*, 2003, (67), 1230-1238.

[199] Nussbaumer, P., Lehr, P., Billich, A. *Journal of Medicinal Chemistry*, 2002, (45), 4310-4320.

[200] Trost, B. M., Chan, D.M.T. *Journal of the American Chemical Society*, 1981, (103), 5972-5974.

[201] Simoni, D., Grisolia, G., Giannini, G., Roberti, M. *Journal of Medicinal Chemistry*, 2005 (48), 723-736.

[202] Li, J.J. *Name Reactions; a collection of detailed reaction mechanisms*, 2003, Springer-Verlag publishing, Berlin

[203] Wiberg, K.B., Snoonian, J.R. *Journal of Organic Chemistry,* 1998, (63), 1390-1401.

[204] Mino, T., Masuda, S., Nishio, M., Yamashita, M. *Journal of Organic Chemistry*, 1997, (62), 2633-2635.

[205] De Luca, L., Giacomelli, G., Porcheddu. *Journal of Organic Chemistry*, 2002, (67), 6272-6274.

[206] Chandrasekhar, S., Gopalaiah, K. *Tetrahedron Letters*, 2002, (43), 2455-2457.

[207] Thakur, A.J., Boruah, A., Prajapati, D., Sandhu, J.S. *Synthetic Communications*, 2000, (30), 2105.

[208] Chandrasekhar, S., Gopalaiah, K. *Tetrahedron Letters*, 2003, (44), 755-756.

[209] Paul, S. *Tetrahedron Letters*, 2004, (45), 8825-8829.

[210] Lysek, R., Wozny, E. *Tetrahedron Letters*, 2003, (44), 7541-7544.

[211] Ashton, P.R., Heiss, A.M., Pasini, D., Raymo, F.M. *European Journal of Organic Chemistry,* 1999, 995-1004.

[212] Czech, B. P., Bartsch, R.A. *Journal of Organic Chemistry,* 1984, (49), 4076-4078.

[213] Greene, T.W., *Protective groups in organic synthesis,* 1981, John Wiley, New York

[214] Corey, E. J., Venkateswarlu, A. *Journal of the American Chemical Society*, 1972, (94), 6190-6191.

[215] Cox, D. P., Terpinski, J. *Journal of the American Chemical Society*, 1984, (49), 3216-3219.

[216] Tan, Z. P., Wang, L., Wang, J.B. *Chinese Chemical Letters*, 2000, (11), 753-756.

[217] Cai, S. X., Nquyen, B. *Journal of Medical Chemistry,* 2003, (46), 2474-2481.

[218] Flekhter, O. B., Karachurina, L.T. *Russian Journal of Bioorganic Chemistry*, 2002, (28), 494-500.

[219] Grosshans, P., Jouaiti, A., Bulach, V., Planeix, J.M., Hosseini, M.W., Nicoud, J.F. *CrystEngComm,* 2003, (5), 73, 414-416.

[220] Grosshans, P., Jouaiti, A., Bulach, V., Planeix, J.M., Hosseini, M.W., Nicoud, J.F. *Comptes rendus de l'Acadamie des sciences*, 2004, (7), 189-196

[221] Ichikawa, A., Ono, H., Harada, N. *Chirality*, 2004, (16), 559-567.

[222] Gilmer, J.F., Byrne, W. *World Intellectual Property Organization*, 1998, Publication no. WO/1998/017673. [http://www.wipo.int/portal/index.html.en]

[223] Ye, T., McKervey, M.A. *Journal of the Chemical Society; Perkin Transitions 1*, 1995, 1373-1379.

[224] Varkey, J. T. *Journal of Peptide Science*, 1999, (5), 577-581.

[225] Battersby, A. R., Cardwell, K.S. *Journal of the Chemical Society; Perkin Transitions 1*, 1986, 1565-1580.

[226] El-Moselhy, T. F., Avor, K.S. *Archiv der Pharmazie*, 2001, (334), 275-8.

[227] El-Moselhy, T., Avor, K.S. *European Journal of Medicinal Chemistry,* 2002, (37), 171-176.

[228] Arienti, K., Brunmark, A., Axe, F.U. *Journal of Medicinal Chemistry*, 2005, (48), 1873-1885.

[229] Bourne, Y., Radic, Z. [Crystal structure of mouse AChE complexed with tri-methyl tri-fluoro acetophenone] from http://www/pdb.org (Protein Data Bank). Article in publication.

[230] Atkinson, R. F., Balko, T.W. *Journal of Organic Chemistry*, 1981, (46), 2804-2806.

[231] Brooks, G., Burgess, W., Colthurst, D. *Bioorganic and Medicinal Chemistry*, 2001, (9), 1221-1231.

[232] Ponaras, A. A., Zaim, O. *Journal of Organic Chemistry*, 1987, (52), 5630-5633.

[233] Orlemans, E. O. M., Verboom, W. *Journal of Medical Chemistry,* 1989, (32), 1612-1620.

[234] D'Souza, M., Kevill, D.N. *Journal of Organic Chemistry*, 1995, (60), 1632-1637.

[235] Lee, L., Hossain, M. *Journal of Clinical Pharmacology*, 2004, (44), 599-604.

[236] Atack, J.R., Yu, Q.S. *Journal of Pharmacology and Experimental Therapeutics*, 1989, (241), 194-204.

APPENDIX

Table A.1: Cholinesterase inhibition, elemental analysis and HRMS characterisation data for test compounds.

No.	Name	% BuChE 100 μM	% AChE 100 μM	IC_{50} (μM) BuChE	IC_{50} (μM) AChE	Elemental Analysis and HRMS
	Chapter 2 compounds Reverse carbamates					
	2-BOC					
93	5-hyroxyl	13.8	3.5	-	-	Req. C, 53.87; H, 7.81; N, 5.71: Fnd. C, 53.54; H, 7.72; N, 5.95.
97	5-benzoyl	2.0	5.7	-	-	Req. C, 61.88; H, 6.64; N, 4.01: Fnd. C, 62.03; H, 6.51; N, 3.78
98	5-*p*-nitrobenzoyl	9.5	4.6	-	-	Req. C, 54.82; H, 5.62; N, 7.10: Fnd. C, 54.57; H, 5.68; N, 6.91
99	5-*p*-bromobenzoyl-	9.6	2.8	-	-	Req. C, 50.48; H, 5.18; N, 3.27: Fnd. C, 50.55, H, 4.88; N, 3.40
	2-Ethyl					
94	2-hydroxyl	9.3	2.0	-	-	HRMS (M + 23) Req.240.0849; Fnd. 240.0050.
100	5-benzoyl	29.3	0.8	-	-	Req. C, 59.81; H, 5.96; N, 4.36: Fnd. C, 59.99; H, 6.03; N, 4.01
101	5-*p*-nitrobenzoyl	35.6	0.4	-	-	Req. C, 52.46; H, 4.95; N, 7.65: Fnd. C, 52.13; H, 4.66; N, 7.28
	2-Phenyl					
95	5-hydroxyl	25.0	0.8	-	-	Req. C, 58.86; H, 5.70; N, 5.28: Fnd. C, 58.70; H, 5.62; N, 5.38
102	5-benzoyl	78.5	0.4	32.8	-	Req. C, 65.03; H, 5.18; N, 3.79: Fnd. C, 65.35; H, 5.03; N, 3.52
103	5- *p*-nitrobenzoyl	92.1	45.4	16.1	-	Req. C, 57.97; H, 4.38; N, 6.76: Fnd. C, 57.82; H, 4.50; N, 6.48
104	5- *p*-bromobenzoyl	89.4	68.1	21.9	-	Req. C, 53.59; H, 4.05; N, 3.12: Fnd. C, 53.96; H, 4.22; N, 3.27
105	5-2,6 dichlorobenzoyl	5.1	45.3	-	-	Req. C, 54.81; H, 3.91; N, 3.20: Fnd. C, 55.03; H, 4.11; N, 3.40
106	5-*o*-methylbenzoyl	95.2	1.0	1.5	-	Req. C, 65.79; H, 5.52; N, 3.65: Fnd. C, 65.38; H, 5.45; N, 3.78
107	5-*o*-methyloxybenzoyl	65.3	0.3	-	-	HRMS (M + 23) Req. 422.1217; Fnd 422.2633.
	2-Benzyl					
96	5-hydroxyl	3.5	0	-	-	Req: C, 60.21; H, 6.14; N, 5.02: Fnd: C, 60.56; H, 5.99; N, 4.78.
108	5-benzoyl	8.3	65.0	-	-	Req: C, 65.79; H, 5.52; N, 3.65: Fnd C, 65.51; H, 5.62; N, 3.59.
109	5-*o*-methylbenzoyl	4.3	45.2	-	-	Req. C, 66.69; H, 5.83; N, 3.52: Fnd. C, 66.35; H, 5.71; N, 3.33.

No.	Name	% BuChE 100 μM	% AChE 100 μM	IC_{50} (nM) BuChE	IC_{50} (μM) AChE	Elemental Analysis and HRMS
	Chapter 3 compounds					
	Non-carbamate compound					
150	2-*O*-benzyl-5-*O*-nitro	4.0	9.4	-	-	HRMS (M + 23) Req, 304.0798: Fnd, 304.0861.
	5 -non-substituted compounds					
112	5-hydroxyl	94.5	14.8	3720	-	
131	5-deoxy-5-azido -1,4:3,6-dianhydro-L-iditol	50.4	4.6	-	-	Req C, 55.26; H, 5.30; N, 18.41: Fnd C, 55.58; H, 5.10; N, 17.99
119	5-deoxy-L-xylohex-6-enitol	91.8	9.8	10250	-	-
	5-aliphatic ester substituted compounds					
114	5-*O*-acetyl	96.7	11.9	4018	-	Req. C, 59.81; H, 5.96; N, 4.36: Fnd. C, 59.92; H, 5.96; N, 4.36
115	5-*O*-propionyl	96.7	4.8	986.14	-	. Req. C, 60.89; H, 6.31; N, 4.18: Fnd. C, 60.89; H, 6.29; N, 4.17
116	5-*O*-valeryl	95.2	4.3	701.79	-	Req. C, 62.80; H, 6.93; N, 3.85: Fnd. C, 62.87; H, 6.93; N, 3.78
	5-cycloalkyl ester substituted compounds					
120	5-(cyclopropylcarbonyloxy-)	96.8	54.8	334.62	-	Req. C, 62.24; H, 6.09; N, 4.03: Fnd. C, 62.20; H, 6.09; N, 3.97
121	5-(cyclopentylcarbonyloxy-)	99.3	87.7	5.77	56.23	Req. C, 63.99; H, 6.71; N, 3.73: Fnd. C, 63.98; H, 6.76; N, 3.64
	5-sulfonyl ester substituted					
118	5-*O*-trifluoromethanesulfonyl	85.1	5.8	358.93	-	Req. C, 43.80; H, 3.92; N, 3.41: Fnd. C, 43.82; H, 3.89; N, 3.19
119	5-*O*-methanesulfonyl	9.8	2.7	-	-	Req. C, 50.41; H, 5.36; N, 3.92: Fnd. C, 50.43; H, 5.33; N, 3.75
130	5-*O*-toluenesulfonyl	25.2	65.9	-	-	HRMS (M + 23) Req. 456.1108; Fnd, 456.1093.
	5-aromatic ester substituted compounds					
122	5-*O*-1-naphthoyl	98.6	86.0	28.21	37.60	Req. C, 69.27; H, 5.35; N, 3.23: Fnd. C, 68.96; H, 5.25; N, 2.94
123	5-*O*-2-naphthoyl	99.6	78.1	31.75	43.42	. Req. C, 69.27; H, 5.35; N, 3.23:. Fnd. C, 69.10; H, 5.31; N, 3.08
124	5-(*p*-phenyl-phenylcarbonyloxy-)	98.6	89.3	12.33	51.64	Req. C, 70.58; H, 5.48; N, 3.05: Fnd. C, 70.29; H, 5.46; N, 2.96
128	5-*O*-nicotinoyl	99.4	32.9	57.33	-	Req. C, 62.49; H, 5.24; N, 7.29; Fnd. C, 62.42; H, 5.22; N, 6.96
129	5-*O*-isonicotinoyl	96.9	23.9	88.30	-	HRMS(M + 23) Req, 407.1233; Fnd, 407.1219.
125	5-*O*- cinnamoyl	95.5	82.8	137.02	-	HRMS (M + 23) Req: 409.1423; Fnd, 409.1440.
126	5-(*p*-heptyloxyphenylcarbonyloxy-)	79.2	-	2787	-	Req. C, 67.59; H, 7.09; N, 2.81: Fnd. C, 67.60; H, 7.09; N, 2.74
127	5-(coumarincarbonyloxy-)	99.8	80.7	72.64	60.41	Req. C, 63.85; H, 4.69; N, 3.10: Fnd. C, 63.59; H, 4.69; N, 2.98.

No.	Name	% BuChE 100 μM	% AChE 100 μM	IC_{50} (nM) BuChE	IC_{50} (μM) AChE	Elemental Analysis and HRMS
	5- aromatic ether substituted compounds					
140	5-*O*-benzyl	92.8	54.9	52.29	-	Req. C, 68.28; H, 6.28; N, 3.79: Fnd. C, 68.22; H, 6.27; N, 3.69
148	5-*O*-(phenylpropyloxy-)	97.8	50.9	201.45	-	Req C, 69.50; H, 6.85; N, 3.52: Fnd C, 69.46; H, 6.88; N, 3.39.
	5-amide substituted compound					
154	5-benzylamide	88.1	46.1	-	-	HRMS (M + 23);Req, 405.1427; Fnd, 405.1367.
	Ketone derivatives					
113	5-ketone	46.1	-	-	-	Req. C, 60.64; H, 5.45; N, 5.05: Fnd. C, 60.73; H, 5.43; N, 4.67.
132	5-oxime	40.1	-	-	-	HRMS (M + 23) Req, 315.0959: Fnd, 315.0957.
133	5-oxime benzoate ester	64.8	-	-	-	Req. C, 63.63; H, 5.09; N, 7.07: Fnd. C, 63.69; H, 4.92; N, 6.77
134	5-alkene ester [Wittig reaction]	91.3		4838	-	Req. C, 62.24; H, 6.09; N, 4.03: Fnd. C, 62.20; H, 6.08, N, 3.98
137	Lactone [Baeyer-Villiger reaction]	56.5	-	-	-	HRMS (M + 23);Req, 316.0798: Fnd, 316.0881.
139	Lactam [Beckmann reaction]	63.4	-	-	-	HRMS (M + 23) Req, 329.1115: Fnd, 329.1212.
	Chapter 4 compounds					
	5-substituted benzoyl esters					
162	5-(salicyloyl)	99.8	62.6	0.179	10.4	Req. C, 63.15; H, 5.30; N, 3.51: Fnd. C, 62.96; H, 5.29; N, 3.41.
171	5-(*m*-hydroxy benzoyl)	99.2	0	16.34	-	HRMS (M + 23) Req, 422.1216; Fnd, 422.1215
172	5-(*p*-hydroxy benzoyl)	98.5	0	323.38	-	HRMS (M + 23);Req, 422.1216; Fnd, 422.1207.
173	5-(*o*-nitro) benzoate	99.3	0	18.04	-	HRMS (M + 23) Req, 451.1117: Fnd, 451.1132.
174	5-(*m*-nitro benzoyl)	96.1	0	32.00	-	HRMS (M + 23) Req, 451.1117; Fnd, 451.1104
175	5-(*p*-nitro benzoyl)	99.1	58.7	117.00	-	Req. C, 58.88; H, 4.71; N, 6.54: Fnd. C, 58.65; H, 4.71; N, 6.36.
178	5-(*o*-amino benzoyl)	98.6	0	27.43	-	HRMS (M + 23) Req, 421.1393; Fnd, 421.1376.
179	5-(*m*-amino benzoyl)	98.1	0	85.73	-	HRMS (M + 23) Req, 421.1393; Fnd, 421.1376.
180	5-(*p*-amino benzoyl)	99.4	98.1	297.06	-	Req. C, 63.31; H, 5.57; N, 7.03:. Fnd. C, 62.91; H, 5.39; N, 6.57
176	5-(*o*-nitroso benzoyl)	99.7	0	38.80	-	HRMS (M + 23) Req, 435.1169; Fnd, 435.1270.

No.	Name	% BuChE	% AChE	IC_{50} (nM)	IC_{50} (μM)	IC_{50} (nM)
		100 μM	100 μM	BuChE	AChE	huBuChE
	2-carbamate analogues					
187	2-ethyl	97.0	15.9	9.61	-	Req. C, 56.97; H, 5.68; N, 4.15:. Fnd. C, 56.77; H, 5.69; N, 4.02
188	2-dimethyl	99.8	0	6.40	-	HRMS (M + 23): Req, 360.1054: Fnd 360.1059.
189	2-diethyl	95.0	0	2609	-	HRMS (M + 23): Req, 388.1373: Fnd 388.1361.
190	2-morpholine	94.8	12.0	1057	-	Req. C, 56.99; H, 5.58; N, 3.69:. Fnd. C, 56.68; H, 5.63; N, 3.69
	Chapter-5 compounds					
192	2-benzoyl carbamate 5-benzoyl isomannide	66.8	92.3	-	-	Req. C, 65.79; H, 5.52; N, 3.65: Fnd. C, 65.66; H, 5.66; N, 3.38
193	2-benzoyl carbamate 5-benzoyl isoiodide	41.7	89.7	-	-	Req. C, 65.79; H, 5.52; N, 3.65: Fnd. C, 65.56; H, 5.63; N, 3.54.
194	2-benzoyl 5-benzyl carbamate isosorbide	50.6	81.7	-	-	Req. C, 65.79; H, 5.52; N, 3.65: Fnd. C, 65.90; H, 5.52; N, 3.57

PUBLISHED ARTICLES

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemico-Biological Interactions
ELSEVIER
Chemico-Biological Interactions 157–158 (2005) 317–319
www.elsevier.com/locate/chembioint

Short communication

Novel isosorbide-based substrates for human butyrylcholinesterase

J.F. Gilmer[a,*], M.N. Lally[a], P. Gardiner[a], G. Dillon[a], J.M. Gaynor[b], S. Reidy[b]

[a] *School of Pharmacy, Trinity College Dublin, Ireland*
[b] *School of Science, Athlone Institute of Technology, Ireland*

Available online 2 November 2005

Abstract

Butyrylcholinesterase [EC 3.1.1.8] present widely in mammalian tissue does not have a precisely defined biological function or known endogenous substrate. However, it plays an important role in the detoxification of certain xenobiotics and is an established vector for the systemic liberation of other drugs from their prodrugs. While investigating a series of isosorbide-based prodrugs, we discovered that BuChE catalyses the hydrolysis of esters of the simple sugar isosorbide with unusually rapidity and in some cases with remarkable regioselectivity. In this study, a series of isosorbide esters were synthesised and their rates of hydrolysis measured by HPLC following incubation in diluted plasma solution. In general, little hydrolysis of the 5-ester group could be observed but the 2-ester group was usually hydrolysed very rapidly and the hydrolysis rate exhibited an unusual dependence on the identity of the 5-group. The results indicate that while the 5-ester group is not itself hydrolysed it is important for productive binding in isosorbide diesters.

Keywords: Butyrylcholinesterase; Substrate; Isosorbide esters; Human plasma

1. Introduction

Both acetylcholinesterase (AChE EC 3.1.1.7) and butyrylcholinesterase (BuChE 3.1.1.8) catalyse the hydrolysis of choline esters, the latter enzyme being generally distinguished by its capacity to hydrolyse larger esters such as butyrylcholine, benzoylcholine and propionylcholine. BuChE also catalyses the hydrolysis of many xenobiotics, but not with notable efficiency: prodrugs designed to mimic the structural features of the choline unit (e.g. glycolamide) [1] and conceptually related soft drugs may be an exception (Fig. 1).

While investigating potential prodrugs of aspirin we discovered that certain isosorbide esters are hydrolysed with remarkable regioselectivity and rapidity in the presence of BuChE. ISMNA 2 is hydrolysed exclusively at the 2-benzoate ester* in rabbit plasma ($t_{1/2}$ ~4 min in 10% plasma solution). In addition, the diaspirinate analogue 3 is hydrolysed predominantly at the 2-benzoate* in the presence of three other esters ($t_{1/2}$ ~1 min in 30% human plasma $K_{m,app} = 5e^{-5}$) [2]. The K_m values for **2** and **3** are similar to butyrylcholine under these conditions, indicating similar affinity. These two compounds are among the very few aspirin esters observed to undergo productive hydrolysis liberating aspirin. The aim of this study was to survey *h*BuChE hydrolysis efficiency towards isosorbide esters in general. It was expected that this would help in the design of isosorbide-based inhibitors and potentially in revealing the identity of unknown endogenous substrates.

* Corresponding author.

0009-2797/$ – see front matter
doi:10.1016/j.cbi.2005.10.095

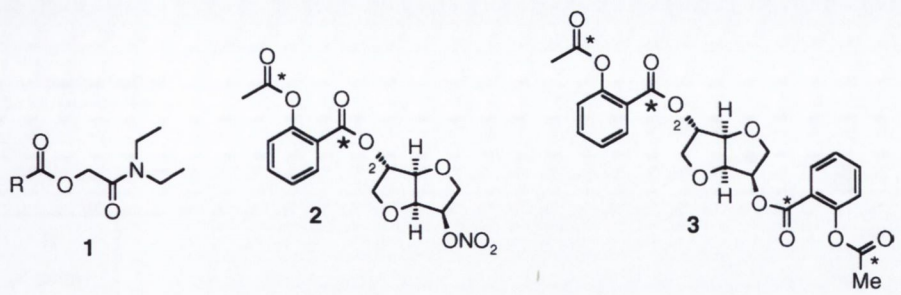


Fig. 1. Glycolamide and isosorbide-based esters.

2. Experimental

2.1. Synthesis of isosorbide-based substrates

Isosorbide-2-esters were generated from isosorbide mononitrate (ISMN; **4**) by treatment with the appropriate acid chloride in the presence of a tertiary base in dichloromethane (Fig. 2). Some esters were further elaborated by reduction of the nitrate ester and esterification using the appropriate acid chloride and a tertiary base. Compounds were characterised by ^{1}H and ^{13}C NMR, MS, TLC and HPLC.

2.2. Substrate hydrolysis experiments

Ester substrate compounds ($2e^{-4}$ M) were incubated in pH 7.4 buffered human plasma (37 °C) and the hydrolysis reaction monitored by RP-HPLC (C8: MeCN/pH 2.5 buffer 50:50) [1,2]. Disappearance kinetics were strictly first-order. The first order rate constant was determined from the slope of plot of ln remaining compounds versus concentration and half-lives were estimated using the expression $t_{1/2} = 0.693/k_{obs}$. Hydrolysis was also determined using phosphate-buffered solutions containing purified human BuChE [Sigma] at 0.015 mg/ml in pH 7.4 buffer (37 °C). The BuChE activity of human plasma was determined using the Ellman assay with butyrylthiocholine as substrate.

2.3. Molecular modelling

An ester substrate was minimised in MacroModel 6.5 using a Monte Carlo search (1000 iterations, PRCG) and the MM3 forcefield. The minimised structure was imported into Insight II and backbone-aligned to the synthetic substrate butyrylcholine, docked within a homology model of *h*BuChE kindly supplied by Harel et al. [3]. The isosorbide-2-ester carbonyl and the Ser-200 residue were tethered to their original positions. The ester and enzyme were then flexibly minimised to remove any high-energy interactions by using a simulated annealing run in Insight II. High energy conformations discovered in the protein were resolved by a further repetition of the minimisation process. The resulting protein structure was then backbone aligned to the original PDB file and the conformation for the substrate overlaid with that of butyrylcholine.

3. Results

3.1. Hydrolysis studies

Twenty-four Esters were synthesised and BuChE-mediated hydrolysis monitored by HPLC: data for a representative group are presented in Fig. 3. Very rapid hydrolysis of the 2-ester group was observed in all cases with half-lives in 10% buffered plasma ranging from 20 s to 25 min depending on the ester structure and the substituent at the 5-position.

The rapid hydrolysis of the isosorbide ester family suggests a quite specific interaction with *h*BuChE. This impression is reinforced by the observation that no hydrolysis of the 5-ester group was observed over the time course of the reactions here. Although 5-esters are not susceptible to hydrolysis, the data indi-

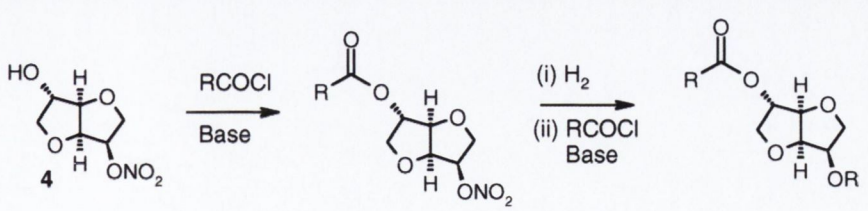
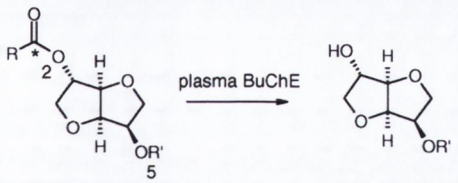


Fig. 2. Synthesis of isosorbide-based substrates.



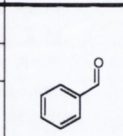
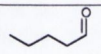
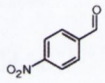
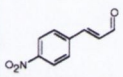
Compound	R (2-ester)	R'	$t_{1/2}$(sec) 10% plasma (50%)
5		H	1260 (357)
6		NO_2	210 (33)
7			72 (19)
8		NO_2	39
9		NO_2	192
10		NO_2	850

Fig. 3. Hydrolysis of isosorbide-based esters and corresponding half-lives in diluted plasma (pH 7.4) and 37 °C.

cates that 5-group makes an important contribution to enzyme–substrate contacts. The rate of hydrolysis at position 2 significantly increases through the series; 2-benzoate-5-OH 5 (k_{obs}, $5.5e^{-4}$ min^{-1}); 2-benzoate-5-nitrate 6 (k_{obs}, $3.3e^{-3}$ min^{-1}); 2–5-dibenzoate 7 (k_{obs}, $9.6e^{-3}$ min^{-1}), i.e. the relatively remote 5-group effects a 20-fold increase in rate across the series 5-OH, 5-ONO_2, 5-benzoate. The $K_{m,app}$ values associated with the hydrolysis also diminish across the series, indicating that the 5-group is important for productive binding. The turnover number for the dibenzoate in the presence of purified BuChE was estimated at 4.04 μmol/mg protein/min; ester hydrolysis under these conditions followed the same rank order as in human plasma.

There were no significant differences between the rates of hydrolysis for simple benzoates and alkyl esters at the 2-position (for compounds bearing similar 5-esters). However, bulkier esters such as the cinnamate 10, or the ibuprofen ester (not shown) underwent somewhat slower hydrolysis.

3.2. Modelling

The ester substrates modelled in Macromodel exhibited an unexpectedly folded conformation due the orientations of the 2-*exo* and 5-*endo* esters. When superimposed on the synthetic substrate butyrylcholine, the 2-benzoate overlapped with the butyryl ester while the choline group overlapped with the 5-ester group. In the protein ligand-complex, the 5-oxygen penetrates a π-pocket bounded by Tyr 442, Trp 84 and Tyr 334 with the 5-aryl ester pointing back out the channel. The bulky 2-ester group is accommodated in the so-called acyl pocket, making hydrophobic interactions with Leu 288 but also with Trp 233. The ester–BuChE contacts were analysed using LPC, which indicated a high overall surface complementarity (score of 0.94).

4. Conclusions

Isosorbide-based 2-esters are hydrolysed unusually rapidly, and specifically by *h*BuChE. The hydrolysis exhibits a 20-fold rate enhancement by esters present in the 5-position, which appears to be due to the promotion of enzyme–ligand binding. The modelling indicates that isosorbide compounds are structurally similar to the butyrylcholine substrate and bind in a similar manner. The work suggests that isosorbide might be used as a template for BuChE inhibitor design and conformational analysis of choline esters.

References

[1] J.F. Gilmer, L.M. Moriarty, M.N. Lally, J.M. Clancy, Isosorbide-based aspirin prodrugs. Part II. Hydrolysis kinetics of isosorbide diaspirinate., Eur. J. Pharm. Sci. 16 (2002) 297–304.

[2] J.F. Gilmer, L.M. Moriarty, D.F. McCafferty, J.M. Clancy, Synthesis, hydrolysis kinetics and antiplatelet effects of isosorbide mononitrate derivatives of aspirin, Eur. J. Pharm. Sci. 14 (3) (2001) 221–227.

[3] M. Harel, J.L. Sussman, E. Krejci, S. Bon, P. Chanal, J. Massoulie, I. Silman, Conversion of acetylcholinesterase to butyrylcholinesterase: modeling and mutagenesis, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 10827–10831.

References

[1] G. Benzi, A. Moretti, Is there a rationale for the use of acetylcholinesterase inhibitors in the therapy of Alzheimer's disease? Eur. J. Pharmacol. 346 (1989) 1–13.
[2] J.F. Trujillo, J. Espinosa, R. Santillán, N. Farfán, H. Hôpfl, Reversible and irreversible inhibitory activity of succinic and maleic acids derivatives on acetylcholinesterase, Eur. J. Pharm. Sci. 18 (2003) 313–322.
[3] J.L. Carter, S.J. Brimijoin, Effects of acute and chronic denervation on release of acetylcholinesterase and its molecular forms in rat diaphragms, J. Neurochem. 36 (1981) 1018–1025.
[4] S.L. Bonting, R.M. Featherstone, Ultramicro assay of the cholinesterase, Arch. Biochem. Biophys. 61 (1956) 89–98.
[5] P.A. Lehmann, Dosis letal o efectiva media: un cálculo fácil y rápido, Arch. Farmacol. Toxicol. VII (1980) 161–177.
[6] Z. Radic, N.A. Pickering, D.C. Vellom, S. Camp, P. Taylor, Three distinct domains in the cholinesterase molecule confer selectivity for acetyl- and butyrylcholinesterase-inhibitors, Biochemistry 32 (1993) 12074–12084.

doi:10.1016/j.cbi.2005.10.062

(18)

Synthesis and structure acitivity relationships (SAR) of a new class of potent and selectivie butyrylcholinesterase inhibitors

Joanne. M. Gaynor[a,*], Gerald. P. Dillon[b,*], Sean Reidy[a], John. F. Gilmer[b]

[a] *School of Science, Athlone I.T. Co Westmeath, Ireland*
[b] *School of Pharmacy, Trinity College, Co Dublin, Ireland*

Abstract

Reported here is the synthesis and SAR of novel group of highly potent and selective inhibitors of human plasma butyrylcholinesterase (BuChE; EC 3.1.1.8). The design is based on the discovery that isosorbide 2-esters are hydrolysed by BuChE at exceptionally rapid rates. Two families of carbamates were synthesised in which the vulnerable 2-ester was replaced with a carbamate or reversed carabamate. Several compounds in one of the families are among the most potent and selective BuChE inhibitors reported.

Keywords: Butyrylcholinesterase; Inhibition; Isosorbide

* Corresponding author.
E-mail addresses: jgaynor@ait.ie (J.M. Gaynor), dillongp@tcd.ie (G.P. Dillon).

1. Experimental

Inhibitor potency and selectivity for BuChE and Acetylcholinesterase (AChE; EC 3.1.1.7) were evaluated using the Ellman assay [1]. Human plasma was used as a source of BuChE with butyrylthiocholine iodide (0.5 mM) as substrate. Electric Eel AChE type III or human RBC AChE (both Sigma) was employed with acetylthiocholine idodide (0.2 mM) as substrate and 5,5′-dithiobis(2-nitrobenzoic acid) (0.3 mM). AChE and BuChE activities were determined spectrophotometrically at 412 nm in 0.1 M sodium phosphate buffer, pH 8.0. Compounds were preincubated with the buffered enzyme at 37 °C. % Inhibition was initially measured at 100 μM and expressed as a percentage of the activity seen in the absence of inhibitor. IC_{50} values were determined for compounds exhibiting significant activity at 100 μM using Xlfit 4.0. Potent compounds were further evaluated using purified human serum BuChE and human erythrocyte (RBC) AChE. Approx 90 compounds were evaluated: around 70 from Group 1 and 20 from Group 2:

2. Results

Inhibition results for some representative compounds are presented in Table 1: In general compounds from Group 1 were potent and selective inhibitors of human plasma BuChE. A small number of compounds in this group were moderate inhibitors of AChE (~μM), but not especially selective. Compounds in Group 2 were much less potent inhibitors of either enzyme (Table 2).

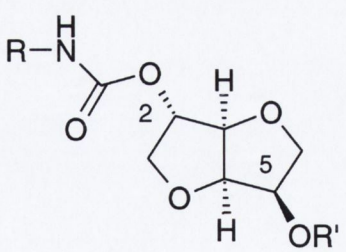


Plate 1. Group 1 2-carbamate ester 5-aryl ester.

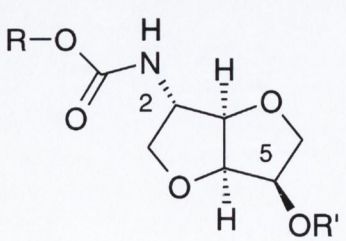


Plate 2. Group 2 Reversed carbamates.

Table 1
IC_{50} values and selectivity of Group 1 compounds

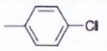
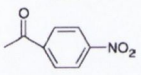
R	R′	Electric Eel AChE	Human RBC AChE	Human plasma BuChE (purified)	Selectivity
	NO_2	34 μM	868 μM	>100 μM	–
		1.14 mM	401	102 (35 nm)	11457
	NO_2	35%@ 100 μM	–	50 nm	>2000

Table 2
%Inhibition and IC_{50} values of Group 2 compounds

R	R′	%AChE inhibition (100 μM)	%BuChE inhibition (100 μM)	IC_{50} plasma (μM)
	H	0.82	25.04	–
		0.42	78.54	32.77
		45.43	92.13	16.05

3. Conclusions

Isosorbide can be used in the construction of potent and highly selective inhibitors of BuChE. Less potent and but moderately AChE selective inhibitors can also be produced. The two groups belong to a new class of carbamate inhibitor type, which should be useful in probing the role of the cholinesterases in normal and diseased tissue. In particular, the BuChE-selective molecules are lipophilic and unionized at physiological pH.

doi:10.1016/j.cbi.2005.10.063

(19)

Inhibition of guinea pig hemi-diaphragm acetylcholinesterase activity by pyridostigmine bromide and protection against soman toxicity☆

Julian R. Haigh[a,*,1], Scott R. Johnston[a], Brian M. Peters[a], Bhupendra P. Doctor[a], Richard K. Gordon[a], Michael Adler[b], Kenneth J. Gall[b], Sharad S. Deshpande[b]

[a] *Division of Biochemistry, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA*
[b] *United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010, USA*

Organophosphate (OP) chemical warfare agents (CWAs) such as soman (GD) exert their toxic effects by inhibiting acetylcholinesterase (AChE), which is

☆ Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition.

* Corresponding author. Tel.: +1 301 319 9844; fax: +1 301 319 9571.
E-mail address: julian.haigh@amedd.army.mil (J.R. Haigh).

[1] The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the U.S. Department of the Army or the Department of Defense.