The Design and Synthesis of Novel APIs Based Upon Topiramate

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Preface

This thesis describes research that was undertaken as part of an Engineering Doctorate which was carried out in collaboration with Onyx Scientific Limited and sponsored by the Engineering and Physical Sciences Research Council.

Being an industrially focused Engineering Doctorate, the work undertaken reflects the requirements and research challenges identified by Onyx Scientific Limited.

Abstract

The research project, conducted in collaboration with Onyx Scientific Limited and Newcastle University, is focused on the anti-convulsant drug topiramate that is best known as a treatment for epilepsy. The established data on the efficacy and limitations of topiramate highlighted it as a promising drug candidate for reprofiling, repurposing or modification. The interest in topiramate stems primarily from its multi-factorial mode of biological action that results in a complex combination of pharmacological effects. This is manifested by the drug having a complex side effects profile with the most common being related to the central nervous system, weight loss and gastrointestinal disorders.

The drug influences the activity of a number of *in vivo* sites including certain types of voltage activated sodium and calcium ion channels, AMPA glutamate receptors and particular isozymes of the enzyme carbonic anhydrase (CA). Of particular interest to this work is the specific inhibition of mitochondrial CAs, CAVA and CAVB by topiramate. The inhibition of these mitochondrial CAs is thought to contribute to the weight loss effects observed in patients being treated with topiramate. The project aims to develop novel selective CA inhibitors (CAIs) that have inhibitory properties towards only particular and relevant CAs. It is hoped that this will enable the pharmacological activity of topiramate to be optimised and certain of its aspects positively exploited whilst limiting toxicity and off-target biological effects.

The objective of the project is to identify potential new APIs by assessing the biological activity of known derivatives of topiramate on the CA targets of interest and by creating novel molecules that introduce structural modifications to topiramate that may increase the specificity for certain CAs and vary the drug's pharmacological effects. The approach of the project is to undertake a classical drug design cycle using modern drug discovery techniques. *In silico* models were used to identify compounds, both known and novel, which show promising interactions with one or more of the target active sites and which therefore may demonstrate a selective biological response. The *in silico* hits generated from modelling will be the primary synthetic targets and are the focus of this research.

Acknowledgements

The author would like to acknowledge supervisors and colleagues for their support in completing this thesis.

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Abbreviations

Ac Acetyl

aq Aqueous

Ar Aromatic (in NMR data)

Ala Alanine

Arg Arginine

Asn Asparagine

Asp Aspartate

Bn Benzyl

$BnNH_2$ Benzylamine

Boc tert-Butoxycarbonyl

br Broad (in IR and NMR data)

BzCl Benzoyl chloride

BnBr Benzyl bromide

C Carbon; Celsius

CA Carbonic anhydrase

CAI Carbonic anhydrase inhibitor

CBz Benzyloxy carbamate

COSY Correlation spectroscopy

Cys Cysteine

d Days; doublet (in NMR data)

DCM Dichloromethane

DEPT Distortionless Enhancement by Polarisation Transfer

$(DHQ)_2PHAL$ Hydroquinine 1,4-phthalazinediyl ether

DIPEA *N*,*N*-Diisopropylethylamine

DMA *N*,*N*-Dimethylacetamide

DMF Dimethylformamide

DMAP 4-Dimethylaminopyridine

DME 1,2-Dimethoxyethane

DMSO Dimethylsulfoxide

ESI Electrospray ionisation

Et Ethyl

EtOH Ethanol

F Fluorine

Glu Glutamate

Gln Glutamine

Gly Glycine

h Hours

His Histidine

HCl Hydrochloric acid

HMBC Heteronuclear multiple bond correlation

HSQC Heteronuclear Single Quantum Correlation

HPLC High performance liquid chromatography

Ile Isoleucine

IPA Isopropanol

IR Infrared

LCMS Liquid chromatography – mass spectrometry

Leu Leucine

Lys Lysine

M Molar

m Multiplet (in NMR data)

m-CPBA *meta*-chloroperoxybenzoic acid

MeI methyl iodide

Met Methionine

MS Mass spectrometry; Molecular ieves

m.p. Melting point

Me Methyl

Mesylate Methanesulfonate

min Minutes

MsCl Methanesulfonyl chloride

nM nano molar

$NaBH_4$ Sodium borohydride

NCS *N*-chlorosuccinimide

NMP *N*-Methyl-2-pyrrolidone

NMR Nuclear magnetic resonance

NOE Nuclear Overhauser effect

OsO_4 Osmium tetraoxide

Pd Palladium

Phe Phenylalanine

Ph Phenyl

ppm Parts per million

Pro Proline

PTFE Polytetrafluoroethylene

Py Pyridine

q Quartet (in NMR data)

RT Room temperature

s Singlet (in NMR data)

Ser Serine

SN2 Bimolecular nucleophilic substitution

t Triplet (in NMR data)

TBDMSCl *tert*-butyldimethyl silyl chloride

TESCl Triethylsilyl chloride

Tf_2O Trifluoroacetic anhydride

Thr Threonine

TMSMgCl Trimethylsilyl magnesium chloride

Trp Tryptophan

Tyr Tyrosine

Et_3N Triethylamine

Tf Trifluoromethanesulfonyl

TFA Trifluoroacetic acid

THF Tetrahydrofuran

TLC Thin layer chromatography

Tosylate p-Toluenesulfonate

Triflate Trifluoromethanesulfonate

TsCl *p*-Toluenesulfonyl chloride

Val Valine

CHAPTER 1. INTRODUCTION

1.1. Topiramate

1.1.1. Introduction

Topiramate **1**, 2,3:4,5-di-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate, is derived from the naturally occurring monosaccharide *D*-fructose bearing a sulfamate (-OSO_2NH_2) group.

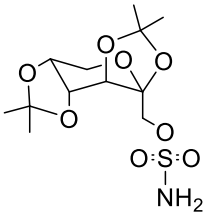

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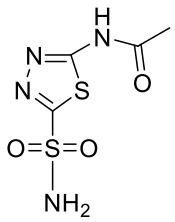
Figure 1: Chemical structure of topiramate (2,3:4,5-di-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate)

Topiramate is a drug best known for the treatment of epilepsy. [1] It was originally synthesised as part of a research project to discover structural analogues of fructose-1,6-diphosphate.[2, 3] The subsequent discovery of topiramate's anticonvulsant properties was first reported by Bruce E. Maryanoff *et al* in 1987.[1] Topiramate was evaluated for potential anticonvulsant effects due to the structural resemblance of its sulfamate group to the sulfonamide moiety in acetazolamide **2** and other arene sulphonamides, such as benzenesulfonamide **3**, that were known to be potent anticonvulsants.[1] Topiramate was found to be highly active in the traditional maximal electroshock seizure (MES) test in mice and rats[1] and a range of other seizure models.[4] The drug has since undergone extensive clinical trials and was found to be clinically effective as both a monotherapy and as an adjunctive to other drugs in the treatment of various forms of epilepsy.[5] Topiramate is approved as an anticonvulsant medication in over 95 countries.[6] In addition, it is approved for migraine prophylaxis in both the UK [5] and US.[7] In 2012, the US Food and Drug Administration approved the use of Qsymia, a combination of phentermine, an amphetamine derivative, and topiramate, for chronic weight management in obese and overweight patients.[8, 9]



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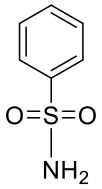

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Figure 2: Chemical structures of acetazolamide 2 and benzenesulfonamide 3

Topiramate is FDA approved [7] and other studies have subsequently been carried out to consider the longer term effects of using the drug.[10] In all cases, the most common side effects occurring in over 30% of patients were related to the central nervous system (e.g. headache, dizziness, fatigue and somnolence) and gastrointestinal disorders (e.g. nausea).[11] Another recognised side effect is a decrease in body weight with recorded weight loss being greatest among patients with the highest body weight at baseline. [11]

1.1.2. Pharmacokinetics

Topiramate is taken orally and its pharmacokinetics are characterised by rapid and linear absorption, a half-life of 19-25 hours and low oral clearance, which is predominantly through renal excretion.[12] It distributes to all tissues including the brain.[11] Topiramate is not extensively metabolised in humans (*ca*. 20%) and is eliminated predominantly unchanged in the urine.[13] Six minor metabolites **4-9** have been isolated and characterised and result from metabolic pathways involving hydroxylation and hydrolysis of the two isopropylidene groups.[3] The metabolites are present in plasma and urine in very small quantities; **9** and **4** were identified as the most prominent urinary metabolites, with a recovery accounting for 4% of the administered topiramate dose. The remaining metabolites account for less than 1% of the original dose.[6] The metabolites are cleared from the body quickly without accumulation.[13] None of the metabolites are thought to play a role in anticonvulsant activity.[14]

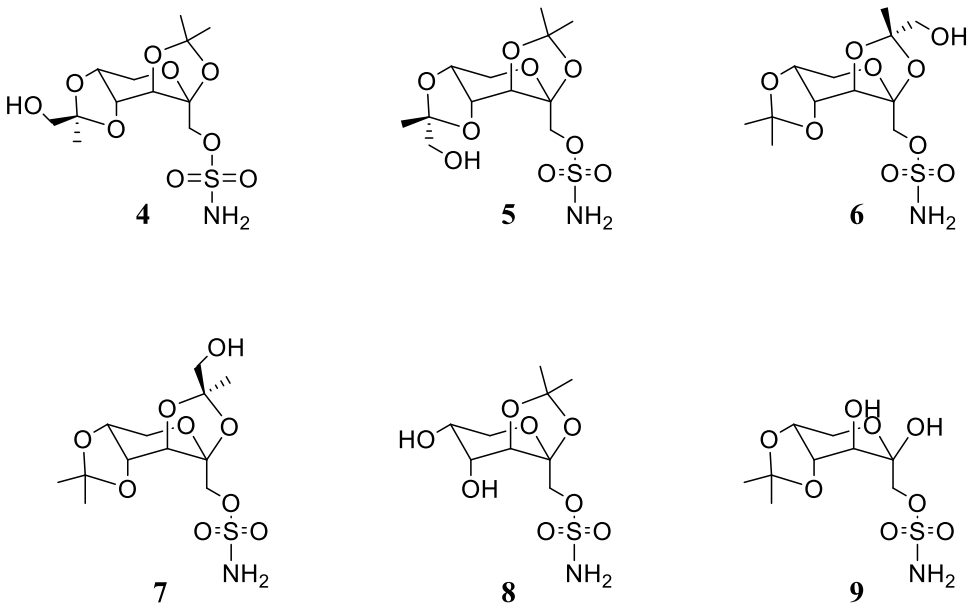


Figure 3: The structure of the six known metabolites of topiramate

1.1.3. Pharmacology

The pharmacology of topiramate has been extensively researched in order to elucidate and study its mode of action in the control of seizures. The precise mechanism by which topiramate exerts its antiseizure and migraine prophylaxis effects are unknown.[5] However, evidence suggests that the drug possesses a unique combination of pharmacological properties resulting in multiple mechanisms of action.[1, 14] A number of electrophysical and biochemical studies on cultured neurons have identified three properties that may contribute to the antiepileptic efficacy of topiramate.[15] [16] [17]

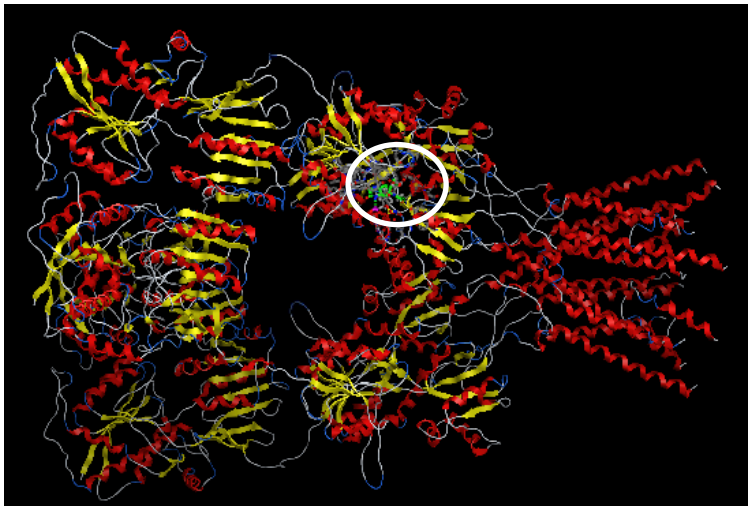
- *the inhibition of voltage sensitive sodium channels*

Action potentials, or nerve impulses, are electrical signals produced by the flow of ions across the plasma membrane of a neuron. They are mediated by transient changes in the permeability of the cell to sodium and potassium ions. The neuronal membrane contains specialised proteins which form pores in the membrane that are selectively permeable to particular ions. The activation of these voltage sensitive channels results in the diffusion of ions into and out of the neuron. In a resting neuron, there is a higher concentration of sodium ions outside the neuron with a higher concentration of potassium ions within. This gives rise to the membrane potential. Action potentials occur due to depolarisation of the neuronal membrane that

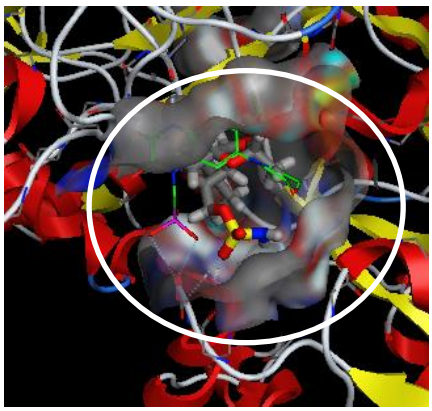
propagates along the nerve. Local depolarisation leads to the flow of positively charged ions across the neuronal membrane. Sodium channels in the neuronal membrane are opened in response to a small depolarisation of the membrane potential. If sodium channels are opened, positively charged sodium ions diffuse into the neuron, making the inside of the neuron momentarily positively charged and the membrane becomes depolarised. The sodium channels spontaneously close and potassium channels begin to open, leading to a reversal in the membrane potential. The potassium channels then also inactivate and the membrane equilibrates to its resting potential. Topiramate has been found to reduce the frequency at which action potentials are generated when neurons are subjected to sustained depolarisation. The amplitude of the inward ion currents via voltage gated sodium channels was found to be reduced by the drug.[15, 18] In particular, topiramate was found to be capable of significantly inhibiting both the fast and persistent components of sodium currents and sustained neuronal depolarisation that feature in seizure initiation and activity.[19]

- *the antagonism of the activity of kainate/ AMPA subtype of glutamate receptor*

Neuroexcitatory function mediated by glutamate, a neurotransmitter, through AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) and kainate receptors is critically involved in normal synaptic function in the brain and central nervous system. It is also critical in the generation of the activity underlying epileptic seizures.[20] Calcium is an important intracellular regulator of neuronal excitability and calcium currents may play an important role in seizure generation.[21] Topiramate has been found to selectivity antagonise calcium ion permeable AMPA receptors and/ or kainate receptors.[20] [16] This finding can be rationalised by examination of the active docking interactions of topiramate within AMPA (Figure 4). It can be envisioned how reducing excess activation of these AMPA and kainate receptors during epileptic seizure activity could result in a powerful antiepileptic mechanism.[16]



A



B

Figure 4: Cartoon diagram of AMPA (A) showing topiramate docked into active site; the white ellipse (B) denotes the binding sites of other known AMPA antagonists (e.g. perampanel). (The structure was drawn in pyMOL using the crystal structure PDB:3KG2)

- *the enhancement of the activity of GABA (γ-aminobutyric acid) at some types of GABA receptors*

γ-Aminobutyric acid, or GABA, is produced by the decarboxylation of glutamic acid and is a potent inhibitory neurotransmitter. It plays a role in regulating neuronal excitability throughout the central nervous system, mediating inhibitory synaptic transmission. $GABA_A$ receptors are part of a ligand-gated ion channel complex that influence the flow of ions into and out of cells and affects the transmembrane potential. Topiramate has been found to have a modulatory effect on these $GABA_A$ receptors increasing the frequency of channel activation and the flux of chloride ions into neurons.[17, 22]

- *the inhibition of carbonic anhydrase*

Topiramate is also known to inhibit all isozymes of the enzyme carbonic anhydrase (CA).[23] Studies have shown that topiramate, selectively, but not specifically inhibits the isozymes CAII, CAIV, CAVA and CAVB more effectively than other CAs.[14, 24, 25] Topiramate has been shown to have particularly effective inhibitory activity against CAII (K_i=10nM), CAVA (K_i=63nM) and CAVB (K_i=30nM).[25, 26] Evidence suggests that the drug's inhibition of certain isozymes of CA contributes to its properties as an anticonvulsant but that it is not the dominant factor in this particular therapeutic area.[14] The inhibition of CAII, CAVA and CAVB in humans by topiramate has a potency that is sufficient to have an appreciable effect on the activity of these CA isozymes at therapeutically relevant doses.[24] Particular side effects experienced by patients using topiramate can be attributed to its potent inhibition of CAs, such as weight loss, transient myopia and renal nephrolithiasis.[11, 23, 24, 27] Topiramate's mode of action as a carbonic anhydrase inhibitor (CAI) is detailed in section 1.3.5.

1.2. Carbonic Anhydrase (CA)

1.2.1. Introduction

CAs are enzymes that are ubiquitous throughout virtually all living organisms. Five genetically distinct classes of CAs are known, α, β, γ, δ and ζ. The α-class of CAs are found in vertebrates and are the most well characterised and researched. α–CAs are the only type present in humans and other mammals. β-CAs are present in higher plants and some prokaryotes, γ-CAs are observed only in *Archaebacteria*, and δ and ζ have only been found in marine diatoms. These classes of CAs differ from each other in their primary amino acid sequences and 3D tertiary structure (α, β, γ).[28] All are metalloenzymes; α-, β-, and δ-CAs contain a Zn^{2+} ion within the active site. γ-CAs are thought to contain a Fe^{2+} ion within the active site, but they are also active with bound Zn^{2+} or Co^{2+} ions, whereas Cd^{2+} or Zn^{2+} ions are present in ζ-CAs. Many representatives of all these enzyme classes, except the δ-CAs, have been crystallised and characterised in detail.[27]

All classes of CAs regulate the concentrations of CO_2, bicarbonate ions and protons by catalysing the reversible hydration of carbon dioxide to yield a bicarbonate anion and a proton:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

The reaction is biologically important and, as such, CAs are expressed variably in different species, tissues and cells. As well as the critical role CAs play in the transport of CO_2 and HCO_3^-, they also influence a diverse range of physiological and pathological processes. In vertebrates, the role of CAs in pH regulation, CO_2 homeostasis, electrolyte secretion, biosynthetic processes, such as gluconeogenesis, lipogenesis, and ureagenesis, bone resorption and calcification have been thoroughly studied.[29, 30] In algae, plants, and some bacteria they play an important role in photosynthesis and other biosynthetic reactions. In diatoms δ- and ζ-CAs play a crucial role in carbon dioxide fixation.[27]

1.2.2. α-Carbonic Anhydrases

At present, there are 15 known forms of α-CAs found in humans differing in their molecular features, their distribution in organs and tissues, their kinetic properties and their response to different types of inhibitors (Table 1).[28] Of these, 13 are catalytically active and three (CAVIII, CAX and CAXI) are known as CA-related proteins (CARPs) and do not show catalytic activity due to the lack of histidine residues required for the coordination of a zinc ion within the active site.[31]

Isozyme	Organ/ tissue distribution	Subcellular location
CAI	erythrocytes, gastrointestinal tract, eye	cytosol
CAII	erythrocytes, gastrointestinal tract, eye, lung, bone, testis, brain, osteoglasts, kidney	cytosol
CAIII	skeletal muscle, adipocytes	cytosol
CAIV	kidney, lung, pancreas, brain capillaries, colon, heart muscle, eye	membrane-bound
CAVA	Liver	mitochondrial
CAVB	heart and skeletal muscle, pancreas, kidney, spinal cord, gastrointestinal tract	mitochondrial
CAVI	salivary and mammary glands	secreted in saliva and milk
CAVII	central nervous system	cytosol
CAVIII	central nervous system	cytosol
CAIX	tumours, gastrointestinal mucosa	transmembrane
CAX	central nervous system	cytosol
CAXI	central nervous system	cytosol
CAXII	kidney, intestine, reproductive epithelia, eye, tumours	transmembrane
CAXIII	kidney, brain, lung, gut, reproductive tract	cytosol
CAXIV	kidney, brain, liver, eye	transmembrane

Table 1: Organ/ tissue distribution, subcellular location of the 15 α-CA isozymes found in humans [28]

To date X-ray crystallographic structures of all isoforms of human CA except CAVB are available (an example is presented in Figure 5).[28] These structures indicate high sequence

homology throughout the isozymes with a high level of conservation of key residues within the active site. The enzymes present a similar structure that is characterised by a central twisted β-sheet surrounded by helical connections and additional β-strands. Despite the high sequence and structural homology of the active sites of the α-CA isozymes, there are some differences in the quaternary structure. Most of the isozymes are monomeric apart from three isozymes, CAIX, CAXII and CAVI that are dimeric although this is not thought to influence catalytic activity in any way.[28]

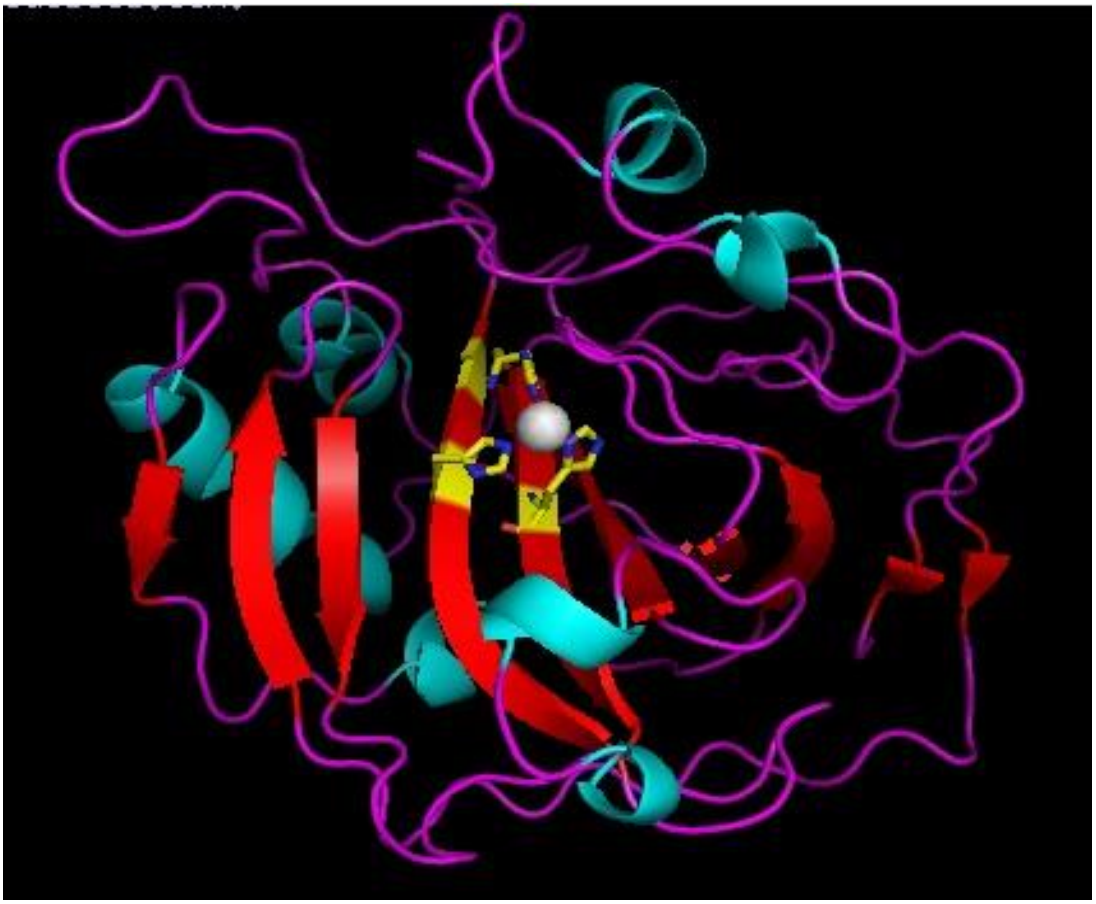


Figure 5: The structure of human CAII shown in cartoon representation showing the zinc ion as a grey sphere and the three coordinating histidine ligands (the structure was drawn in pyMOL using the crystal structure PDB:1CA2)

The active site of the enzyme is located within a conical cleft approximately 15Å deep from the surface of the enzyme to the centre of the molecule. The catalytic zinc ion is located at the base of this cavity in a distorted tetrahedral coordination liganded to three conserved histidine residues (His 94, His 96, His 119) and an oxygen atom of a water molecule that ionises to a hydroxide ion at physiological pH (Figure 7).[30] In all of the α-CA isozymes, the active site cavity has two distinct regions; a hydrophobic region delimited by a group of hydrophobic

amino acids and an adjacent area comprising of hydrophilic residues (Figure 6). This particular active site environment is important to the catalytic activity of the enzyme. The hydrophobic binding site is important in the facilitation of CO_2 into the active site and the hydrophilic residues form a network of hydrogen bonds that maintain an active catalytic structure (Figure 7).[28] In particular, a threonine residue (Thr 199) forms a hydrogen bond with the zinc bound hydroxide ion and a glutamic acid residue (Glu 106) orientates the hydroxide ion in the optimal position for nucleophilic attack on the CO_2 substrate held in the hydrophobic pocket (see Figures 7 and 8). The electrostatic environment, and thus the reactivity of the zinc bound hydroxide ion, is further modulated by "second shell" or indirect ligand residues which are defined as those that hydrogen bond with direct metal ligands, for example, the three histidine residues.[30] "Deep water" (Figure 7) refers to an ordered water molecule positioned near the hydrophobic pocket which plays a role in the enzyme's catalytic activity. Figure 7 shows three such residues interacting with the zinc coordinated histidines, a glutamine (Gln 92), glutamic acid (Glu 117) and an asparagine (Asn 244).

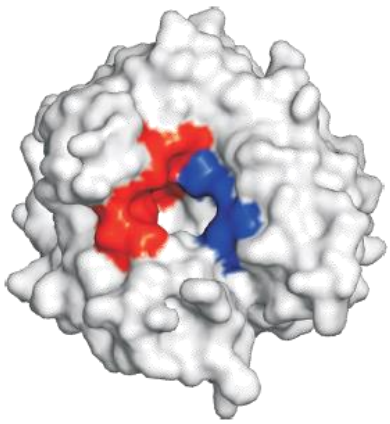


Figure 6: Surface representation of human CAII showing active site cleft. Hydrophobic residues are shown in red and hydrophilic in blue (the structure was drawn in pyMOL using the crystal structure PDB:1CA2)

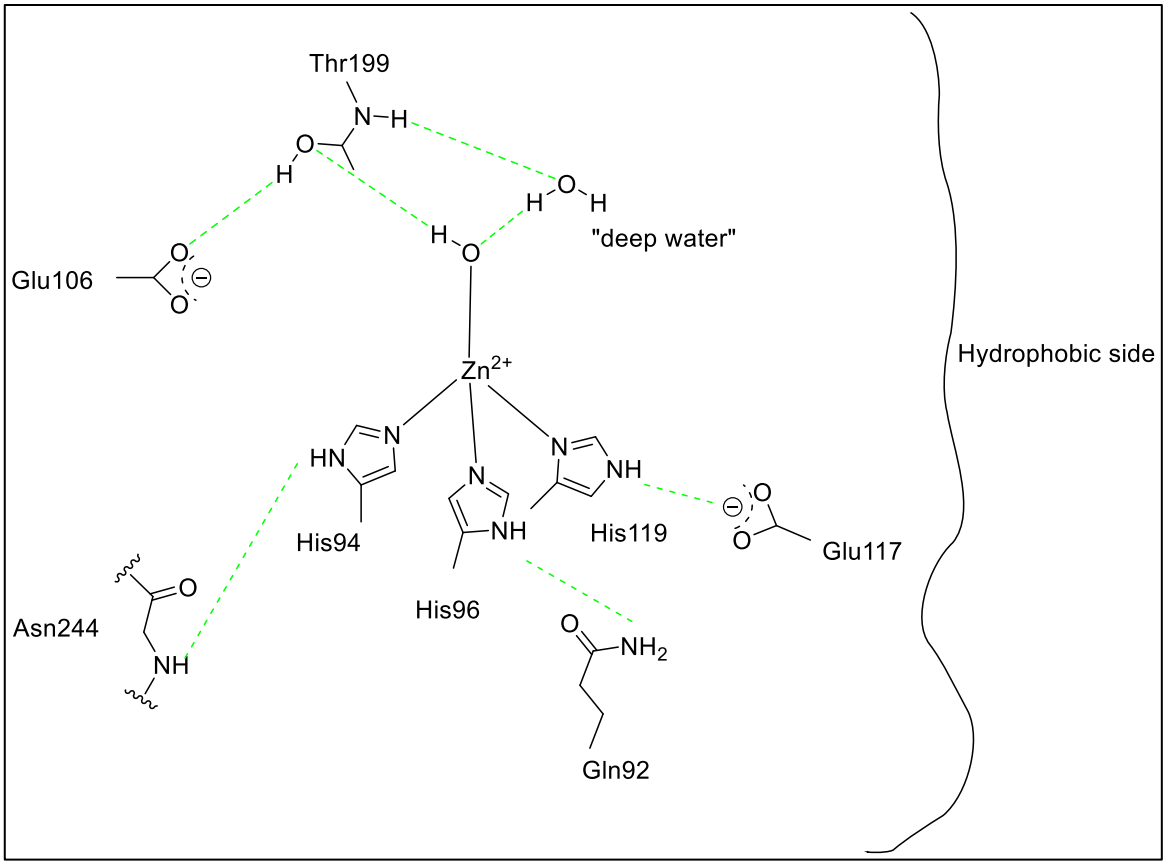


Figure 7: The active site of human CAII (which has been chosen as a representative CA) showing the scheme of hydrogen bond networks, represented by green dashed lines

1.2.3. Catalytic Mechanism

CAs are one of the most efficient biological catalysts known with several isozymes having catalytic efficiency approaching the limit of diffusion control.[30] The kinetics and catalytic mechanism of CA has been extensively studied using human CAII as a model. However, it is thought that all α-CAs exhibit the same general mechanistic scheme where the reversible hydration of carbon dioxide proceeds via a tandem chemical reaction (Figure 8).[32]

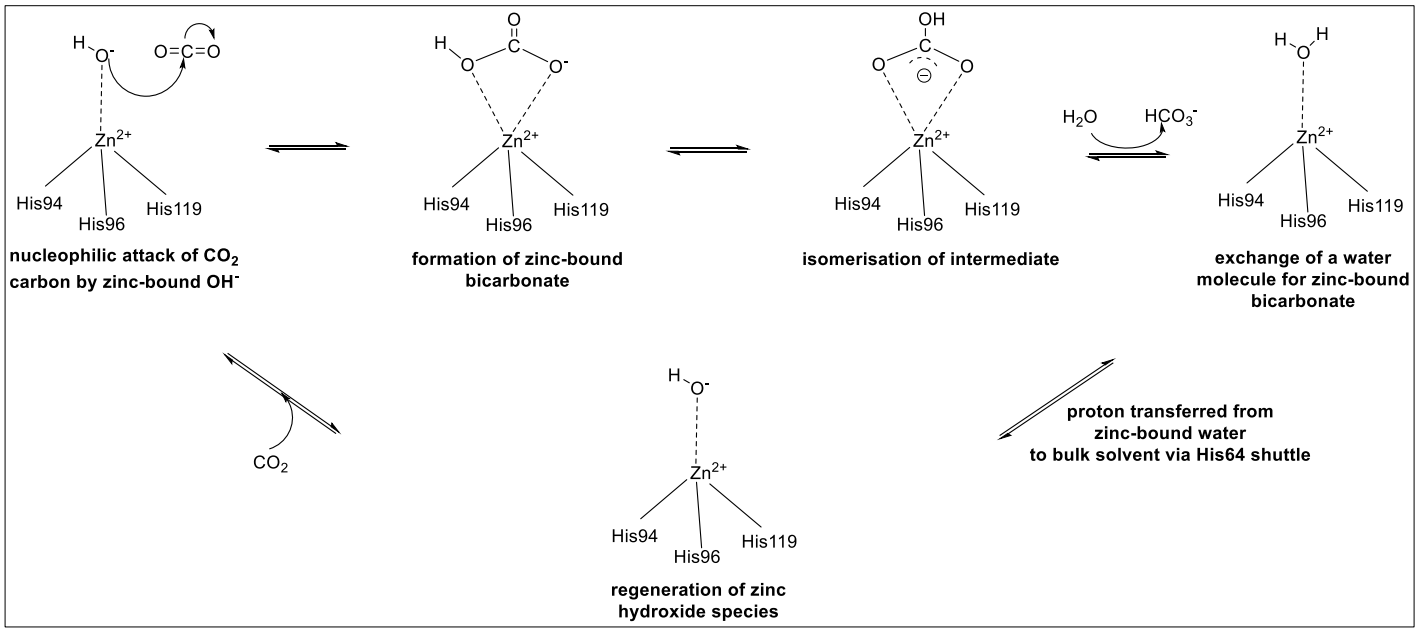


Figure 8: General mechanism for the catalysis of CO_2 hydration by CAs

The active form of the enzyme has a hydroxide ion bound to the zinc ion's fourth coordinating position. This nucleophilic zinc bound hydroxide attacks the enzymatically bound CO_2 molecule and converts it to a bicabonate ion that coordinates to the zinc. The bicarbonate ion is then displaced by a water molecule and is liberated into solution. The transfer of a proton from the zinc bound water molecule from the active site to the bulk solvent is needed to regenerate the active enzymatic environment. This is the rate limiting step of the entire catalytic process.[29] In certain CA isozymes (I, II, IV, VI, VII, IX and XII – XIV) the proton transfer reaction is assisted by a histidine residue (His 64) whereas in others, buffers present in the medium play a role.[27] In those isozymes where His 64 is present, the ionisation reaction is achieved via a series of proton hops across a hydrogen bonded solvent network or proton wire (Figure 9) in which the His 64 and the zinc bound water are linked by two water molecules.[33]

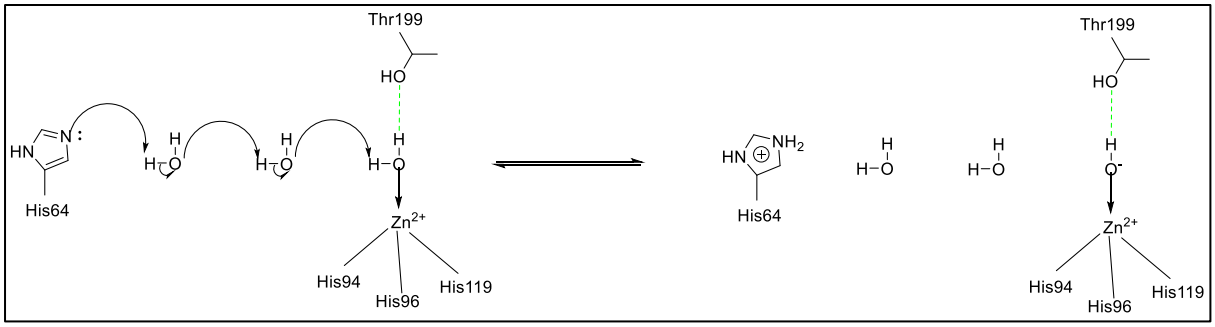


Figure 9: Mechanism of ionisation of zinc bound water in human CAII via a proton shuffle assisted by His 64 to regenerate a nucleophilic zinc bound hydroxide ion

1.2.4. Carbonic Anhydrase Inhibitors

CAs play a role in a number of important physiological processes and thus CA isozymes are important targets for the development of new pharmacological applications. Extensive research into the drug design of both CA inhibitors (CAIs) and CA activators has been undertaken during the last 30 years.[26, 27] CA activators may have pharmacological applications in pathologies in which learning and memory are impaired, such as Alzheimer's disease or ageing.[34] The inhibition of CAs have potential therapeutic value in treating a range of disorders including oedema, glaucoma, obesity, cancer and epilepsy (Table 2), as well as in the emerging areas of antifungal or antibacterial agents.[27, 35, 36]

Isozyme	Disease in which isozyme is involved
CAI	Retinal/ cerebral oedema
CAII	Glaucoma, oedema, epilepsy, altitude sickness
CAIII	Oxidative stress
CAIV	Glaucoma, retinitis pigmentosa, stoke
CAVA	Obesity
CAVB	Obesity
CAVI	Cariogenesis
CAVII	Epilepsy
CAVIII	Neurodegeneration/ cancer
CAIX	Cancer
CAXII	Cancer/ glaucoma
CAXIII	Sterility
CAXIV	Epilepsy, retinopathy

Table 2: Human CA isozymes as drug targets in various diseases. (No data is available in the literature on the involvement of CAX and CAXI in diseases) [28]

As specific isozymes of CAs are responsible for different biological responses, the challenge is to design isozyme-specific CAIs to selectively target particular pathologies and minimise off target biological activities that manifest as unwanted side effects or toxicity. This poses certain difficulties; there are 13 catalytically active human CA isozymes spread diffusely throughout the tissues and organs of the body, the isozymes have a high level of structural similarity within their active sites and each isozyme functions via the same catalytic mechanism. This explains why none of the CAIs currently used clinically show selectivity for a specific isozyme but indiscriminately inhibit several CA isozymes, behaviour that is shared by topiramate.[29]

CAIs generally bind to the active site of the enzyme and prevent or hinder the sequence of reactions detailed in Figure 8 from occurring. CAIs can be divided into two main classes, those that contain a zinc binding group and those that bind to the active site of CA but have no direct

interaction with the zinc ion. Sulfonamides ($R\text{-}SO_2NH_2$) and their isosteres, sulfamates ($R\text{-}OSO_2NH_2$) and sulfamides ($R\text{-}NHSO_2NH_2$), constitute the main class of CAIs and fall into the first category.[29] Several compounds such as acetazolamide **2**, methazolamide **10**, ethoxzolamide **11**, sulthiame **12**, dichlorophenamide **13**, dorzolamide **14**, brinzolamide **15**, sulpiride **16** and zonisamide **17** have been in clinical use for many years, as diuretics, antiglaucoma agents, as well as antiepileptics.[29] Sulfamates including topiramate **1** and irosustat **18** although developed independently of their potential CA inhibitory properties, are also potent CAIs. Irosustat **18** is a first-in-class steroid sulfatase inhibitor to be used clinically in patients with advanced hormone-dependent cancers.[28]

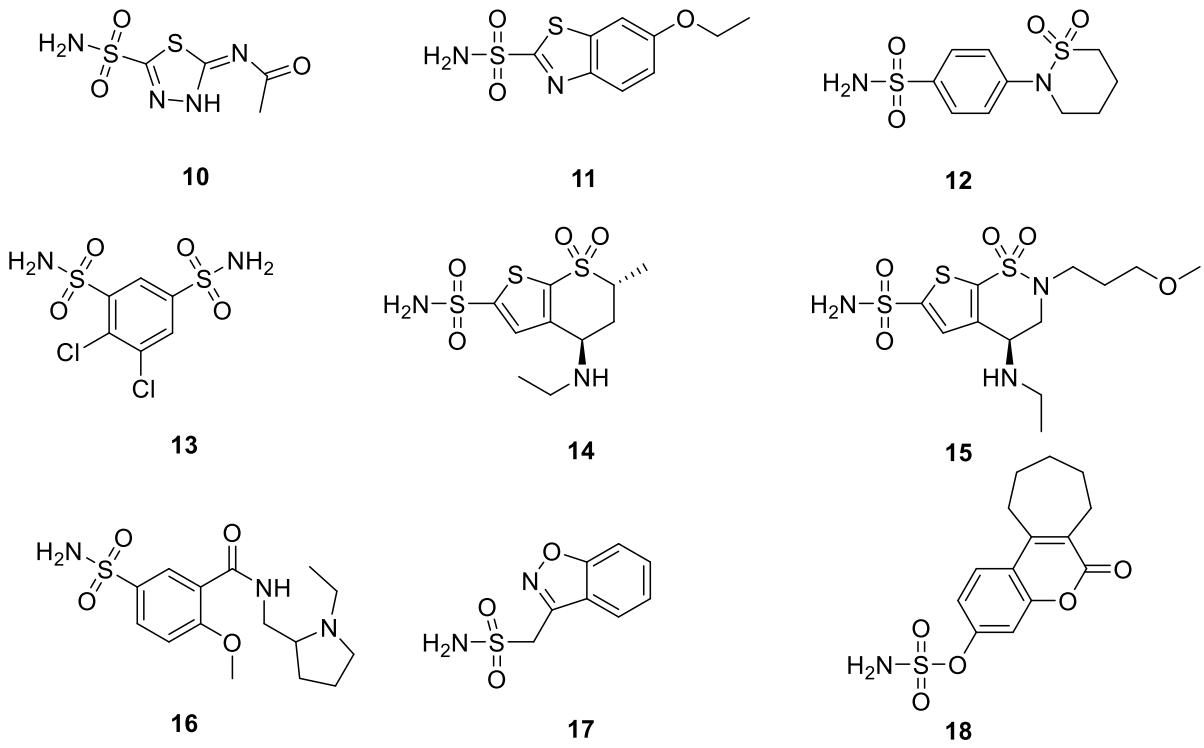


Figure 10: Structures of sulfonamide and sulfamate CAIs currently in clinical use

Both sulfonamides and sulfamates bind to the zinc ion in the enzyme active site in their deprotonated form as anions. Figure 11 shows the key binding interactions between a generic sulfonamide inhibitor and the active site of human CAII. Crystallographic studies are available for many sulfonamide adducts with several CA isozymes and this has enabled the elucidation of the key factors responsible for the binding of the sulfonamide moiety to the CA active site.[28] In all of the studied adducts the binding of the sulfonamide derivatives is primarily by the

coordination of the deprotonated nitrogen atom of the sulfonamide to the catalytic zinc ion leading to the substitution of the zinc bound water molecule. Additionally, two hydrogen bonds between the sulfonamide and the important Thr199 residue form and, depending on the nature of the sulfonamide's molecular scaffold (depicted as R in Figure 11), interactions with the hydrophilic and/or hydrophobic region of the active site can be also present. Sulfamates show a similar pattern of interactions with the active site of human CAII.[28]

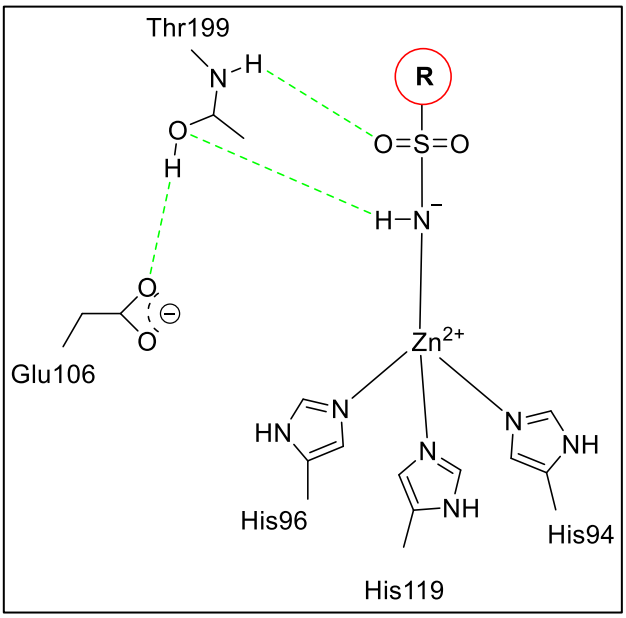


Figure 11: Schematic illustration of the key interactions between a generic sulfonamide inhibitor and the human CAII active site with hydrogen bonds represented by green dashed lines [28]

Other families of CAIs possess different mechanisms of action in which they do not directly interact with the zinc ion. Phenols (e.g. phenol **19**) anchor to the zinc bound water molecule/ hydroxide ion within the active site through two hydrogen bonds with the phenolate group.[28] Polyamines (e.g. spermine **20**) were found to have a similar mechanism of inhibition as phenol with one of the terminal ammonium moieties anchored to the zinc-coordinated water molecule/hydroxide ion by means of a hydrogen bond.[37] Coumarins (e.g. **21**) and thiocoumarins (e.g. **22**) have a mechanism of action independent of the catalytical zinc ion. They act as "prodrug" inhibitors, binding in their hydrolysed form. Crystallographic studies of adducts of coumarins and several of their derivatives with various CA isozymes showed that these compounds bind to the enzyme at the entrance of the CA active site cavity in a region

where only CA activators had been observed earlier.[29] The result is the partial occlusion of the entrance to the active site.

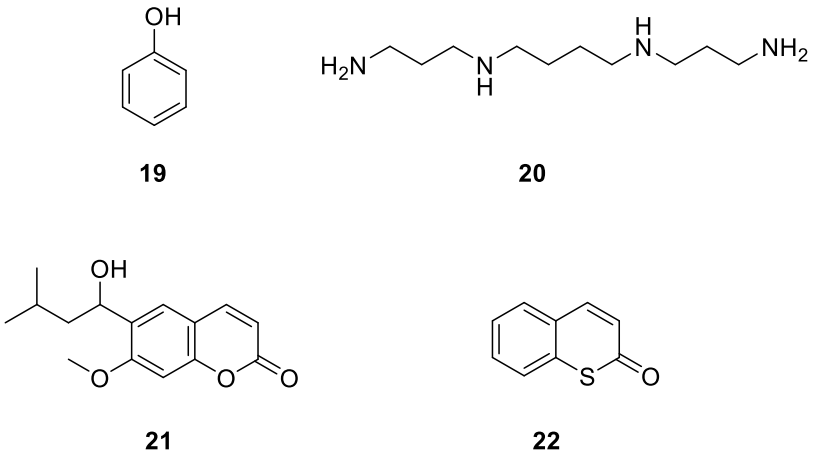


Figure 12: Structures of various CAIs which have a different mode of inhibition than sulfonamides and related compounds

1.2.5. Topiramate as a CAI

The enzyme inhibition interactions between topiramate and the active site of human CAII can be confidently represented, as shown in Figure 13, as a known crystal structure of the complex exists.[38] The 3D enzyme-inhibitor structure reveals the molecular interactions that readily account for the good CAII inhibition observed for topiramate.[39] The sulfamate group of topiramate acts as a zinc binding group and participates in a network of hydrogen bond interactions with residues within the active site. The ionised nitrogen atom of the sulfamate coordinates to the zinc and displaces a hydroxide ion. In addition, this nitrogen atom donates a hydrogen bond to the hydroxyl group of residue Thr 199. One of the sulfamate oxygen atoms accepts a hydrogen bond from the backbone NH group of Thr 199.[38] The sugar scaffold plays an important role in securing the molecule into the appropriate position within the enzyme active site. The oxygen of the pyranose ring makes a hydrogen bond with Thr 200 whilst the methyl groups of the two acetal groups of the sugar are positioned appropriately to interact with the hydrophobic pocket of the active site and form Van der Waals interactions with the active site cleft. Additionally, the oxygen atoms of the two acetal groups form polar interactions with active site residues Gln 92 and Asn 62.[28]

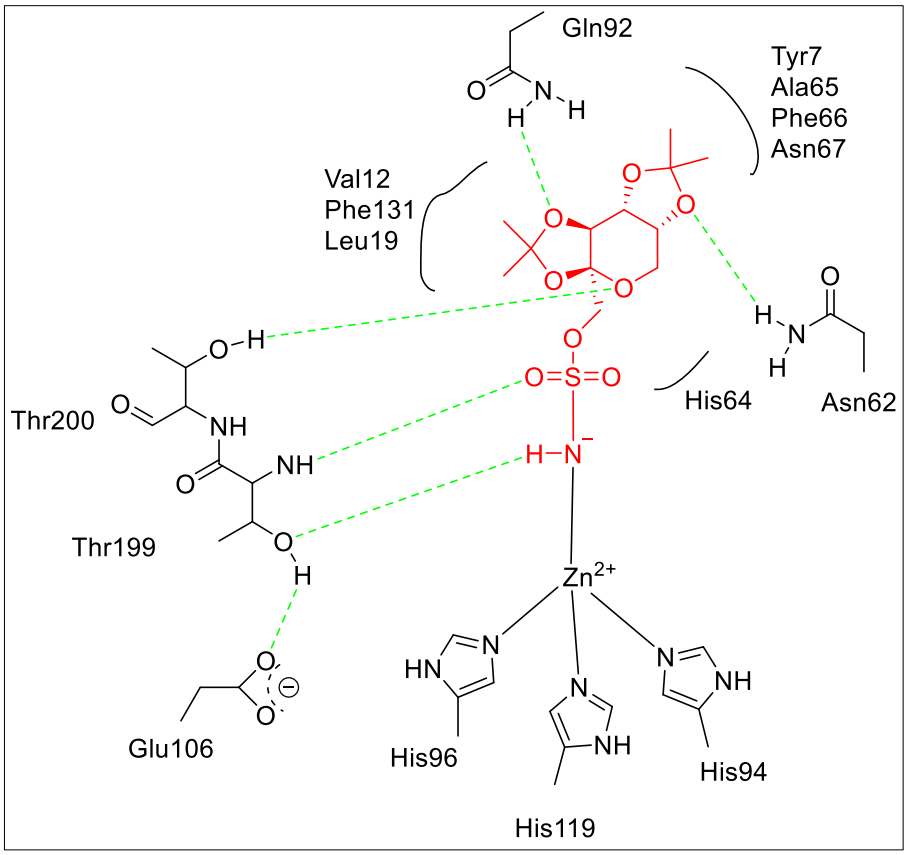


Figure 13: Schematic diagram of the active site of the human CAII/ topiramate adduct crystal structure (with topiramate shown in red and hydrogen bonds represented by green dashed lines) (PDB: 3HKU) [28]

Of particular interest to this project is the inhibitory effect of topiramate **1** on the two mitochondrial CA isozymes, CAVA and CAVB. A side-effect observed in epileptic patients treated with topiramate was that they experienced weight loss.[11, 40] A new obesity therapy consisting of a combination of topirimate and phentermine, branded as Qsymia, was approved by the US Food and Drug Administration in 2012 and has been found to have robust efficacy for weight loss.[8, 9] Although, the pharmacological explanation for the weight loss observed with topiramate is still under investigation, it is thought to be a result of its inhibition of CAVA and CAVB.[26] Dysfunctional mitochondrial activity can be a fundamental cause of obesity and it is thought that topiramate mediates mitochondrial metabolism pathways via its inhibition of CA isozymes.[27] Figure 14 shows the binding interactions between topiramate and human CAVA. Although there is no known crystal structure of the topirimate/CAVA adduct, the schematic diagram has been proposed following *in silico* structural and sequence homology studies of the known crystal structures of murine CAVA with that of human CAII.[41] Figure

14 shows that the tetrahedral geometry of the zinc binding site and the key hydrogen bonds between the sulfamate group of topiramate and the active site are all retained in the topiramate/CAVA complex. The extended network of hydrogen bonds and Van der Waals interactions between the sugar scaffold and the active site cleft are also retained, although the pattern is different, and exert a stabilising effect as observed in CAII.[41]

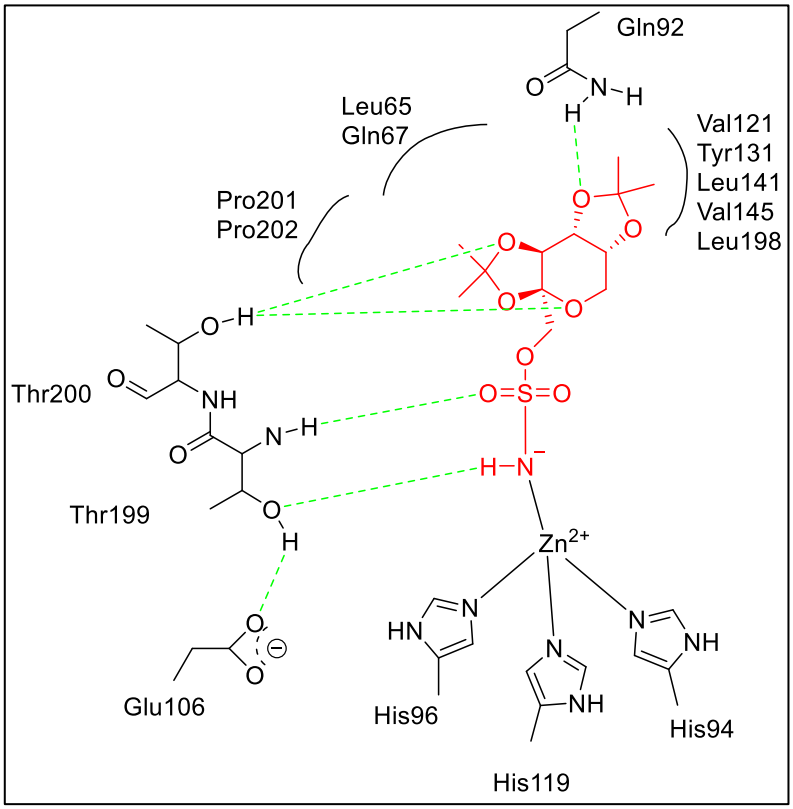


Figure 14: Schematic diagram of the active site of the human CAVA/ topiramate adduct crystal structure (with topiramate shown in red and hydrogen bonds represented by green dashed lines) based upon homology studies [41]

CAVA and CAVB are involved in several biosynthetic processes including ureagenesis, gluconeogenesis and lipogenesis. The hydration reaction catalysed by CAVA and CAVB within mitochondria, assisted by CAII in the cytosol, ensures the provision of sufficient bicarbonate ions for several key processes involving pyruvate carboxylase, acetyl coenzyme A (acetyl CoA) and carbamoyl synthetases.[26] CAVA and CAVB are thought to be critical to the synthesis of fatty acids and the action of CAIs on these particular isozymes leads to the potent inhibition of lipogenesis.[39] This has been rationalised by considering the role of CA isozymes

within fatty acid synthesis, as shown schematically in Figure 15. Fatty acid synthesis takes place in the cytosol whereas acetyl CoA, the building block of fatty acids, is formed in mitochondria from pyruvate. Acetyl CoA must therefore be transferred from mitochondria to the cytosol. However, mitochondria are not readily permeable to acetyl CoA and this barrier is bypassed by citrate which carries acetyl groups across the mitochondrial membrane. Citrate is formed from pyruvate within the mitochondrial matrix by a pathway involving the enzyme pyruvate carboxylase. Citrate is translocated from mitochondria to the cytoplasm and a further biosynthetic pathway leads to *de novo* lipogenesis. Bicarbonate ions are required in two key steps; within the mitochondria the carboxylation of pyruvate to oxaloacetate, the precursor to citrate, is carried out in the presence of bicarbonate. The mitochondrial isozymes CAVA and CAVB will thus influence this reaction. Bicarbonate ions also play a key role in the carboxylation of acetyl CoA to malonyl coenzyme A (malonyl CoA) within the cytosol and it is suggested that this is mediated by CAII, the cytosolic isozyme.[27]

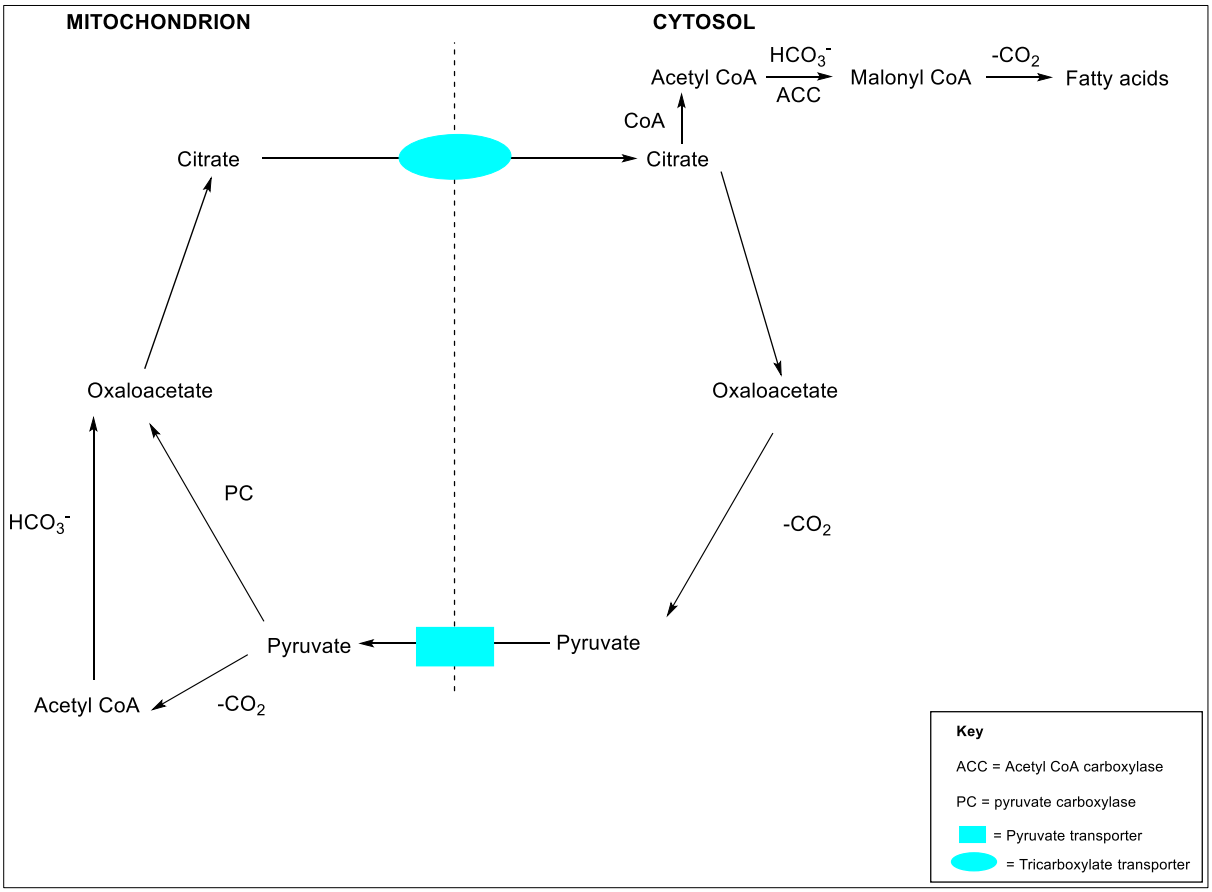


Figure 15: Fatty-acid biosynthesis and the role of CA isozymes [26]

1.2.6. Development of novel CAIs based on topiramate

Topiramate is known to inhibit both CAII and the mitochondrial CA isozymes, CAVA and CAVB.[28] As such it represents a valid pharmacophore from which to consider the design of isozyme specific CAIs. Further to the discovery and characterisation of the mitochondrial isozymes human CAVA and CAVB, they are now considered as "druggable" targets and that novel sulfonamide and sulfamate CAIs directed specifically against these isozymes may have applications for the development of antiobesity drugs.[26, 39] It is hypothesised that designing a novel agent with a specific inhibition profile for CAVA and CAVB will enable the weight loss effects associated with inhibition of these isozymes to be positively exploited whilst minimising off-target biological effects. The project has adopted a rational drug design process with the active sites of CAII, CAVA and CAVB being the initial biological targets against which potential drug candidates, both known and novel compounds, will be assessed (Figure 16). *In silico* modelling has been used to identify and predict which of the metabolites and postulated analogues of topiramate will act as specific CAIs. The *in silico* hits generated from modelling will be the primary synthetic targets and a first round has been completed. These compounds will be used for biological testing to act as either a positive, or negative, test of the computational models and to guide further *in silico* development work.

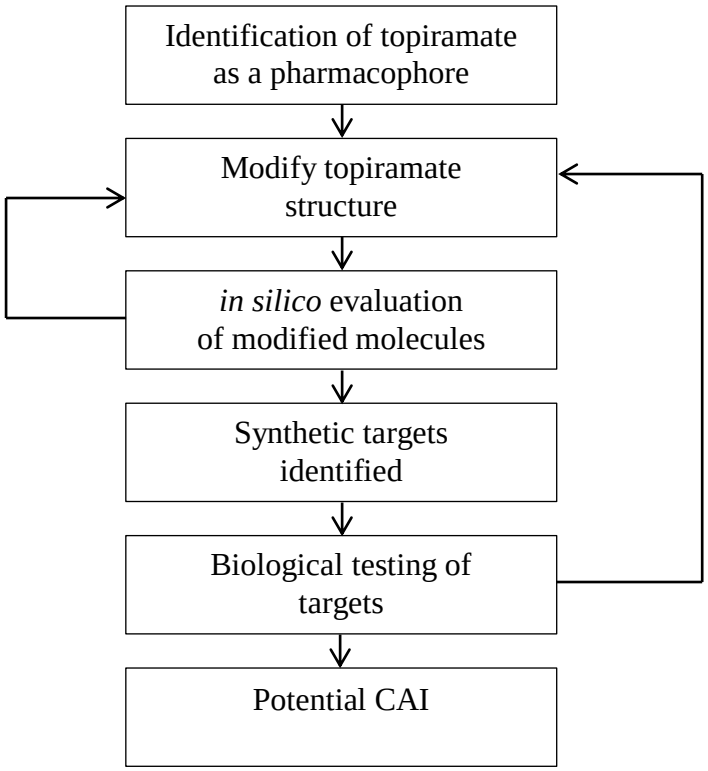


Figure 16: The approach to isozyme specific drug design and modulation of the inhibition profile of topiramate

1.3. *In silico* Evaluation Study

The computational modelling study has been carried out by Prosarix Limited, a Cambridge based biotechnology firm that specialises in modern drug design methods. The information presented in the following section is adapted from a confidential report prepared for the research partnership by Prosarix Limited.

The objectives of the initial *in silico* work were to:

- analyse the likely activities of topiramate metabolites (**4** – **9**) on CAII, CAVA and CAVB in order to identify which may retain CA inhibitory properties;
- model iminosugar analogues of topiramate on CAII, CAVA and CAVB;
- predict the likely activities of such compounds on these targets.

A number of molecular studies were conducted. The Molecular Operating Environment (MOE) software suite developed by Chemical Computing Environment (CCG Inc.) was used to visualise and understand the details of the active sites of the relevant CAs and receptor ligand

interactions. The crystal structure of human CAII complexed with topiramate was analysed with MOE in order to understand the important interactions present in the inhibitor complex. Accurately mapping the network of hydrogen bonds and hydrophobic interactions between topiramate and the active site cleft of CAII can then be used to inform subsequent models which introduce the active sites of other CA isozymes and topiramate-like compounds.

As no crystal structures of human CAVA and CAVB exist, models of the active sites of these CA isozymes were constructed based upon a murine CAVA template. Murine CAVA has high sequence homology to human CAVA sharing 76% sequence identity.[41] Homology modelling tools within MOE were used to prepare an initial sequence alignment of murine CAVA with human CAVA and human CAVB followed by detailed model construction. Various refinements were made to the models to account for solvent electrostatics, molecular mechanics and energy minimisation calculations. The optimised models of human CAVA and CAVB were structurally aligned with the human CAII structure to enable analysis of the active site differences and predict the likely major binding site differences. Topiramate was docked into the active site models of CAVA and CAVB and the pose and interactions of the molecule with active site residues were analysed. Prosarix employed their proprietary software, Protoscreen and Protodiscovery, to explore binding site flexibility to assess all possible binding site conformations of both active site residues and the ligand.

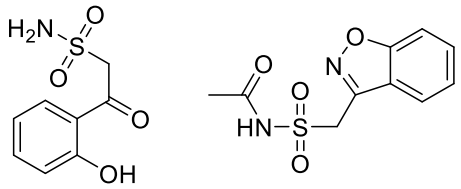
The results for the CAVA/ topiramate receptor–ligand interactions agreed with those depicted in Figure 14 with several important features exhibited. Mutations of a number of residues within an α-helix loop region adjacent to the zinc ion affects its orientation. [41] Of particular influence is the change from Ala 64 in CAII to a bulkier leucine residue in CAVA which projects into the binding site. As a consequence, the active site cleft is narrower in human CAVA than in human CAII. This reduction in the active site volume does not accommodate topiramate in the CAII binding mode and enforces an altered residue conformation. In addition, Phe 131 in CAII is a tyrosine residue in CAVA which projects a hydroxyl group into the binding site resulting in a clash with one of the geminal methyl groups of topiramate when it is aligned in its CAII conformation and thus an alternative conformation of topiramate is required within the CAVA active site.

The results of the CAVB docking studies suggest that topiramate adopts a similar binding pose with CAVB as is shown with CAII. The reason for this is twofold; the Ala 64 residue in CAII is replaced by a serine group in CAVB which results in a similar active site volume, also Phe

131 is conserved in CAVB. This means that the enforced changes in the binding mode of topiramate observed in CAVA when Phe 131 is replaced by a tyrosine residue does not occur in CAVB. A further binding site difference in CAVB is residue 92 which is glutamine in CAII, asparagine in CAVA but is a leucine residue in CAVB. In CAVB, this residue is in a position that could potentially allow for interactions to one of the oxygen atoms of the acetal groups.

Armed with an understanding of the likely binding modes of topiramate in both human CAVA and CAVB models and the knowledge of a binding mode between topiramate and CAII, the next stage of the modelling process was to consider molecular variants of topiramate. Topiramate metabolites **4**, **5**, **8** and **9** were docked into the models of the active sites of CAII, CAVA and CAVB. The metabolites **4** and **5** are predicted to exhibit good affinity with CAVB but not with CAVA and CAII. This difference is due to the residue at position 65 in each isozyme. In CAII it is Ala 65, in CAVA it is Leu 65 and Ser 65 in CAVB. The presence of the serine in CAVB active site is predicted to interact specifically with the hydroxyl groups of one of the acetals whereas these hydroxyl groups present energetically unfavourable interactions with Ala 65 in CAII and Leu 65 in CAVA. This suggestion of selectivity towards only one isoform of CA warrants further investigation.

In addition, further *in silico* models in which two compounds related to the anti-epileptic drug zonisamide **17** were docked into the CA receptor models were developed. 2-sulfamoylacetyl phenol **23** is a reductive metabolite of zonisamide and N-acetyl zonisamide **24** results from the acetylation of zonisamide.[42] It was of interest to explore the likely binding modes and affinities of both molecules to assess whether there was any scope of metabolites and analogues of zonisamide as inhibitors of CAVA and/or CAVB. The modelling predicted that both **23** and **24** did not present interesting leads on which to extend the scope of the project. However, **24** was considered to have utility as a reference for biological evaluation as it would provide an interesting comparative data set to topiramate and related compounds.



23 **24**

Figure 17: Compounds related to zonisamide; 2-sulfamoylacetyl phenol **23** and N-acetyl zonisamide **24**

Table 3 summarises the predicted affinities of metabolites of topiramate and zonisamide with various isozymes of carbonic anhydrase. Within the table, predicted positive affinity is represented by "+", weaker predicted affinity by "-" and likely weak affinity by "- -". Interestingly, topiramate metabolites **4** and **5** are predicted to exhibit weaker affinities with respect to CAII and considerably weaker to CAVA, but higher affinities to CAVB. This suggestion of selectivity for one isozyme above others identified **4** and **5** as interesting targets for synthesis in order that biological testing could be carried out to assess their pharmacological properties and support the modelling process. The metabolites are known compounds having been isolated, characterised and synthesised [3] but their pharmaceutical potential has not been fully explored.

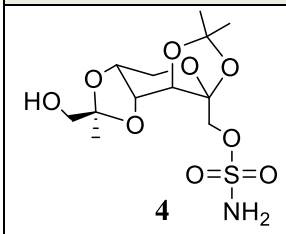
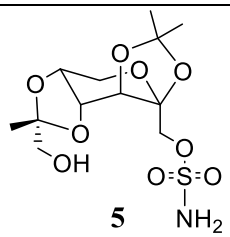
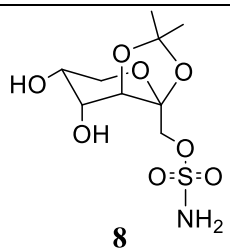
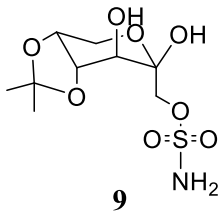
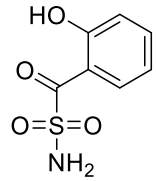
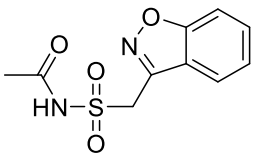
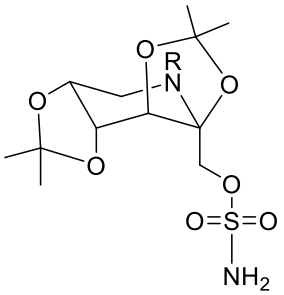
Compound	CAII	CAVA	CAVB
 **4**	-	--	+
 **5**	-	--	+
 **8**	--	-	-
 **9**	-	--	-
 **23**	-	-	-
 **24**	--	--	--

Table 3: Predicted affinities of various compounds to isozymes of carbonic anhydrase

(Key to affinity predictions: + = positive/++ = highly positive/- = negative/-- = highly negative)

The novel compounds under consideration are iminosugar analogues of topiramate, the basic structure of which is shown in Figure 18. All *in silico* models developed to date initially considered the analogue **25**. However, further substitution of the endocyclic nitrogen may be beneficial to the affinity of the molecule for the active site, may enhance isozyme specificity or may be necessary to afford the molecule with the necessary stability for *in vivo* application. Compounds **26** – **29** may be subject to further modelling work. However, the synthetic complexity in terms of construction of such scaffolds is worthy of mention and is a known issue within this field of chemistry.



25 R=H, **26** R=Me, **27** R=CF_3, **28** R=OH, **29** R=OMe

Figure 18: Iminosugar analogues of topiramate

The iminosugar analogue of topiramate **25** is predicted to bind with CAII in an almost identical binding pose to topiramate as depicted in Figure 13. The key difference is that the hydrogen bond between the oxygen atom of the pyranose ring of topiramate and the hydroxyl group of Thr 200 is replaced by a hydrogen bond donated by the secondary amine nitrogen in the new ligand to the hydroxyl group of Thr 200. In the protonated form, the new amine can also donate its other hydrogen to form a hydrogen bond with a complexed water present within the CAII active site (Figure 19).

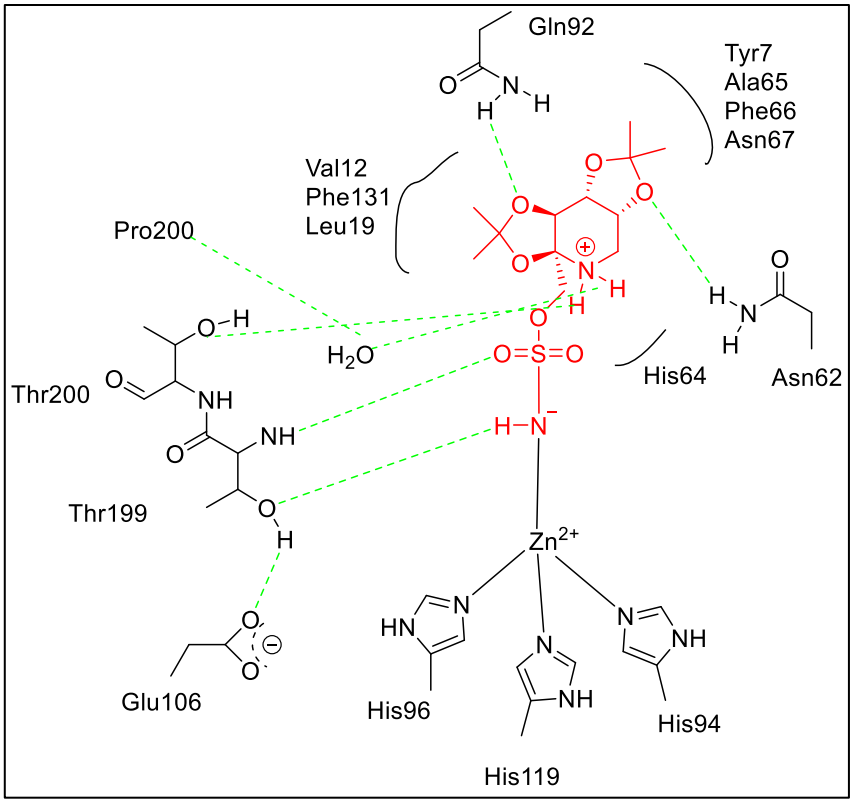


Figure 19: Schematic diagram of the predicted active site of human CAII and an iminosugar analogue of topiramate (with the ligand shown in red and hydrogen bonds represented by green dashed lines)

Overall, the iminosugar is predicted to bind with reduced affinity to CAII versus topiramate. This is related to increased energetics of docking due to structural differences in the iminosugar compared to its topiramate equivalent that will slightly penalise the binding of the ligand. It is proposed that the iminosugar will exhibit increased strain energy compared to that of topiramate. It is expected that the iminosugar analogue will have increased desolvation energy by virtue of the solvation required for a secondary amine versus an ether oxygen, even though the nitrogen atom is partially exposed to solvent and only directly contacts one amino acid. Of course, a prime objective of the research is to try and develop topiramate analogues that exhibit specificity for only particular CA isozymes and not CAII in particular. Thus a compound that has less affinity for CAII may be of benefit in that it may avoid CAII inhibition activity. The predicted reduced affinity of the iminosugar analogue may be compensated by other advantageous features of the ligand such as in its pharmacokinetics or increased solubility.

Introducing alternative functionalisation to the nitrogen atom of the iminosugar as in **26 - 29** affords opportunity to improve affinity and selectivity and this will be further assessed.

The binding modes of the iminosugar analogue **25** with CAVA is similar to topiramate. Of interest is that the proposed hydrogen bond interactions of the protonated amine with both the Thr 200 residue and a water molecule held within the active site is predicted to be conserved between CAII and CAVA. Similar to CAII, the compound exhibits slightly more strain energy than its topiramate counterpart and also slightly higher desolvation energy. Despite confirmation strain energy and desolvation energy increases, **25**, is predicted to achieve a positive binding affinity for CAVA and CAVB. It is also of note that modelling suggests there is increased scope to introduce additional functionality/structural motifs onto the sugar scaffold which could in turn lead to advantageous isozyme differentiation. The specifics of this were intended as part of the second iteration of compounds once positive conformation of affinity, through *in vivo* testing, was obtained.

CHAPTER 2. Synthesis and Biological Evaluation of Metabolites and Isoteres of Topiramate and N-acetyl Zonisamide

The *in silico* modelling work as detailed in Section 1.3 suggested that several metabolites of topiramate **1** may have potential as CAIs with the ability to demonstrate a greater specificity than topiramate for the CA isozymes CAVA and CAVB. Compounds **4**, **5** and **8** were identified as initial synthetic targets with the objective of obtaining CA inhibitory data on these compounds that would be used to evaluate the predictions generated by the *in silico* modelling and to further develop the computational models to identify other possible CAIs.

A second group of compounds, **30** to **34** (Figure 20), were also of interest and were identified as additional synthetic targets. In respect of this range of compounds, the sulfamate group that is present in topiramate **1** is replaced with various five membered heterocycles that have a loose isosteric relationship to the sulfamate group. It was hypothesised that the heteroatoms present within the five membered rings would provide comparable opportunities for hydrogen bond interactions with residues within the active sites of CA isozymes to those observed for the sulfamate group. This may lead to alternative binding modes to that of the lead compound topiramate **1** which may generate favourable biological results in terms of CA affinity and specificity. In addition, measurement of the biological activity of these compounds would provide additional and useful indicators for the ongoing development of selective CAIs.

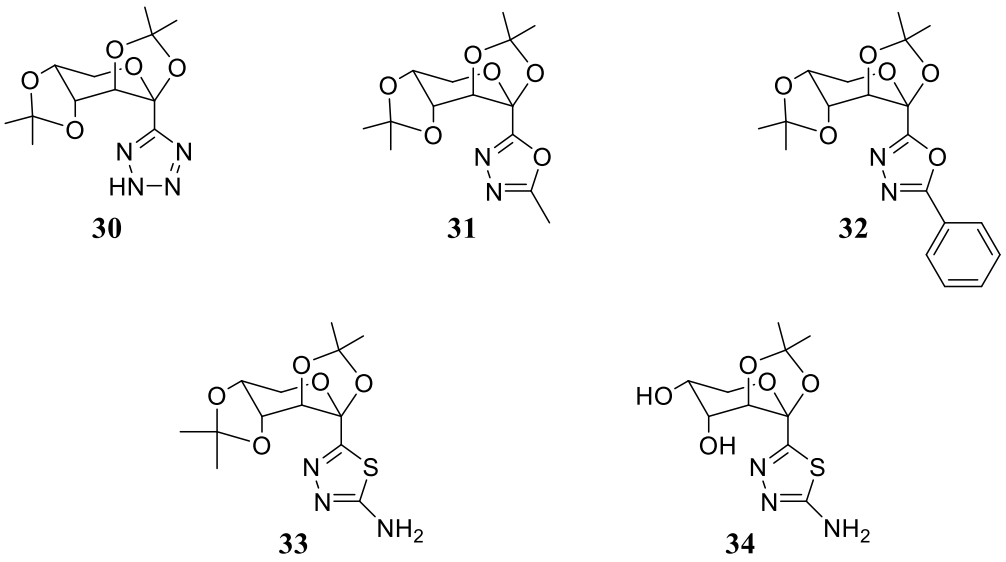


Figure 20: Heterocyclic analogues of topiramate

A second tranche of related novel compounds, **35** to **38** (Figure 21), in which the linker between the heterocycle and the pyranose ring is extended by a methylene group were also of synthetic interest. The heterocycles offer hydrogen bonding opportunities and the additional methylene group at the anomeric position of the pyranose ring affords greater conformational flexibility. Whilst this greater flexibility may incur an energy penalty in terms of affording a fixed binding conformation, this was likely to be offset by the potential to allow increased interactions with key amino acid residues within the active site as well as with zinc.

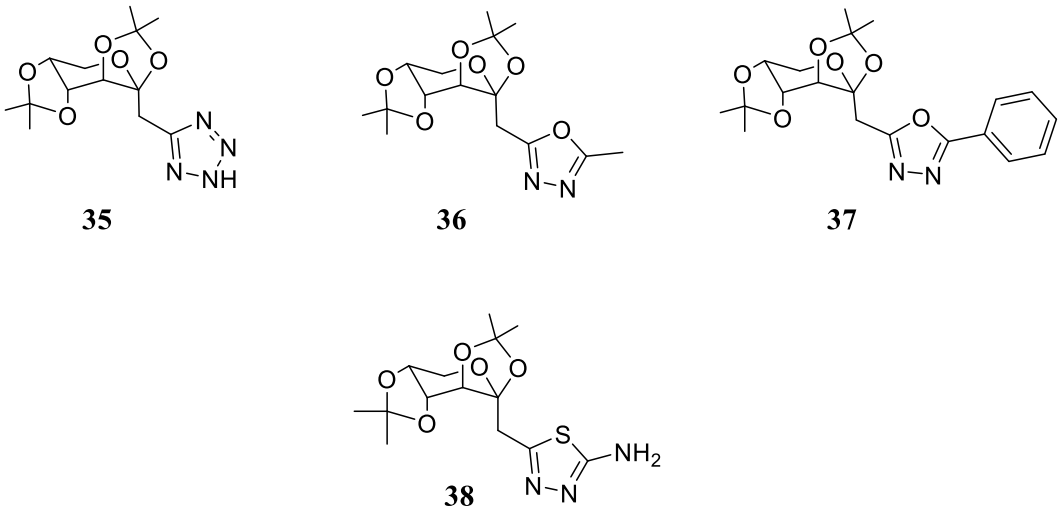


Figure 21: Additional heterocyclic analogues of topiramate

The synthesis of *N*-acetyl zonisamide **24** (Figure 22) was also supported. Although the *in silico* modelling work predicted that **24** was likely to have limited affinity for the CA isozymes of interest, obtaining biological data for this compound would provide a useful reference to support the ongoing programme of *in silico* modelling. In addition, any data collected was useful to assess the ongoing potential of derivatives and analogues of zonisamide as selective CAIs.

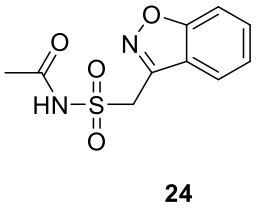
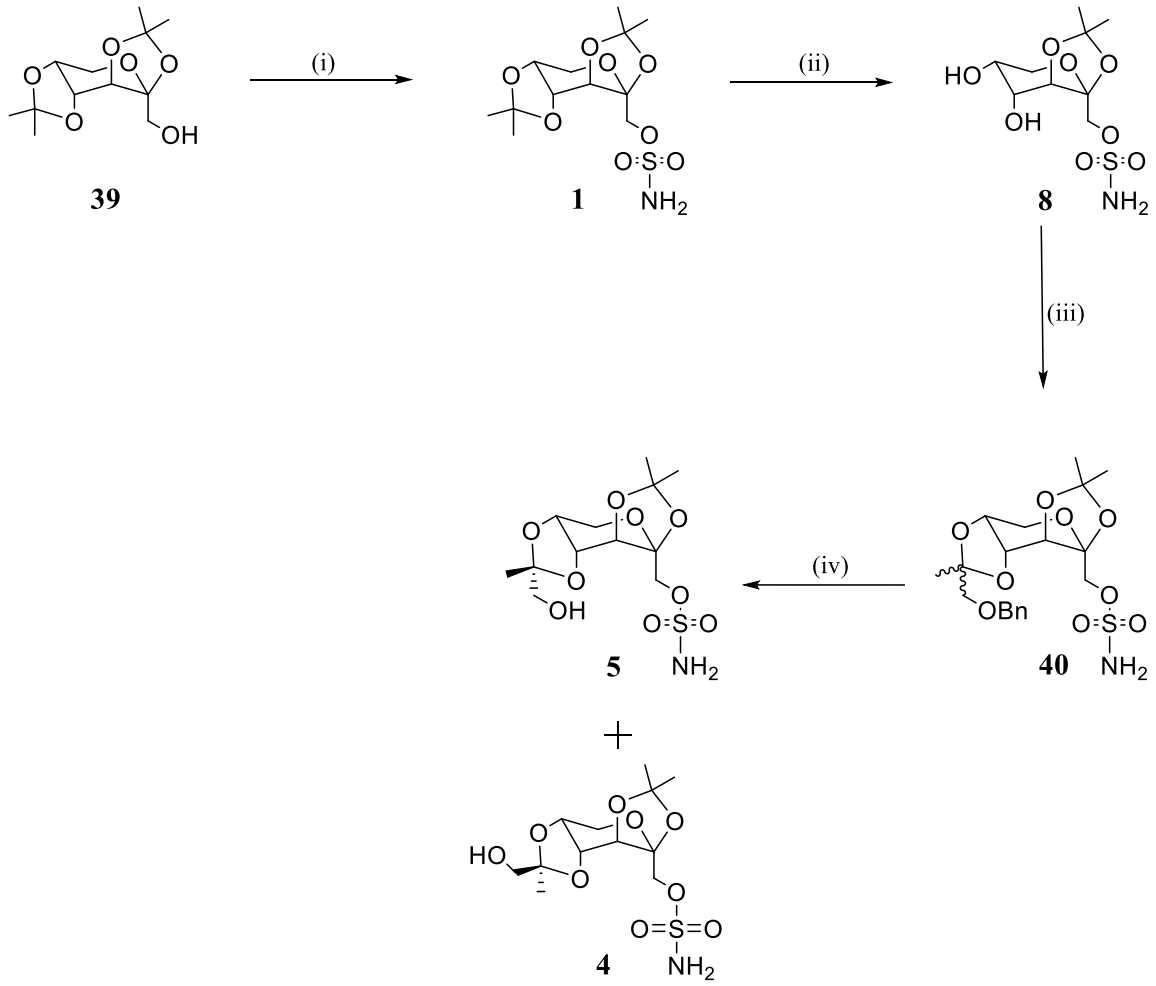


Figure 22: Structure of *N*-acetyl zonisamide

2.1. Synthesis of Topiramate Metabolites

Scheme 1 details the proposed preparative route to the hydroxylated diastereomeric metabolites **4** and **5** of topiramate (Scheme 1). The desired third metabolite, **8**, is obtained as an intermediate in the overall synthetic scheme. Scheme 1 was adapted from a literature precedent with several changes introduced to improve product purity and yield at particular stages.[3] Initially, the starting material, 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39**, was readily prepared from the condensation reaction of the monosaccharide *D*-fructose with acetone and concentrated sulphuric acid. However, for later syntheses, a cheap commercial source of **39** was identified and purchased in bulk.



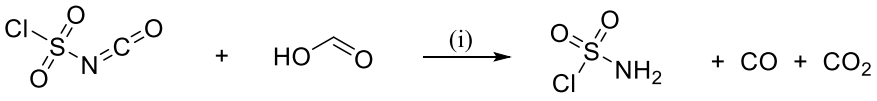
Reagents and conditions: (i) Sulfamoyl chloride, THF, Et_3N, 0^0C to RT; (ii) 3N HCl, THF, 40^0C; (iii) Benzyloxyacetone, ethanol, triethylorthoformate, conc. H_2SO_4, RT, 3h; (iv) H_2, 10% Pd/C, ethanol, RT, 24h.

Scheme 1: Preparation of 4,5-*O*-(1-hydroxymethylethylidene)-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate (*R*/*S*)

The first stage of the synthetic route was to introduce the biologically important sulfamate group to 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39** to obtain topiramate **1**. The reported approaches to installing a sulfamoyl group on alcohols are very limited.[43] The most widely cited procedure for sulfamoylation is to treat the appropriate alcohol, in this case the primary alcohol group attached to the anomeric carbon of **39**, as a solution in an anhydrous aprotic solvent under nitrogen with a suitable base, generally sodium hydride, at low temperature, followed by the addition of sulfamoyl chloride **43**. The resulting reaction mixture is then allowed to warm to room temperature under nitrogen.[44] Sulfamoyl chloride has been

widely used as the reagent to effect this transformation in steroids and other primary alcohols and was considered appropriate for use here. [45] [46]

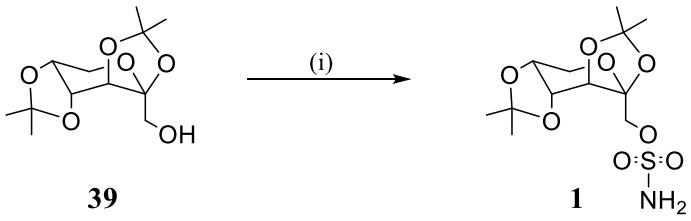
As an inexpensive commercial supply of sulfamoyl chloride was not readily available, it was necessary to prepare the reagent in a quantity sufficient to support the chemistry that was planned. The methodology for preparing sulfamoyl chloride from the reaction of chlorosulfonyl isocyanate **41** and formic acid **42**, Scheme 2, was employed.[47] Several adaptations were introduced to the literature procedure to improve the results obtained and maximise product yield when the reaction was carried out on a larger scale. It was hoped that it would be necessary to only undertake one or two large scale preparations of sulfamoyl chloride to support ongoing chemistry development. Initially, the reaction shown in Scheme 2 was carried out without solvent. On a small scale, *ca* 1g, this was found to work satisfactorily. However, increasing the scale resulted in a viscous and immobile reaction mixture despite the use of overhead agitation. The addition of anhydrous toluene whilst the formic acid was added to the chlorosufonyl isocyanate was found to aid reaction mobility and homogeneity and thus improve reaction progression. Sulfamoyl chloride is often stored and used as a solution in toluene but decomposes rapidly and is generally used *in situ*.[44] [47] This was not readily amenable to the production of a large quantity of sulfamoyl chloride that could be used over a period of time. It was found that a more effective solution on a larger scale was to isolate the sulfamoyl chloride as a solid by removal of the toluene by concentration *in vacuo* at low temperature. No further purification of the sulfamoyl chloride was undertaken and the material was used in its crude form in the subsequent chemistry steps. Careful storage of the resulting solid under nitrogen and at temperatures below 0°C was necessary to prevent decomposition due to ingress of air and moisture. The reaction was successfully completed on a 100g scale producing sulfamoyl chloride considered to be of satisfactory activity and purity. The prepared sulfamoyl chloride was shown to have a good "shelf-life" when stored appropriately with no reduction in activity observed in reactions carried out over six months after its initial production.



41 **42** **43**

Reagents and conditions: (i) Toluene, 0^0C-RT.

Scheme 2: Preparation of sulfamoyl chloride **43**



39 **1**

Reagents and conditions: (i) Sulfamoyl chloride, THF, Et_3N, 0^0C to RT.

Scheme 3: Preparation of topiramate **1** from 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39**

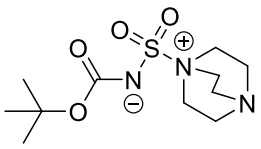
In order to obtain reliable reference data of the initial step of Scheme 1 and confirm reaction outcomes, a model reaction was carried out using a small amount of commercially sourced sulfamoyl chloride. An initial small scale reaction was attempted with 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39** and the commercially sourced sulfamoyl chloride in order to produce reference NMR, mass spectrometry and TLC analysis of **1**. DMF was used as the reaction solvent in accordance with literature procedure[1] although triethylamine was used as base in replacement of sodium hydride. The analytical data of the product obtained from this first reaction conformed to published data.[1] The reaction was repeated under identical conditions using sulfamoyl chloride that had been synthesised in accordance with Scheme 2. It was noted that a slightly greater stoichiometric amount of sulfamoyl chloride was required in the second reaction to push the reaction to completion than was necessary in the first when the commercial supply of sulfamoyl chloride was used. This suggested that the sulfamoyl chloride produced from formic acid and chlorosulfonyl isocyanate was less active, more prone to decomposition under the reaction conditions employed leading to a larger impurity profile. The core analytical data obtained for the resulting product of the reaction was concordant with that of **1** prepared using the commercial supply of sulfamoyl chloride. Active yields of *ca* 40%

were achieved in both experiments which were broadly in line with literature reported yields.[3] However, an impurity was observed in the ^{1}H NMR of the isolated crude product from the second reaction, using prepared sulfamoyl chloride, in an approximately 10% molar quantity. The impurity remained post purification of the crude material by chromatography. The chemical shifts of the impurity suggested that it related to a DMF adduct from the competing reaction between DMF and sulfamoyl chloride, although the identity of the impurity was not readily apparent by mass spectrometry. Similar results have been reported in the literature where DMF was used as the reaction solvent and excess equivalents of strong base were used.[44] As the formation of this impurity was only apparent in the second reaction where sulfamoyl chloride synthesised from chlorosulfonyl isocyante and formic acid was used it may be attributable to it having lower overall purity than that of the commercial supply which meant that it was necessary to use a greater excess of sulfamoyl chloride to achieve reaction completion. To assess whether the impurity would affect the subsequent stages of chemistry of Scheme 1, trial reactions were carried out using a small amount of **1** prepared in the second reaction which contained the 10% impurity. Disappointingly, ^{1}H NMR data showed that the impurity was carried through subsequent reaction stages despite several purification steps in the downstream chemistry.

The use of purchased sulfamoyl chloride was not an available solution to preventing the impurity with the costs being too prohibitive on a larger scale. Purification of sulfamoyl chloride prepared in-house was not a viable option either as the material was too reactive. Methods of optimising the reaction to prepare sulfamoyl chloride were also considered as a means of increasing the purity of the reagent. This proved challenging with very little literature guidance and generally small scope for improvement offered by the reaction. The challenge was, therefore, to attempt to optimise the conditions of the sulfamoylation reaction of 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39** in order to prevent the formation of the impurity while still obtaining a reasonable yield of active material. The first option was to alter the conditions of the reaction of fructopyranose **39** with sulfamoyl chloride from those followed. The most obvious change to be considered was the replacement of DMF as reaction solvent as it was postulated that the impurity was solvent derived. Alternative solvents used as the medium for the sulfamoylation reaction reported in the literature were DMA, NMP, DCM, DME and THF. [44, 45] A trial reaction to prepare **1** with a sample of sulfamoyl chloride from the same batch prepared in accordance with Scheme 2 was carried out in THF and was successful. A similar yield was obtained to that when the reaction was carried out in DMF and

analysis of the product by NMR and mass spectrometry confirmed the desired product had formed. No impurities were evident as in the earlier reaction carried out in DMF representing a very positive result. The reaction was subsequently scaled up successfully applying the same conditions.

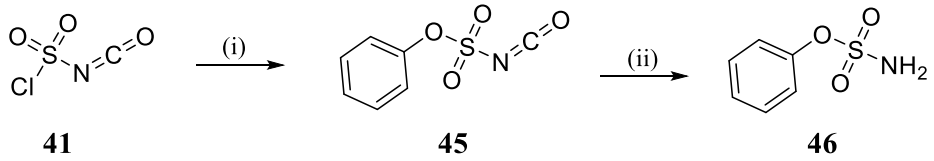
Consideration was also given to the potential of developing alternative sulfamoylation reagents. There has been recent pharmaceutical research interest in a range of antibiotic and anti-tumour agents containing the sulfamate group.[43] This has led to a focus on the development of novel sulfamoylating reagents that improve upon the stability, safety, reactivity and "process-friendliness" of sulfamoyl chloride. [43,48] A recently reported sulfamoylation reagent is the Burgess-type analogue **44** which was demonstrated to readily install a sulfamoyl group on a range of primary and secondary alcohols under relatively mild conditions. [49] [43]



44

Figure 23: Burgess-type sulfamoylation reagent, *N*-(*tert*-butoxycarbonyl)-*N*-[(triethylenediammonium)sulfonyl]azanide

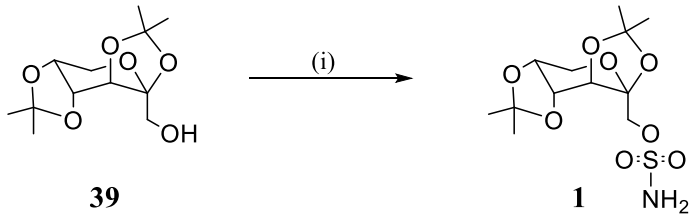
However, the synthesis of this Burgess-type reagent was not trivial and it was considered of interest to pursue other avenues. It was postulated that the replacement of the chlorine atom with a suitable leaving group e.g R–O-SO_2-NH_2 would result in an effective sulfamoylation reagent that would confer improved stability to that of sulfamoyl chloride. The preparation and use of phenyl sulfamate **46** as an alternative sulfamoylation reagent was investigated in the first instance. Scheme 4 outlines the preparative method employed to prepare phenyl sulfamate **46**. Chlorosulfonyl isocyanate **41** was reacted with phenol in toluene to give the intermediate isocyanate **45**. The addition of **45** to water resulted in formation of phenyl sulfamate **46** which was obtained as a white solid. NMR and LCMS data confirmed the desired product had been prepared in good yield and purity.



41 **45** **46**

Reagents and conditions: (i) Phenol, toluene, 100^0C, 22hr; (ii) H_2O

Scheme 4: Preparation of phenyl sulfamate



39 **1**

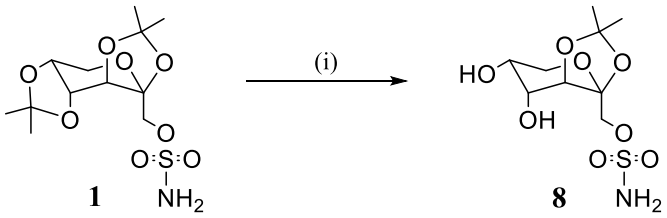
Reagents and conditions: (i) Phenyl sulfamate, toluene, Et_3N, reflux; 24h.

Scheme 5: Preparation of topiramate 1 from 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39** with phenyl sulfamate

The reaction of phenyl sulfamate **46** with 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39** (Scheme 5) resulted in the formation of the desired product **1** without any significant impurities present. The conditions used for the reaction are shown in Scheme 5. Toluene was chosen as the reaction solvent and triethylamine as base. No reaction between **39** and phenyl sulfamate was observed at room temperature. Increasing the reaction temperature to *ca* 100°C did induce some reaction progression with evidence of the presence of phenol as a by-product by TLC. Reaction progression was slow despite the addition of a large excess of phenyl sulfamate to the reaction in an attempt to consume starting material. A poor overall yield of **1**, *ca* 8%, was achieved which was disappointing and the recovery of *ca* 46% of the input fructopyranose **39** confirmed a poor reaction profile under the conditions employed.

The reaction outcomes were positive insofar as analysis of the product obtained from the reaction of fructopyranose **39** and phenyl sulfamate correlated with that obtained from the analogous reaction with sulfamoyl chloride confirming that phenyl sulfamate could be used as an alternative sulfamoylation reagent. However, poor reaction progression and suspected

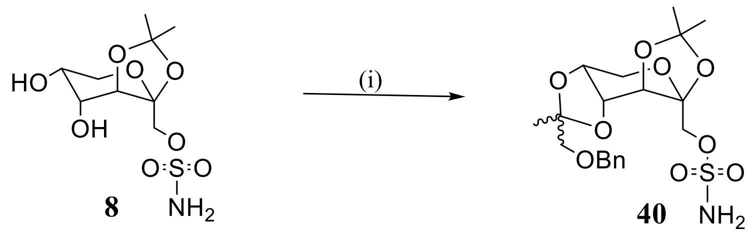
decomposition of phenyl sulfamate at sustained high temperatures resulted in a very low yield. This demonstrated that further development work would be required to sufficiently optimise the reaction conditions in order for phenyl sulfamate to be able to offer a viable alternative to the use of sulfamoyl chloride. Had time permitted, the scope for developing this reaction and of alternative sulfamoylation reagents would have afforded an interesting avenue for the project. The next obvious steps would be a change of solvent and use of a stronger base e.g. sodium hydride. As the ultimate goal of the project was synthesis of the metabolites to obtain *in vitro* data, priority was not given to this interesting side investigation. This is considered as an interesting area for additional development.



Reagents and conditions: (i) 3N HCl, THF, 40^0C, 3.5h.

Scheme 6: Selective deprotection of topiramate **1**

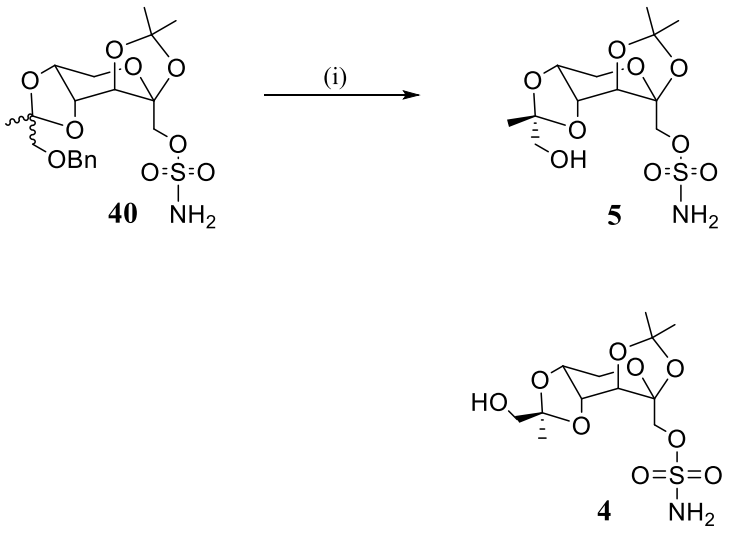
With the sulfamate group successfully installed, the next step in the preparation of topiramate metabolites **4** and **5** was the selective deacetalisation of the dioxolane ring at the 4,5- position around the pyranose ring under acidic conditions to give the diol 2,3-*O*-isopropylidine-*β*-*D*-fructopyranose sulfamate **8** (Scheme 6). It was important to maintain a controlled reaction in order to avoid the hydrolysis of the second acetal group at the 2,3- position. Monitoring of the reaction by TLC and HPLC enabled adequate tracking of reaction progression. It was established that heating the reaction for three and a half hours at *ca* 50°C allowed for sufficient starting material to be selectively deprotected to give the desired product. The product was isolated as a white solid following chromatography in a reasonable yield of 29% in line with that reported in the literature.[50] ^{1}H NMR of the purified product confirmed the presence of only two remaining methyl groups and data conformed to that in the literature.[1] The product of the reaction **8** is a metabolite of topiramate **1** and its submission for biological testing was supported.



Reagents and conditions: (i) Benzyloxyacetone, ethanol, triethylorthoformate, conc. H_2SO_4, THF, RT, 3h.

Scheme 7: Preparation of 4,5-*O*-(1-benzyloxymethylethylidene)-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **40**

The next stage of the synthesis was the formation of 4,5-*O*-(1-benzyloxymethylethylidene)-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **40** from the reaction of 2,3-*O*-isopropylidine-*β*-*D*-fructopyranose sulfamate **8** with benzyloxyacetone in the presence of triethyl orthoformate and concentrated sulphuric acid (Scheme 7). A mixture of *R* and *S* acetals in a 6:1 ratio was reported in the literature.[3] Analysis of the reaction by HPLC and LCMS clearly showed the formation of two products in a similar ratio to that reported confirming successful preparation of the two diastereomers. The separation of the diastereomers by chromatography was challenging and deemed unnecessary at this point. Therefore the isomeric mixture was taken through to the subsequent reaction. An overall yield of for the two isomers of 56% was obtained.



Reagents and conditions: (i) H_2, 10% Pd/C, ethanol, RT, 24h.

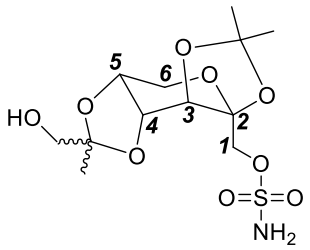
Scheme 8: Preparation of 4,5-*O*-[(1R)-1-hydroxymethylethylidene]-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **5** and 4,5-*O*-[(1S)-1-hydroxymethylethylidene]-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **4**

The final stage of the synthesis (Scheme 8) was the debenzylation of **40** by hydrogenolysis and the separation of the two resulting diastereomers. The reaction proceeded satisfactorily and although the separation of the two products by column chromatography was not trivial, two compounds were successfully obtained in 39% and 6% yields which was comparable to those reported in the literature.[3] It was anticipated from the literature that the ratio of the *R:S* diastereomers would be roughly 6:1. The hydrogenolysis resulted in the isolation of two white solids that were readily distinguishable by their NMR and mass spectrometry data; 762mg of one diastereoisomer and 109mg of the other diastereoisomer were obtained. This result largely reflected the expected ratio of *R*:*S* diastereomers and thus the product obtained in the highest proportion was tentatively assigned as the *R*-isomer **5** and the minor product obtained was proposed to be the *S*-isomer **4**. However, comparison of the ^{1}H NMR data for **4** and **5** to that reported in the literature [3] highlighted some differences in the relative chemical shifts of certain protons that warranted further investigation before the stereochemistry of the two products could be confidently assigned. The data collected for each compound is summarised in Table 4.

	Major Product **Proposed *R*-isomer (5)**	**Minor Product** **Proposed S-isomer (4)**
Melting point	104 - 106°C (Literature: 106 - 108°C)[3]	122 - 124°C (Literature: 136 - 140°C)[3]
^{1}H NMR Assay	97% purity	97% purity
Mass spectra	m/z 378 (MNa^+) 373 (MNH_4^+) 356 (MH^+)	m/z 378 (MNa^+) 373 (MNH_4^+) 356 (MH^+)

Table 4: Summary of analytical data for the major and minor products from the debenzylation reaction of 4,5-*O*-(1-benzyloxymethylethylidene)-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **40**

The melting point recorded for the major product is sufficiently close to the value quoted in the literature to suggest it is the *R* isomer. The melting point for the minor product, however, is markedly lower than the literature value and therefore does not readily assist in confirming the identification of this compound. The impact of impurities or entrapped solvent were postulated as depressing the melting point. The same key peaks were visible in the mass spectrum of each compound, both mass spectra indicated the correct mass for the desired product, 355.36gmol^{-1}. The ^{13}C NMR spectra of the products show the correct number of carbon atoms (12) with appropriate chemical shifts. Unfortunately, ^{13}C NMR data was not included within the published literature so a direct comparison of this data was not possible. The ^{1}H NMR data for both the major and minor products is shown in Figure 24 and
Figure **25** respectively and the NMR data is summarised below. Proton numbering is in accordance with the molecule below.



^{1}H NMR data of the major product from the debenzylation reaction of **40**:^{1}H NMR (DMSO-d_6, 270MHz): δ 1.25, 1.34, 1.46 (3 s, 9H, CH_3), δ 3.34 (s, 2H, CH_2OH), δ 3.62 (d, 1H, J = 13.2Hz, H_6), δ 3.76 (d, 1H, J = 13.2Hz, H_6) δ 3.99 (s, 2H, H_1), δ 4.27(d, 1H, J = 2.7Hz, H_3), δ 4.30 (d, 1H, J = 8.6Hz, H_5), δ 4.64 (dd, 1H, J = 2.7, 8.6Hz, H_4), δ 4.9 (bs, 1H, O*H*), δ 7.62 (bs, 2H, NH_2)

^{1}H NMR data of the minor product from the debenzylation reaction of **40**:^{1}H NMR (DMSO-d_6, 270MHz): δ 1.26, 1.33, 1.46 (3 s, 9H, CH_3), δ 3.29 (dd, 2H, CH_2OH), δ 3.62 (d, 1H, *J* = 13.2Hz, H_6), δ 3.74 (d, 1H, *J* = 13.2Hz, H_6), δ 3.95 – 4.04 (dd, 2H, H_1), δ 4.24 (d, 1H, *J* = 2.7Hz, H_3), δ 4.32 (d, 1H, *J* = 7.8Hz, H_5), δ 4.66 – 4.69 (dd, 1H, *J* = 2.7, 7.8Hz, H_4), δ 4.96 (t, 1H, O*H*), δ 7.64 (bs, 2, NH_2)

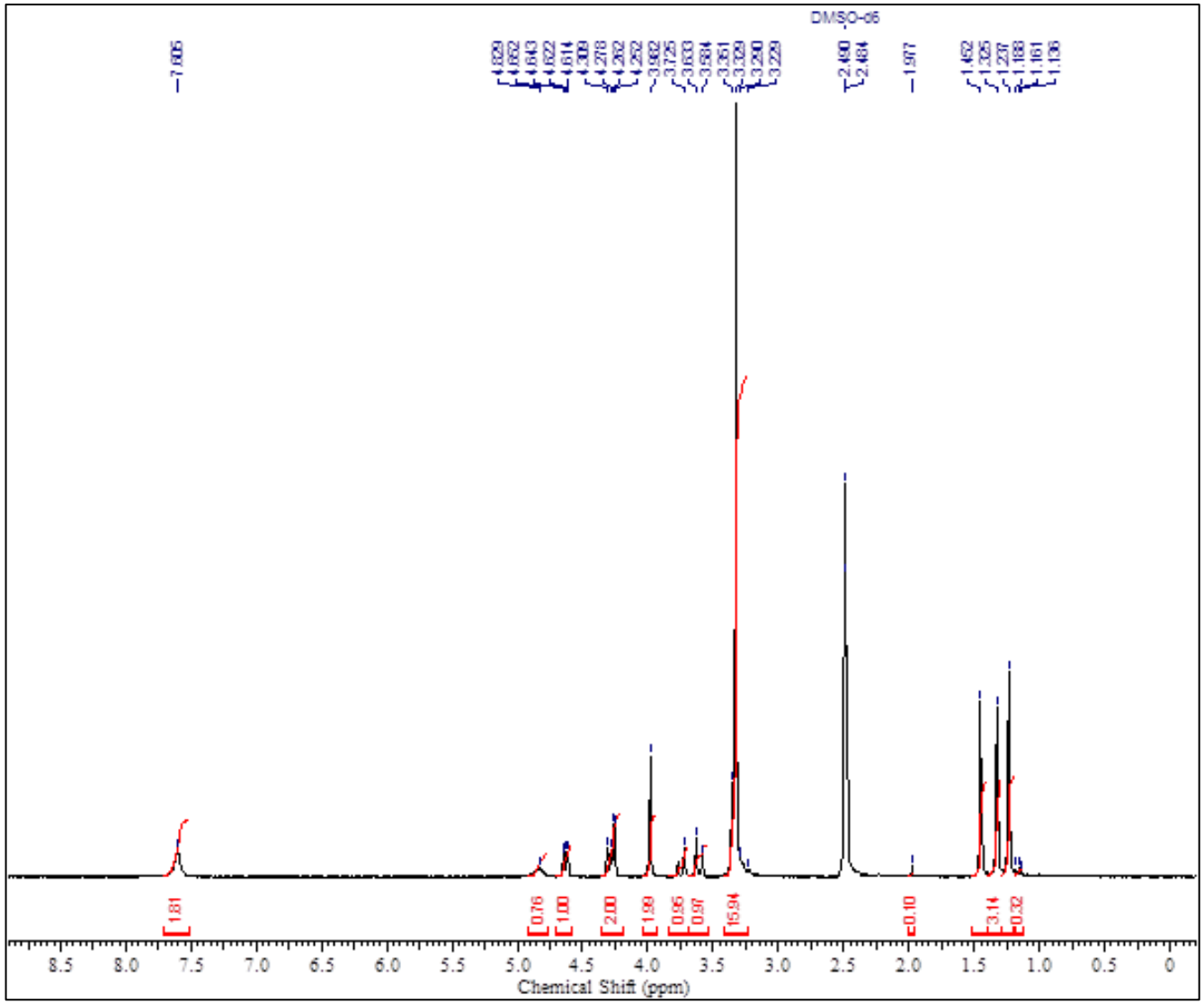


Figure 24: ^{1}H NMR spectrum of the major product from the debenzylation reaction of **40**

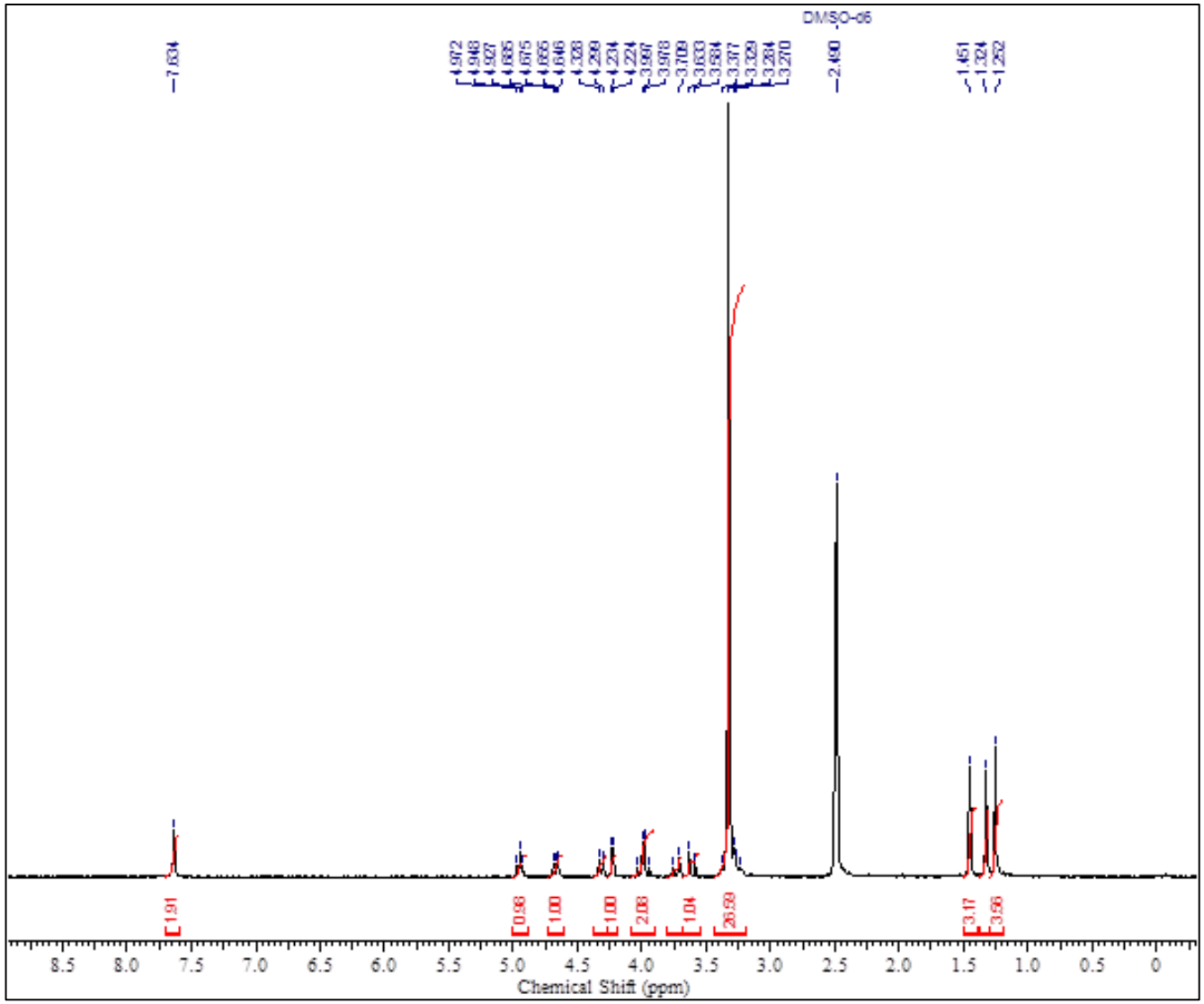


Figure 25: ^{1}H NMR spectrum of the major product from the debenzylation reaction of **40**

A D_2O shake of each sample revealed the position of the primary CH_2-OH group. A 2D COSY NMR experiment confirmed proton assignments. The assignment of the stereochemistry of each isomer was not straightforward by the direct comparison of the ^1H NMR data with that contained in the literature as there were some subtle differences that did not render the compounds readily identifiable.[3] The key differences in the ^1H NMR data were the relative chemical shifts of the protons at positions three (H_3) and five (H_5) on the pyranose ring. Further NMR experiments were undertaken to attempt to elucidate the stereochemistry of each compound and thus make a positive identity. A 2D NOESY NMR experiment was carried out for each compound. The resulting spectrum of a 2D NOESY experiment contains both diagonal and cross peak signals. The diagonal spectrum consists of the 1D spectrum and the cross peak signals arise from protons that are close in space. It thus can provide valuable information about the spatial proximity between protons even if they are not bonded together. It can therefore be a powerful tool in the assessment of molecular geometry and assignment of stereochemistry. Although the 2D NOESY spectra obtained for the two diastereoisomers had low resolution and were relatively difficult to interpret, the spectrum for the major product of the reaction did show a weak correlation between the protons of one of the three methyl groups, presumed to be the single methyl group on the 4,5-acetal group, and the proton at position four on the pyranose ring (H_4). This correlation was not evident in the 2D NOESY spectrum of the minor product. Consideration of the 3D models of each of **5** and **4**, Figure 26 and Figure 27 respectively, a through space interaction between the methyl protons and H_4 would be an expected result for **5** with *R* stereochemistry. The interaction would not be expected in **4** which has *S* stereochemistry.

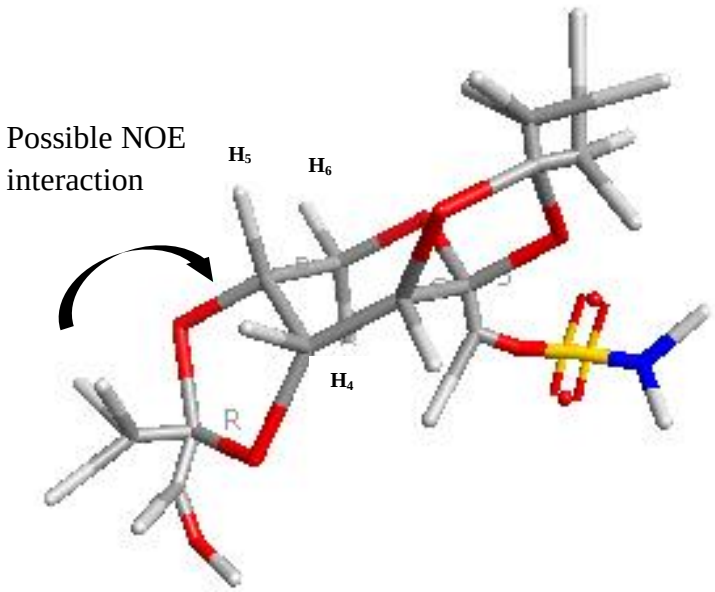


Figure 26: 3D representation of 4,5-*O*-[(1R)-1-hydroxymethylethylidene]-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **5**

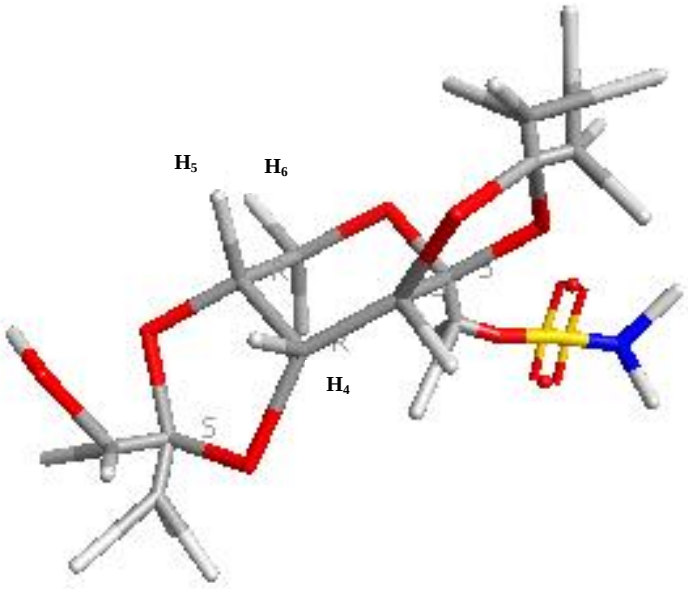


Figure 27: 3D representation of 4,5-*O*-[(1S)-1-hydroxymethylethylidene]-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **4**

Additional NMR experiments were carried out at the Newcastle University NMR Department to provide further data to confirm the structures and stereochemistry of the two compounds prepared. An important outcome of this was the ability to distinguish between the three methyl groups of each isomer so as to make meaningful interpretations of the observed 2D NOESY data. 2D HSQC and 2D HMBC experiments were carried out for both the major and minor product. For HSQC, the resulting 2D spectrum correlates directly bonded carbon and proton nuclei that can be used to map known proton assignments onto their directly attached carbons. An HMBC spectrum is used to identify long-range couplings, generally across two to three bonds, between protons. Consideration of each spectrum in conjunction with the ^{1}H, ^{13}C and 2D COSY NMR data confirmed the correct proton and carbon assignments had been made and enabled the elucidation of particular methyl groups.

Equipped with a clear interpretation of the NMR data collected thus far, several 1D NOE experiments were carried out for each compound. The value of these experiments relies upon the correct assignment of each proton to be able to establish the appropriate experiments to undertake. This method would support the results of the 2D NOESY experiments and provide further salient information about the spatial relationship between the single methyl group on the 4,5-acetal and the protons around the pyranose ring. A 1D NOE experiment differs from its 2D counterpart in that it relies on selective excitation of one peak by irradiation at a specific frequency and observing the NOE at another position within a molecule. The method is particularly useful if the determination of a correlation between a precise pair of protons is required. In the resulting NOE spectrum, a positive interaction with another proton is represented by an inverse peak, the position of which correlates with the chemical shift of that particular proton. The selectively excited peak is shown as a positive peak at the position corresponding to the relevant chemical shift of the excited proton.

For the major isomeric product of the reaction, tentatively proposed as the *R* isomer **5**, irradiation at the frequency of H_4 showed a strong positive interaction with the singular methyl group on the 4,5-acetal as shown by Figure 28. Figure 29 also shows positive correlation with H_3 and H_5 protons. Both of these results would be anticipated for the *R* isomer as confirmed by inspection of Figure 26.

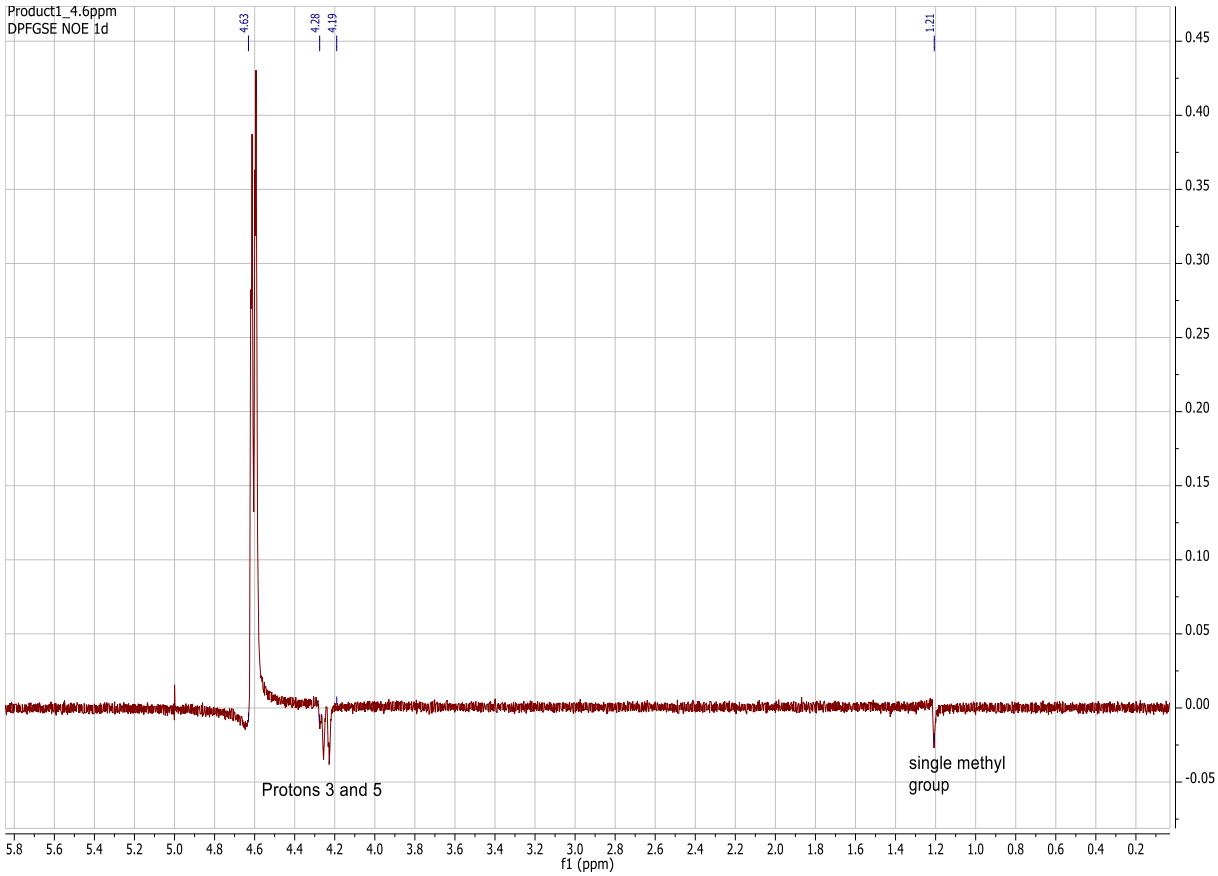


Figure 28: 1D NOE spectrum of major isomer – irradiated at the frequency of H_4

The 1D NOE experiment where the major isomer was irradiated at the frequency of the singular methyl group of the 4,5-acetal gave a positive interaction with protons H_4, H_5 and the methylene group of the hydroxyl group of the 4,5-acetal (Figure 29). This was a promising result if the 3D representation of the molecule (Figure 26) is considered as through space interactions would certainly be expected between each of the H_4 and H_5 proton and the protons of the methylene group within the *R* isomer.

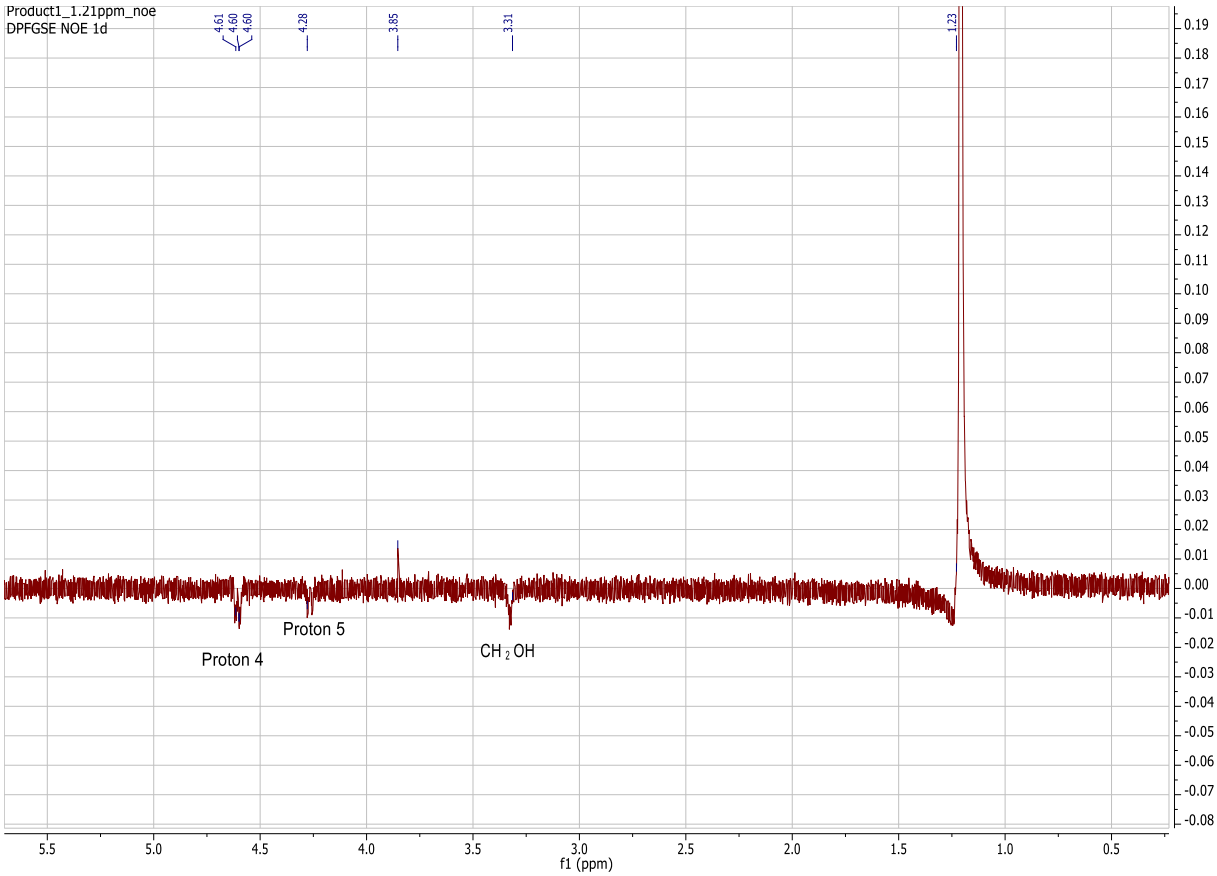


Figure 29: 1D NOE spectrum of major isomer – irradiated at the frequency of the single methyl group

In addition to the previous two experiments, further 1D NOE experiments were conducted in order to test the correct assignment of the three methyl groups. A sample of the major isomer was irradiated at a frequency that was considered to correspond with the chemical shift of one of the methyl groups of the acetal group at the 2,3-position of the pyranose ring. This resulted in a clear interaction with the geminal methyl group and thus confirmed the correct assignment and frequency of the third singular methyl group of the 4,5-acetal.

Similar 1D NOE experiments were conducted for the minor isomer, tentatively proposed as the *S* isomer **4**. The following results were observed:

- irradiation at the frequency of the singular methyl group of the 4,5-acetal gave no interaction with any protons of the pyranose ring as shown in Figure 30. This would appear to be a reasonable result if the 3D representation of the molecule (Figure 27) is considered with the position of the singular methyl group being more remote from the protons around

the pyranose ring. These results support the proposed stereochemical assignment of *S* to the minor isomer.

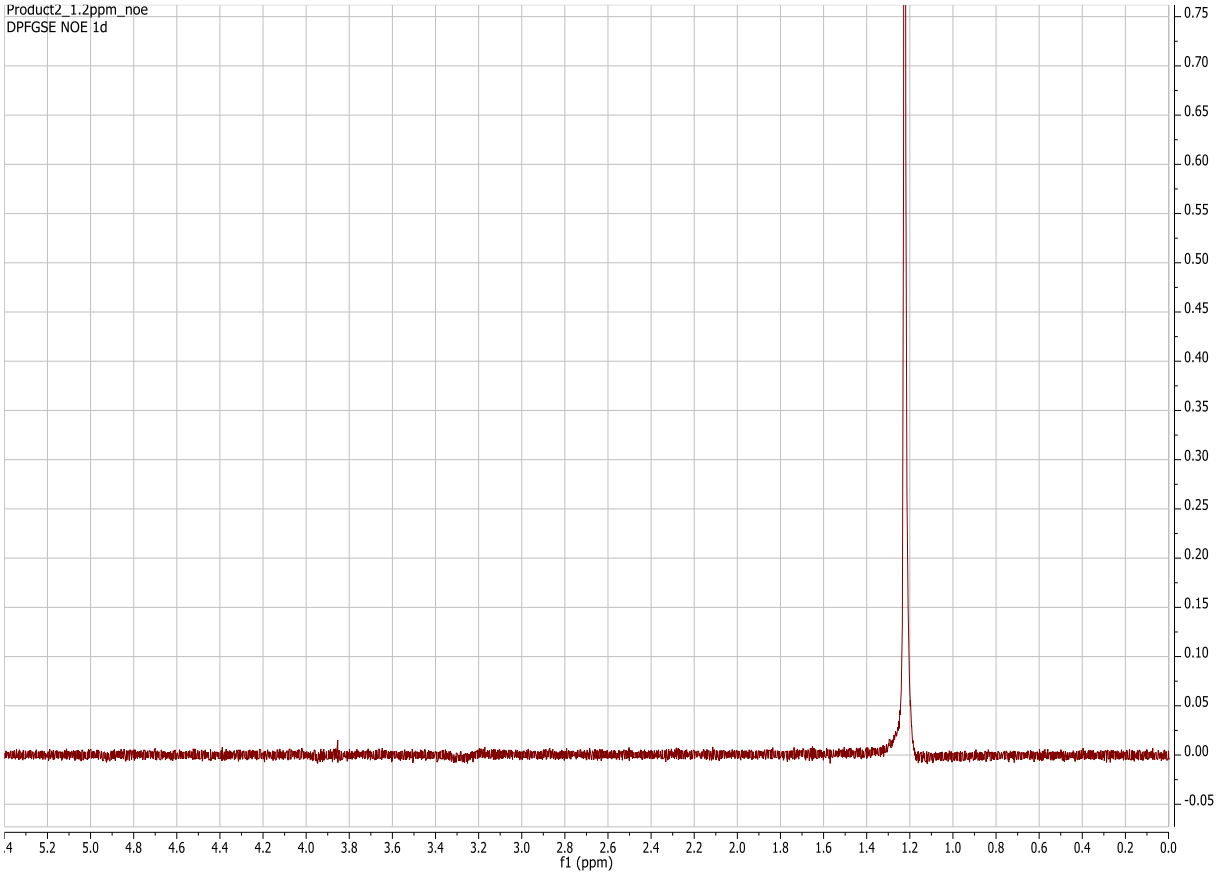


Figure 30: 1D NOE spectrum of minor isomer – irradiated at the frequency of the single methyl group

The extensive analysis undertaken for each of the two final products obtained by following Scheme 1 has enabled the structures of each compound to be defined with some confidence. All data collected confirms the major isomer as the desired metabolite 4,5-*O*-[(1R)-1-hydroxymethylethylidene]-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **5** and the minor isomer as 4,5-*O*-[(1S)-1-hydroxymethylethylidene]-2,3-*O*-isopropylidene-*β*-*D*-fructopyranose sulfamate **4**. It is difficult to account for the differences between the ^{1}H NMR data of the compounds prepared here and that included within the literature but it may be related to slight conformational differences induced by temperature or concentration or differences in the purity profiles of the materials. Analytical data collected for each intermediate compound of Scheme 1 conformed to the published ^{1}H NMR data which meant that any potential problems with the intermediate compounds could be ruled out.

Sufficient quantities of **4**, **5** and **8** were successfully prepared in suitable purity to submit for biological evaluation to determine CA inhibition against a range of CA isozymes.

2.2. Synthesis of five-membered heterocyclic analogues of topiramate

A range of analogues of topiramate, **30** to **34** (Figure 20) have been synthesised in which the sulfamate group has been replaced by various hetereocycles. Although known compounds,[51] they represent an interesting class of compounds from which novel derivatives may be obtained relatively easily. There is currently no known literature on the biological activity of these compounds and as such they may represent an opportunity for the development of novel APIs if any demonstrate CA activity and specificity.

Scheme 9 shows the total synthetic pathway from 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39** to the oxadiazoles **31** and **32**. The efficient methodology was adapted from a literature procedure with the key chemical step being the formation of the tetrazole **30** by 1,3-dipolar cycloaddition.[51, 52] The "diacetone fructose" **47**, was obtained by the oxidation of the primary alcohol of 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39**. Pyridinium chlorochromate, *o*-iodobenzoic acid (IBX), Pfitzner-Moffatt reagent and manganese dioxide have been reported as oxidising agents used to prepare **47**. [51, 53, 54, 1] However, none of these reagents was ideal for reasons of toxicity and safety and so an alternative means of oxidation was sought. Three trial oxidation reactions of fructopyranose **39** were performed; the use of Dess-Martin periodinane, a Swern oxidation and a Corey-Kim oxidation and the reaction outcomes compared (Table 5). Each of the oxidation reactions resulted in the preparation of "diacetone fructose" **47** obtained as a colourless syrup following purification by column chromatography. The NMR spectra of the products of the reactions were concordant although the level of impurities present varied. In each case, the IR spectrum of the product of each reaction showed a clear absorption that was characteristic of an aldehyde C=O bond stretch which had not been present in the starting material **39**. The Corey-Kim conditions using *n*-chlorosuccinimide and dimethyl sulfide produced superior results in terms of reaction profile, product yield and product purity and these conditions were selected for scale up. The Corey-Kim oxidation of fructopyranose **39** was successfully carried out on a 10g scale.

Oxidation methodology	Product Yield
Dess Martin Periodinane *Dess Martin Periodinane (1.1eq)/ DCM/ 0°C to RT*	71%
Swern oxidation *$(COCl)_2$ (1.1eq)/DMSO (2.4eq)/DCM/-5°C/Et_3N/H_2O*	74%
Corey-Kim oxidation *NCS (1.5eq)/ Me_2S (3 eq)/DCM/-25°C/Et_3N*	86%

Table 5: Comparison of product yields from each oxidation reactions to prepare "diacetone fructose" **47**

Figure 31 shows the proton NMR spectrum of the product derived from the Corey-Kim oxidation reaction. The four methyl substituents of the acetal groups surrounding the pyranose ring are clearly visible at *ca.* 1.3 to 1.55ppm as is the proton of the aldehyde at *ca* 9.5ppm. However, the spectrum shows contamination of the aldehyde with varying amounts of hydrate that exist in solution. Additional signals are present in the regions of the spectrum indicative of the methyl groups and ring protons as well as a hydroxyl signal at *ca* 2.2ppm. The presence of this hydrated species did not adversely affect the course of the subsequent reaction.

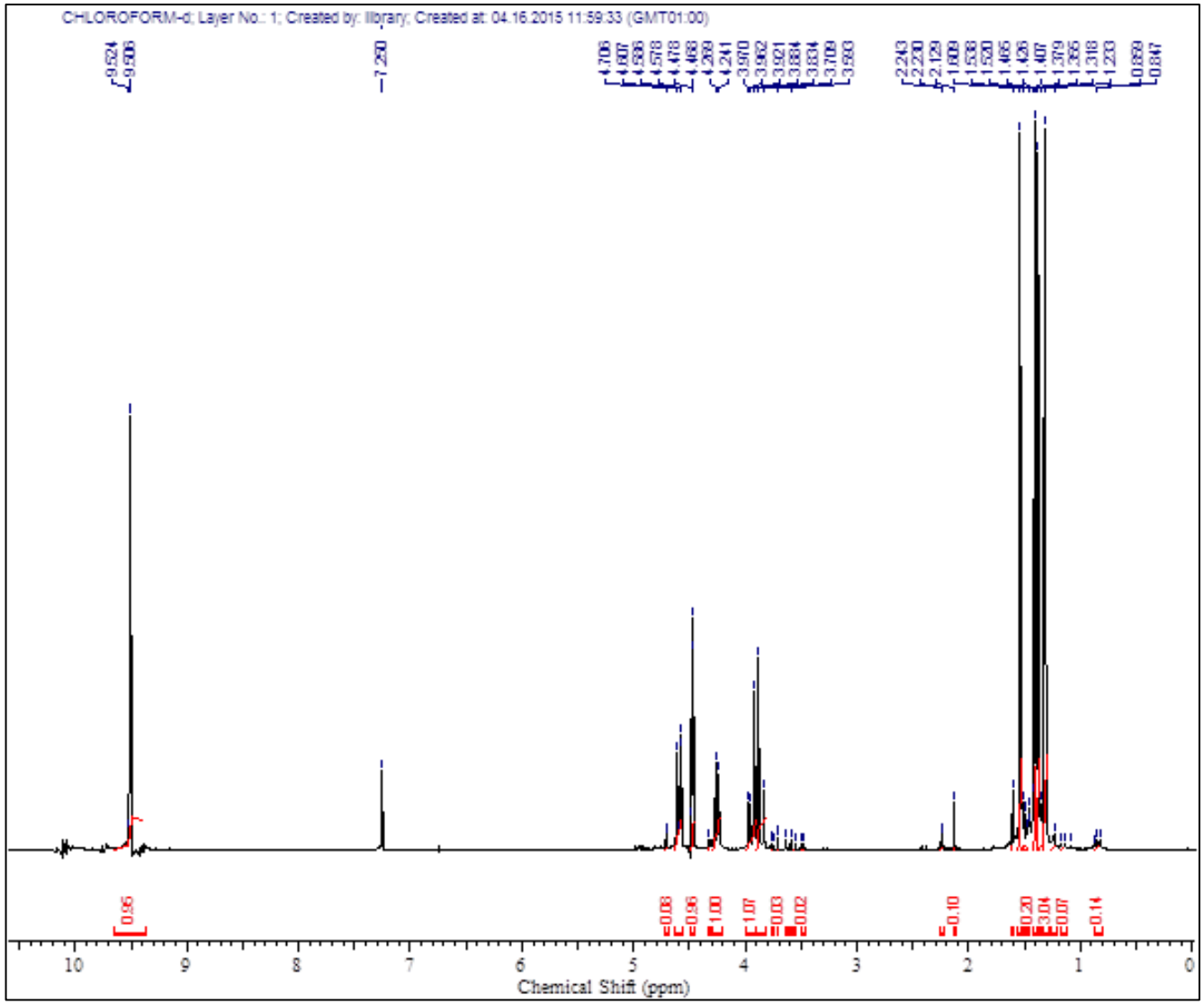
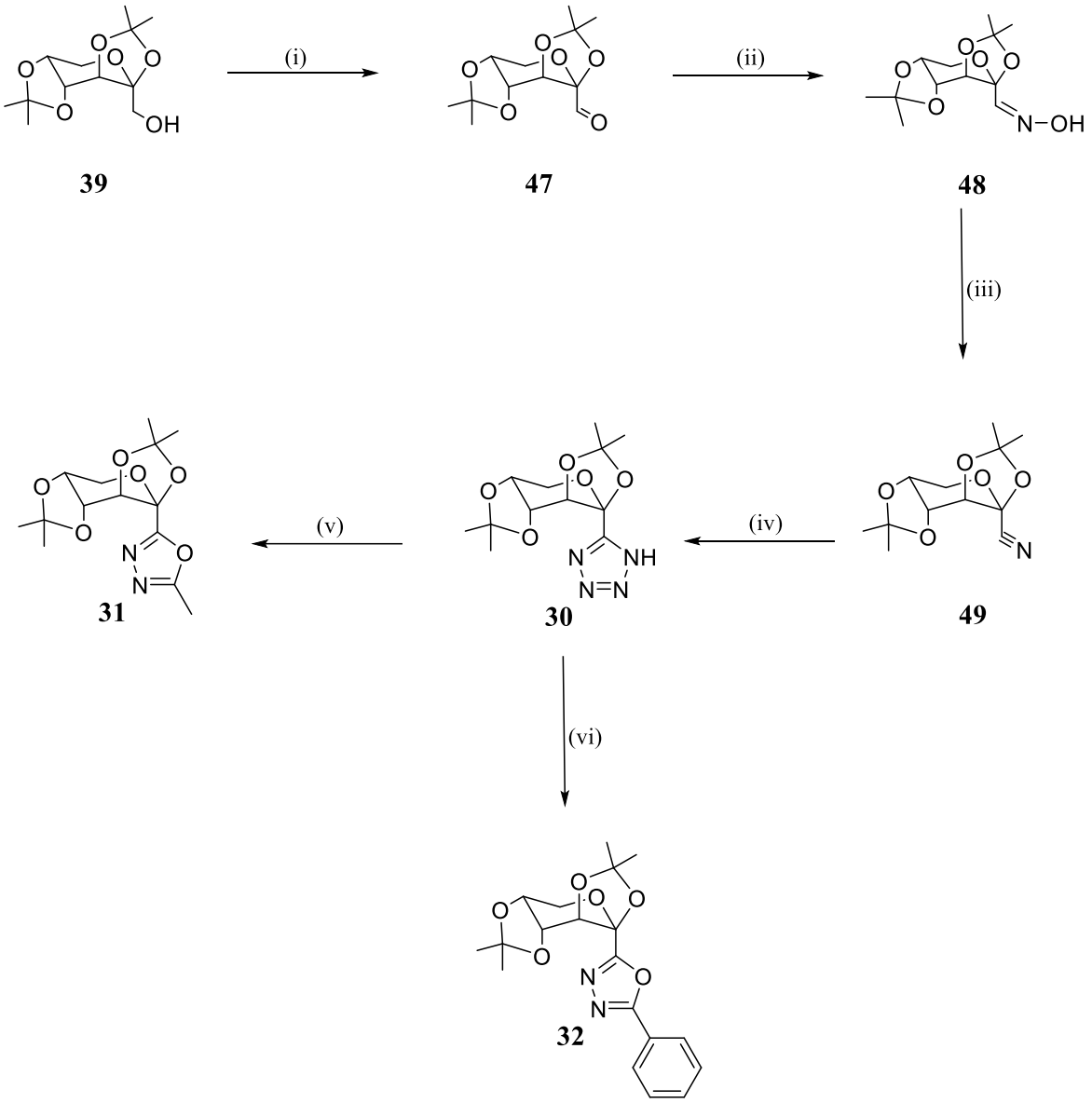


Figure 31: ^{1}H NMR spectrum of "diacetone fructose" **47**

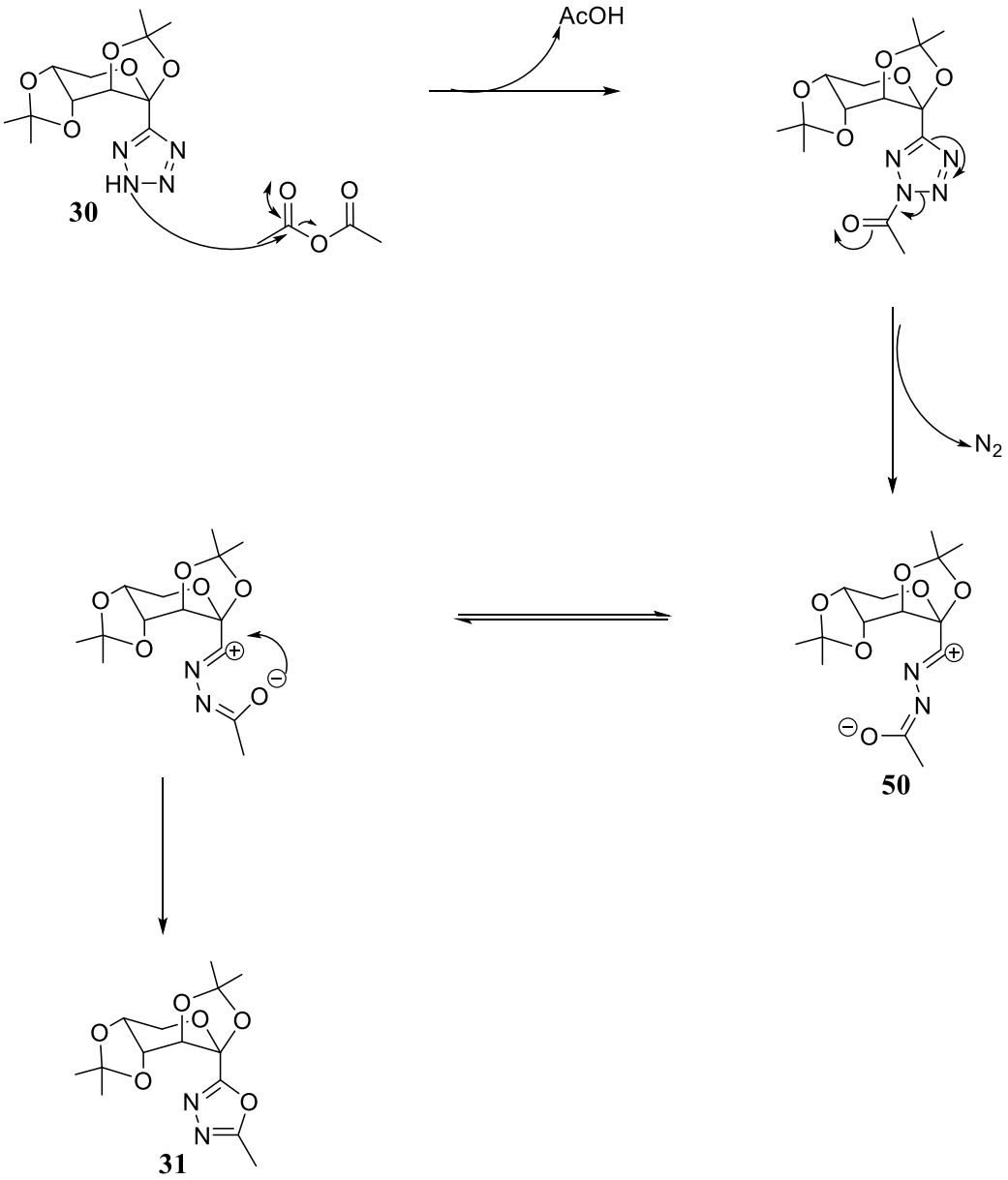


Reagents and conditions: (i) NCS, DCM, dimethyl sulfide, Et_3N, -25^{0}C, 2h; (ii) Hydroxylamine hydrochloride, DIPEA, RT, 16h; (iii) Thionyl chloride, 50^{0}C, 16h; (iv) NaN_3, NH_4Cl, DMF, RT, 72h; (v) Ac_2O, 120^{0}C, 16h; (vi) BzCl, pyridine.

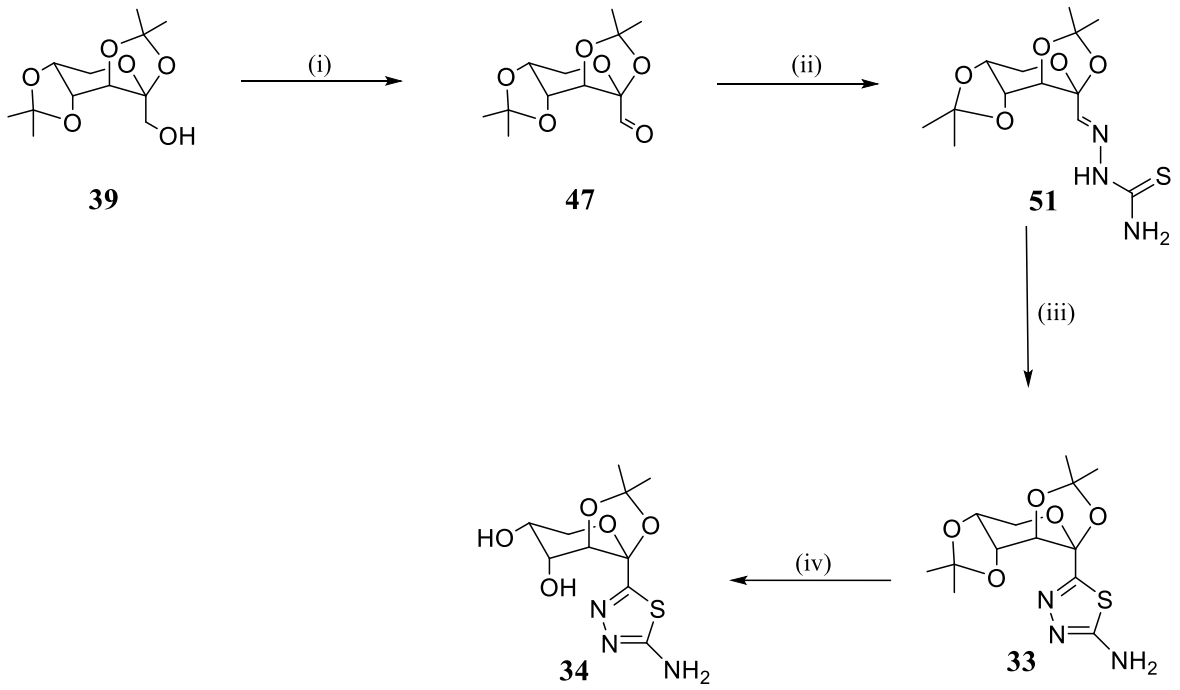
Scheme 9: Preparation of heterocyclic analogues of topiramate; tetrazole **30**, methyl oxadiazole **31** and phenyl oxadiazole **32**

The oxime **48** was prepared by reacting "diacetone fructose" **47** with hydroxylamine hydrochloride in the presence of base at room temperature. The literature procedure suggested the use of sodium bicarbonate as base but using this methodology gave slow reaction

progression and a complicated reaction profile resulted which made purification challenging.[51] Triethylamine and DIPEA were both trialled as alternative bases. The reaction containing DIPEA proved more successful in terms of product yield (*ca* 78%) and ease of product purification. These reaction conditions were adopted. The dehydration of the oxime **48** to give the nitrile **49** was achieved using an acylating mixture of acetic anhydride in pyridine as defined in the literature.[51] The reaction could not be reproduced using these conditions and resulted in an incomplete reaction and a mixture of products. There are several literature examples where thionyl chloride in basic media is used to dehydrate oximes into their corresponding nitriles. Therefore, a trial reaction was carried out in which oxime **48** was reacted with thionyl chloride in pyridine as base and solvent. The reaction outcomes were unsatisfactory despite having been successfully demonstrated on a range of carbohydrate moieties within the literature.[55] Replacing pyridine with THF as reaction solvent resulted in a clean reaction profile. No additional base was required when two equivalents of thionyl chloride was used. NMR and LCMS analysis confirmed the desired nitrile **49** had been obtained in a 90% yield. The 1,3-dipolar cycloaddition of sodium azide and **49** was carried out in DMF in the presence of ammonium chloride and resulted in the formation of tetrazole **30**. The reaction proceeded smoothly in line with the procedure reported in the literature although an extended reaction duration of 16 hours was necessary to achieve reaction completion. Oxadiazoles can be prepared by the reaction of tetrazoles with carboxylic acid derivatives.[52] The proposed mechanism of the reaction is interesting and is shown in Scheme 10.[56] The more nucleophilic nitrogen atom of the tetrazole is acylated first to give the intermediate, **50**, the subsequent removal of a nitrogen molecule furnishes the oxadiazole and is a driving force of the reaction. Thus when the tetrazole **30** was reacted with acetic anhydride, oxadiazole **31** was obtained. Likewise the reaction of tetrazole **30** with benzoyl chloride resulted in the formation of phenyl oxadiazole **32**.



Scheme 10: Proposed mechanism for the reaction of tetrazole **30** with acetic anhydride to form the oxadiazole **31**



Reagents and conditions: (i) NCS, DCM, dimethyl sulfide, Et_3N, -25^{0}C, 2h; (ii) Thiosemicarbazide, ethanol, 78^{0}C, 4h; (iii) $FeCl_3.6H_2O$, ethanol, pyridine, 50^{0}C, 1h; (iv) 5N HCl, THF, 40^{0}C, 4h.

Scheme 11: Preparation of heterocyclic analogues of topiramate, thiadiazole **33** and thiadiazole **34**

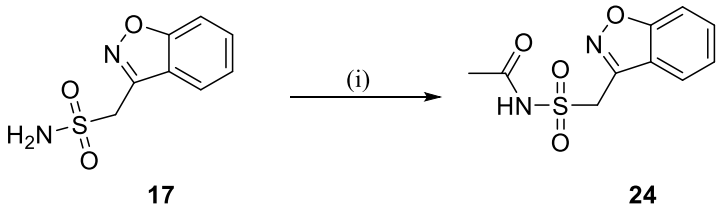
The synthesis of hetereocyclic rings containing sulfur and nitrogen were also of interest due to their wide application in the pharmacological field and to probe for interactions within the CA active sites. Two thiadiazoles **33** and **34** were successfully prepared from "diacetone fructose" **47** in accordance with Scheme 11. The intermediate thiosemicarbazone **51** was prepared by the reaction of "diacetone fructose" **47** with thiosemicarbazide. The reaction was carried out in ethanol and reaction completion was recorded after four hours heating at reflux. The product was obtained in a 93% yield following column chromatography. The cyclic thiadiazole **33** was then obtained by treating thiosemicarbazone **51** with a 2M ethanolic solution of ferric chloride in pyridine. The reaction was incredibly swift with complete consumption of the starting material noted after less than one hour at 50°C. Selective deprotection of the acetal at the 4,5-position of cyclic thiodiazole **33** around the pyranose ring to furnish the novel compound **34** was then considered. Compound **33** was of interest to the project as it would provide a further probe into CA selectivity to support the building of structure activity relationship data. The

reaction conditions selected reflected those successfully employed in the selective acetal deprotection of topiramate **1** to give **8**, but as the stability of the thiadiazole to strong acid was unknown, the reaction was initially carried out using 1M HCl. The reaction needed to be carefully monitored to prevent hydrolysis of the second acetal group at the 2,3-position of the pyranose ring and also to assess whether there was any degradation of **33** during the reaction. Minimal reaction progression was observed with 1M HCl despite gentle heating of the reaction. It was determined by progressively increasing the strength of the hydrochloric acid that 5M HCl at 40°C would lead to the formation of the desired thiadiazole product **34**. As in the reaction to form **8**, once LCMS analysis indicated that *ca* 25% of the desired product was present in the crude reaction mixture the reaction was stopped and neutralised. Following work up, thiadiazole **34** was successfully isolated as a white solid by column chromatography in a 20% yield. Unreacted starting material was recovered and the reaction repeated on this material to increase the amount of **34** available.

Sufficient quantities of tetrazole **30**, oxadiazoles **31** and **32** and thiodiazoles **33** and **34** were prepared in suitable purity to submit for biological evaluation to determine CA inhibition against a range of CA isozymes. The results are presented in Section 2.4.

2.3. Synthesis of *N*-Acetyl Zonisamide

Zonisamide **24** is an anti-epileptic drug that, in common with topiramate, is known to induce weight loss in epilepsy patients. [41] This pharmacological effect is considered to be due to the CA inhibitory action of zonisamide.[41] As a sample of zonisamide was to be submitted for biological testing as a reference standard against which the metabolites and analogues of topiramate were to be tested, it was considered of interest to extend the biological testing to the acetylated derivative of zonisamide **24**. The production of any biological results for **24** would not only provide a useful comparator to zonisamide and the topiramate family of compounds but would help in the development of insights for future design of more selective CA isozyme inhibitors with potential use as anti-obesity drugs possessing a novel mechanism of action.



Reagents and conditions: (i) Ac_2O, DMAP, reflux, 24h.

Scheme 12: Preparation of *N*-acetyl zonisamide **24**

A small commercial supply of zonisamide was purchased from which to prepare a small amount of *N*-acetyl zonisamide **24**. Scheme 12 shows the methodology employed to prepare **24**. The *N*-acetylation of zonisamide using both acetyl chloride and acetic anhydride were initially trialled. Both reactions required the addition of a 10% molar equivalent of DMAP and reflux temperatures to progress. Complete consumption of starting material was observed after 24 hours for the reaction with acetic anhydride. Reaction progression was slower for the acetyl chloride reaction by LCMS monitoring although data suggested that the desired product **24** had successfully formed in both cases. The acetic anhydride reaction was considered to be more favourable and this methodology was selected for scale up. The crude material from the reaction following work up was purified on silica with **24** obtained as a white solid in an 82% yield.

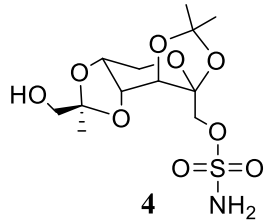
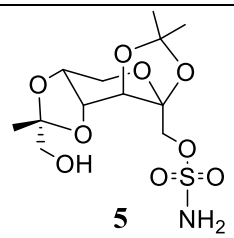
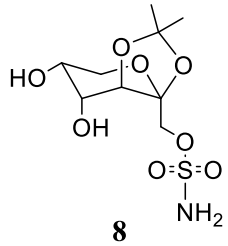
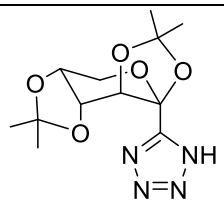
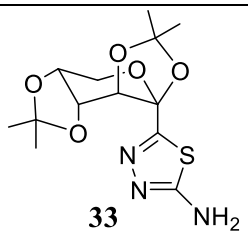
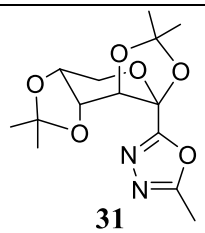
A sufficient quantity of **24** was prepared and with a suitable purity to submit for biological evaluation to determine CA inhibition against a range of CA isozymes. The results are presented in Section 2.4.

2.4. Biological activity of topiramate metabolites and analogues

An initial nine compounds were submitted to the group of Professor C. Supuran at the University of Florence for evaluation of carbonic anhydrase inhibitory activity. The compounds were tested against human isoforms of CAII, CAVA, CAVB and CAIX, by a stopped-flow CO_2 hydrase assay. Details of the methodology used are provided in Section 2.6.

Acetazolamide **2** and topiramate **1** were used as standards. The results in terms of the inhibition constants (K_I) measured for each compound tested are presented in Table 4. The inhibitor

constant is an indication of how potent an inhibitor is and is reflective of the binding affinity of an enzyme and inhibitor. The smaller the K_I, the greater the binding affinity and the smaller the amount of inhibitor required to suppress the activity of the enzyme. Comparison of the K_I values of different isoforms of a particular enzyme can provide useful information about specificity of inhibitors for one isozyme over another. K_I is an important initial measure for designating the likelihood that a particular compound will have potential as a clinically relevant drug.

Compound	K_I (nM)			
	CAII	CAVA	CAVB	CAIX
 **4**	79.9	30.1	85.1	6.3
 **5**	567	426	920	257
 **8**	705	91.5	171	261
 **30**	>10,000	349	878	2,970
 **33**	>10,000	178	2,070	4,620
 **31**	>10,000	81.3	2,345	>10,000

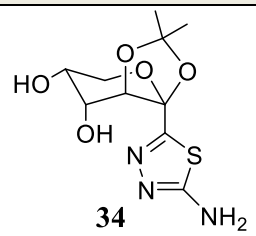
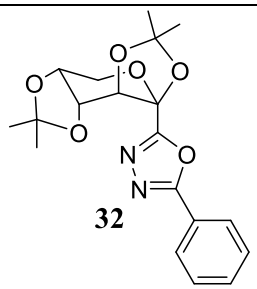
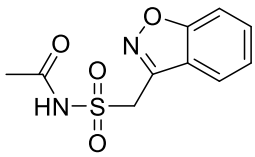
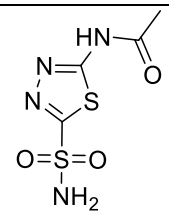
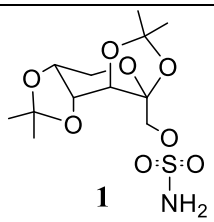
Compound	K_I (nM)			
	CAII	CAVA	CAVB	CAIX
 **34**	>10,000	149	1,790	>10,000
 **32**	>10,000	91	6,430	>10,000
 **24**	>10,000	91.4	162	789
 **2**	11.9	63.1	54.5	25.3
 **1**	9.8	62.8	30.1	58.4

Table 4: Inhibition data of selected compounds against isozymes CAII, CAVA, CAVB and CAIX at 20°C

The inhibition data of the four CA isozymes tested here provides some very interesting findings. The following observations can be made.

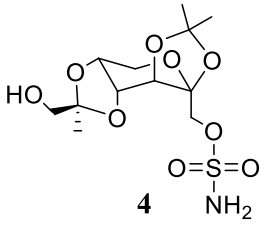
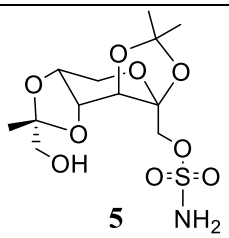
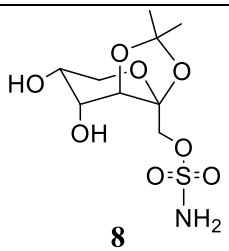
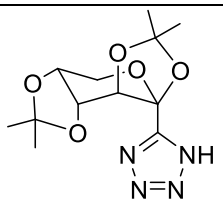
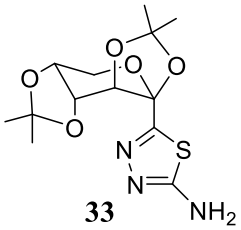
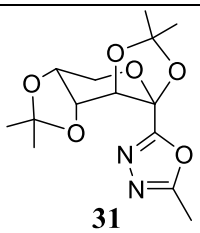
i) Generally, the two mitochondrial enzymes, CAVA and CAVB, were found to be most susceptible to inhibition by the selection of compounds tested. This was a positive result in that the enzymes sensitive to inhibition are the ones of particular interest as they may be useful for the identification and development of new pharmacological applications. **4** was shown to be a potent inhibitor of CAVA with an inhibition constant of 30nM which is lower than the figure recorded for both topiramate **1** and acetazolamide **2**. A second group of compounds, **8**, **24**, **31** and **32** were shown to be effective inhibitors of CAVA with K_I values in the range of *ca* 80 to 90nM. A third group of compounds showed moderate CAVA inhibitory activity with K_I values of 149 to 426nM. Topiramate **1** and acetazolamide **2** were the most effective inhibitors of CAVB followed by **4**. **24** and **8** were moderate inhibitors of CAVB with K_I values of 162nM and 171nM respectively. The remaining compounds were much weaker CAVB inhibitors.

ii) Compound **4** was observed to be a highly potent inhibitor of CAIX with a K_I value of 6.3nM. CAIX is a transmembrane enzyme that is predominantly expressed in the cancer cells of tumours making it a particularly interesting target for the development of anti-tumour agents. [57] Acetazolamide **2** and topiramate **1** were also shown to be effective CAIX inhibitors with K_I values of 25.3nM and 58.4nM. **5** and **8** showed moderate inhibition of CAIX. None of the other compounds tested demonstrated potency as CAIX inhibitors.

iii) The findings confirmed topiramate **1** and acetazolamide **2** as potent inhibitors of CAII. **4** was shown to be an effective CAII inhibitor with a K_I value of 79.9nM. **5** and **8** were found to be weak inhibitors of CAII. The six other compounds tested were ineffective in the inhibition of CAII.

It is important to consider the inhibitory profile of a potential CAI across a range of CA isozymes as this will indicate the general selectivity for particular CAs. A key issue in the development of new CAIs is how to selectively target a specific CA and limit the overall promiscuity for CA isozymes often demonstrated by CAIs. This selectivity will hopefully illicit a much more targeted biological response and thus improve the efficacy of any new drug. Determining the selectivity ratio of a CAI for one CA isozyme over another is a useful indication of selectivity between isozymes.[58] The selectivity ratio of two different

isozymes is the ratio of the corresponding inhibition constants. Table 5 shows the selectivity ratios between isozymes CAII and CAVA, CAII and CAVB and CAII and CAIX of the tested compounds. A selectivity ratio of less than one indicates that a compound better inhibits CAII over the other isozyme whereas a selectivity ratio of greater than one suggests a greater affinity for the other CA isozyme, in this case CAVA, CAVB or CAIX, over the cytosolic enzyme CAII. (It should be noted that when comparing such values, absolute K_I values must always be recalled when defining positive utility).

Compound	Selectivity ratio K_I(CAII)/K_I (CAVA)	Selectivity ratio K_I(CAII)/K_I (CAVB)	Selectivity ratio K_I(CAII)/K_I (CAIX)
 **4**	2.65	0.94	12.68
 **5**	1.33	0.62	2.21
 **8**	7.70	4.12	2.70
 **30**	28.65	11.39	3.37
 **33**	56.18	4.83	2.16
 **31**	123.00	4.26	1.00

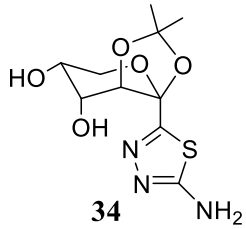
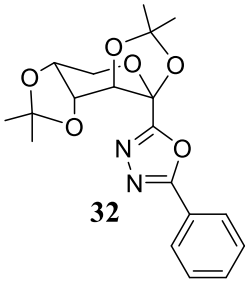
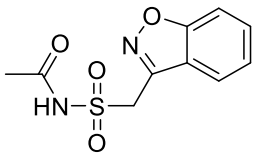
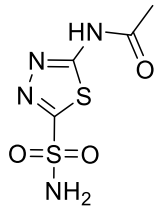
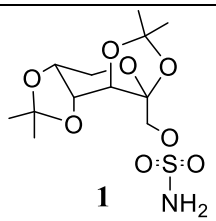
Compound	Selectivity ratio K_I(CAII)/K_I (CAVA)	Selectivity ratio K_I(CAII)/K_I (CAVB)	Selectivity ratio K_I(CAII)/K_I (CAIX)
 **34**	109.89	1.56	1.00
 **32**	67.11	5.59	1.00
 **24**	109.41	61.73	12.67
 **2**	0.19	0.22	0.47
 **1**	0.16	0.33	0.17

Table 5: Selectivity ratios between different CA isozymes for compounds tested

Generally, the selectivity ratios of the heterocyclic compounds tested confirm that they are more effective at inhibiting CAVA and CAVB than CAII. Conversely, both of the drugs in clinical use, topiramate **1** and acetazolamide **2** are more effective at inhibiting CAII than the other CA isozymes tested here as indicated by selectivity ratios that are less than one. An

interesting feature that emerges is that several of the compounds tested **31**, **32**, **24**, **8**, **30**, **33** and **34** show very positive selectivity for CAVA over CAII as evidenced by the high selectivity ratios. Further consideration of the data for each of these compounds shows that they have similar inhibition profiles. Each compound has a markedly lower K_I value for the inhibition of CAVA than those measured for the other CA isozymes. This suggests that there is potential scope for a CAI to be developed that will selectively inhibit CAVA whilst having much lower affinity for other CA isozymes. **31**, **32**, **24** and **8** are of interest as they have been shown to be particularly effective CAVA inhibitors. Compound **4** has high selectivity ratios for both CAVA and CAIX indicating it is a more potent inhibitor of both of these isozymes than of CAII. The data also suggested that not only does **4** have very high affinity for CAIX but demonstrates selectively for this isozyme over the other isozymes tested. This also presents a very exciting prospect.

As the range of compounds tested is relatively limited, it is difficult to arrive at any firm conclusions at this point about structure activity relationships. However, the following comments can be made.

i) CA inhibitory activity is still evident when the sulfamate group is replaced with various five-membered nitrogen containing heterocycles. This suggests a different binding mechanism for the heterocyclic compounds as the lone pair of the primary amine of the sulfamate group is replaced with an anion in the various hetereocycles. The lack of a methylene group between the heterocycle and the sugar scaffold as is in place for the derivatives of topiramate will decrease flexibility and further indicates differences in binding modes.

ii) Altering the hydrophilic nature of the substituents at the four and five positions around the fructose ring affects the inhibitory profile. This is demonstrated by comparison of the inhibition constants for topiramate **1**, **4** and **5** and of the related compounds **34** and **33**. It is postulated that the additional hydroxyl group in for example, compound **4**, must offer another hydrogen bond donor/acceptor site to interact with a near amino acid residue.

The findings from the biological testing will be used to inform further *in silico* modelling and will be used as a test of the predictions generated from the modelling work completed thus far. The results have indicated that the chemical synthesis and subsequent biological testing of

several related compounds **52** to **58** (Figure 31) would be beneficial in facilitating the building of improved structure activity relationship data.

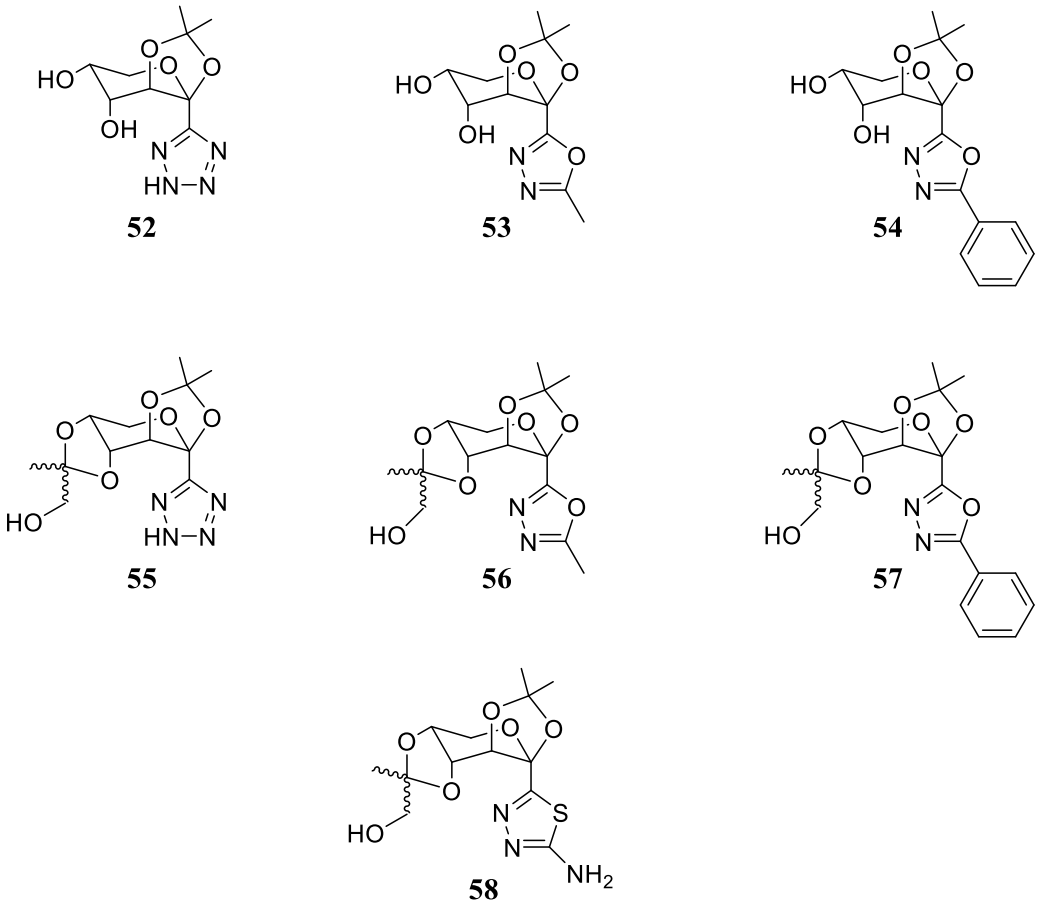


Figure 32: Additional heterocyclic analogues of topiramate for which synthesis is supported

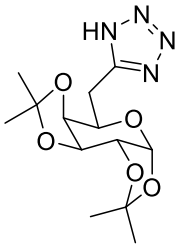
Overall, the results of the biological testing were extremely positive in that an interim goal of the project was achieved. Two "old" drugs, topiramate and zonisamide, have been redefined by structural modification to provide compounds that have demonstrated high selectivity for particular CA isozymes that generates potential in two therapeutic areas. Moving forward with the compounds **4** and **24**, it is anticipated that both will have limited toxicity based upon known human exposure to the parent drugs and associated metabolites. Equivalent data for the heterocyclic analogues of topiramate is not as readily available. A key question for the ongoing development of the project is whether CAVA and CAIX alone are valid and druggable targets for the intended use of any new drugs. There is precedent for the development of specific inhibitors of CAIX for anti-cancer purposes.[26] CAVA is perhaps less well defined, but there

has been recent research interest into this area in the consideration of anti-obesity drugs. [58] The work completed here to date will ultimately inform, and hopefully progress, understanding of selective CAIs.

2.5. Synthesis of additional five-membered heterocyclic analogues of topiramate

The novel compounds, **35** to **38** represent the next round of synthetic targets of this project. The compounds are related to the heterocycles described in Section 2.2 but contain an additional methylene group between the heterocycle and the pyranose ring. The compounds are of particular interest as they retain a heterocycle at the C1 position of the *D*-fructose scaffold. The additional methylene group may afford greater flexibility within the compunds that may result in increased binding opportunities that are more akin to the sulfamate group within topiramate. It has been demonstrated by biological evaluation of various heterocyclic analogues of topiramate that the replacement of the sulfamate group with a heterocycle does not negate CA inhibitory activity but, in certain cases, results in different CA inhibition profiles between the various ioszymes. The extension of the linker from the pyranose ring has been shown to be of pharmacological importance in topiramate.[59] A study into the structure activity relationship of topiramate with several biological targets showed that biological activity can be modulated by altering the distance between the sulfamate group and the pyranose ring.[59] It is therefore of interest whether similar pharmacological effects will be observed if the linker is extended in length when the sulfamate group is replaced by a heterocycle.

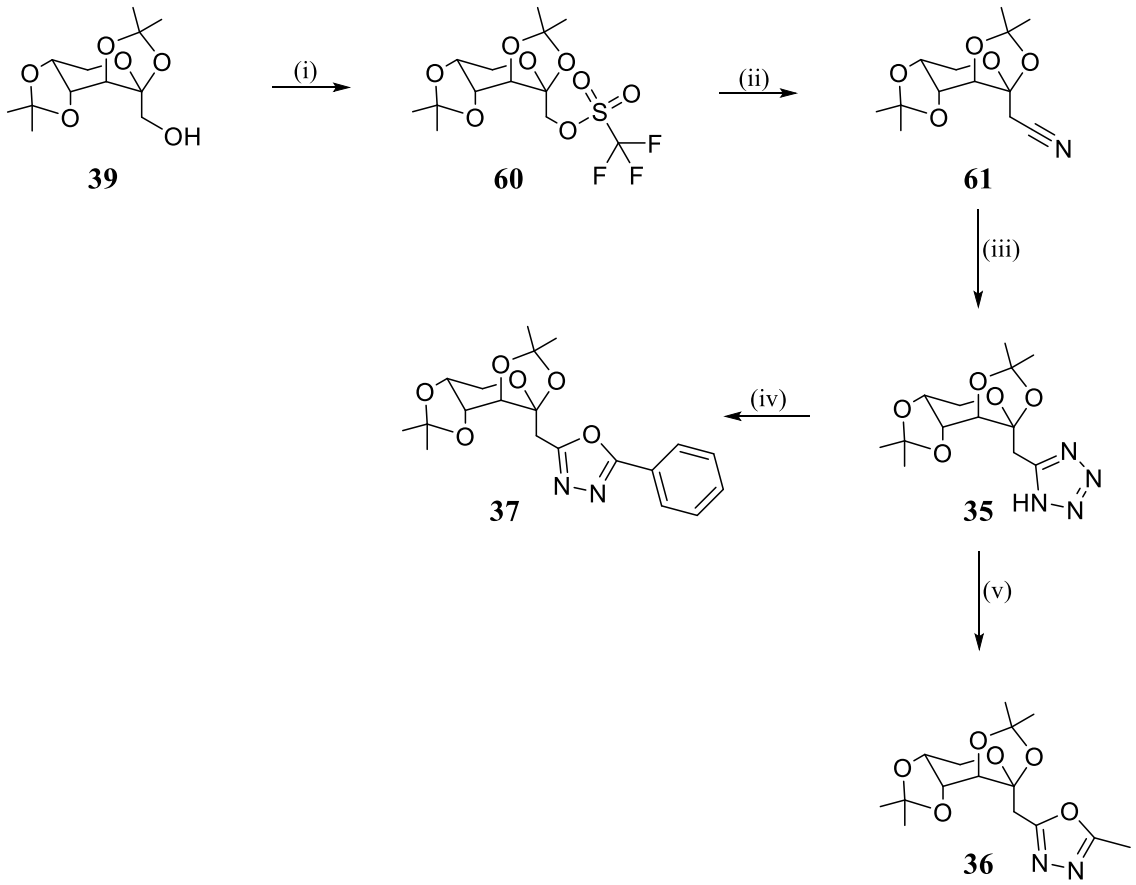
A comprehensive literature review identified that there are no precedents for the synthesis and characterisation of compounds **35** to **38**. A synthetic methodology for the preparation of **59** from *D*-galactose provided a useful starting point from which to devise a preparative route to the key tetrazole **35**.[60] Adapting the chemistry successfully employed in the preparation of oxadiazoles **31** and **32** from tetrazole **30** was also considered a reasonable initial approach.



59

Figure 33: Chemical structure of 5-[6-deoxy-(1,2:3,4-di-O-isopropyliene-α-D-galactopyranose-6-yl)]tetrazole

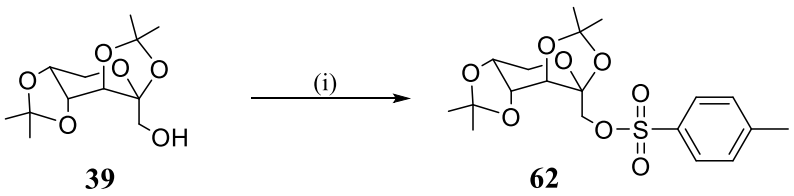
Scheme 13 provides an outline of the proposed preparative route from 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39**. Tetrazole **35** and oxadiazole **36** have been successfully prepared in quantities sufficient to support biological testing utilising the postulated methodology following development of the route.



Reagents and conditions: (i) Tf_2O, Et_3N, 0^0C; (ii) NaCN, DMSO, 80^0C; (iii) NaN_3, NH_4Cl, DMF, 100^0C; (iv) BzCl, pyridine, RT; (v) Ac_2O, 120^0C.

Scheme 13: Synthetic route to heterocyclic analogues of topiramate

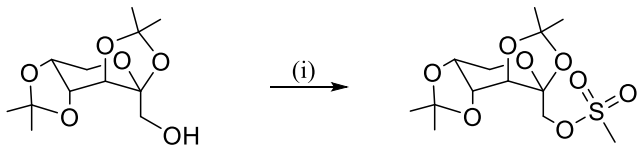
The selection of a suitable group with which to activate the anomeric alcohol group of fructopyranose **39** was the key consideration for stage 1 of Scheme 13. The formation of a sulfonate ester was successfully achieved using both *p*-toulene sulfonyl chloride (tosylate chloride) and methanesulfonyl chloride (mesylate chloride) (Scheme 14). The formation of both the toylsate **62** and mesylate **63** derivatives of **39** was reasonably facile. The reaction with mesylate chloride proceeded at room temperature with the addition of triethylamine as base (Scheme 15). Likewise, the reaction with tosylate chloride was carried out under equivalent conditions (Scheme 14). Both products were obtained as white crystalline solids in good yield and purity.



39 **62**

Reagents and conditions: (i) TsCl, Et_3N, 0^0C.

Scheme 14: Tosylation of 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39**

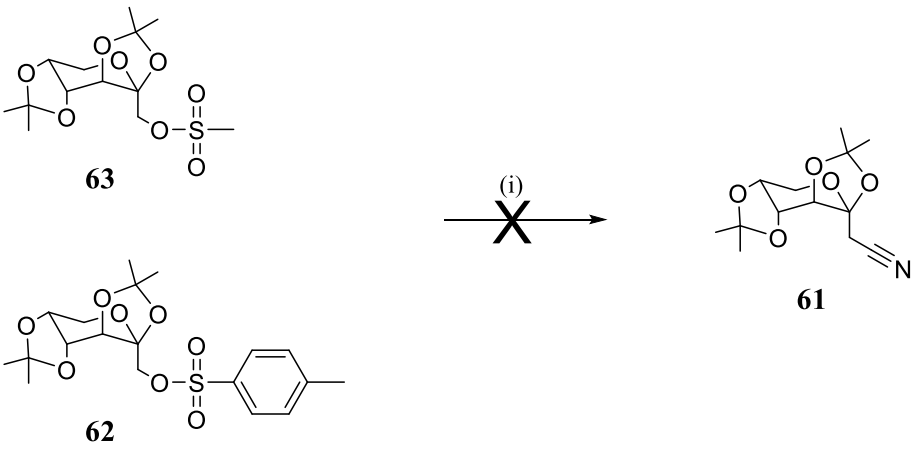


39 **63**

Reagents and conditions: (i) MsCl, Et_3N, 0^0C.

Scheme 15: Mesylation of 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39**

The nucleophilic displacement of the mesylate and/or tosylate group by cyanide to form a nitrile was the next step (Scheme 16). The reaction was attempted on both the mesylate **62** and tosylate **63** substrates in order to assess whether superior results were obtained from one or the other.



Reagents and conditions: (i) KCN, DMF, >120^{0}C.

Scheme 16: Attempted introduction of a nitrile group

The reactions were initially carried out using potassium cyanide with DMF as solvent as these conditions had proved successful in the literature for the analogous *D*-galactose example.[60] However, despite maintaining elevated reaction temperatures in excess of 125°C for prolonged periods, no reaction progression was stimulated with only starting material visible when monitoring by TLC and HPLC. The difference in reactivity between this substrate and *D*-galactose was attributed to the presence of additional hindrance in the *D*-fructose substrate versus *D*-galactose and non-equivalence in the possible angles of attack of the nucleophile. A series of small scales reactions were then carried out in parallel (Table 6) in which various conditions were altered to establish if there was a reaction parameter that was vital for the substitution reaction to proceed satisfactorily.

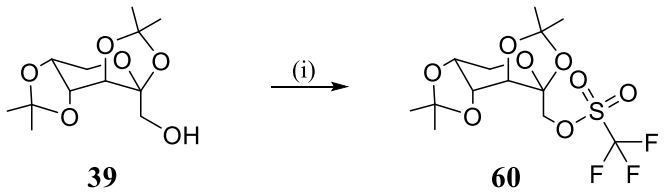
Reaction	Substrate	Molar Ratio of KCN	Molar Ratio of NaCN	Solvent	Temperature	Catalyst	After 72 hours
1	**62**	1:2	-	DMSO	120°C	-	No reaction
2	**62**	1:5	-	DMSO	120°C	-	No reaction
3	**62**	1:2	-	DMF	120°C	18-crown-6 ether	No reaction
4	**62**	1:5	-	DMF	120°C	18-crown-6 ether	No reaction
5	**62**	1:5	-	NMP	120°C	-	No reaction
6	**62**	-	1:2	DMSO	120°C	-	No reaction
7	**62**	-	1:5	DMSO	120°C	-	No reaction
8	**62**	-	1:2	DMF	120°C	15-crown-5-ether	No reaction
9	**62**	-	1:5	DMF	120°C	15-crown-5-ether	No reaction
10	**62**	-	1:5	NMP	120°C	-	No reaction
11	**63**	1:2	-	DMSO	120°C	-	No reaction
12	**63**	1:5	-	DMSO	120°C	-	No reaction
13	**63**	1:2	-	DMF	120°C	18-crown-6 ether	No reaction
14	**63**	1:5	-	DMF	120°C	18-crown-6 ether	No reaction
15	**63**	1:5	-	NMP	120°C	-	No reaction
16	**63**	-	1:2	DMSO	120°C	-	No reaction
17	**63**	-	1:5	DMSO	120°C	-	No reaction
18	**63**	-	1:2	DMF	120°C	15-crown-5-ether	No reaction
19	**63**	-	1:5	DMF	120°C	15-crown-5-ether	No reaction
20	**63**	-	1:5	NMP	120°C	-	No reaction

Table 6: Trial nucleophilic displacement reactions undertaken on mesylate **63** and tosylate **62**

The trial reactions attempted are listed in Table 6 and were carried out on both the mesylate **63** and tosylate **62** substrates. Both DMSO and NMP have been used as solvents in similar reactions and have sufficiently high boiling points to enable vigorous heating.[55] An alternative source of cyanide anion was also considered with sodium cyanide providing an alternative option. Increasing the stoichiometric amount of the cyanide reagent to five equivalents was also considered a reasonable method to improve reaction progression. A widely used methodology to accelerate substitution reactions of this nature is the addition of crown ethers to reactions due to their affinity for metallic cations. The cation forms a ligand within the crown ether cavity generating "naked" anions which are highly nucleophilic. 18-crown-6 ether has a particular affinity for potassium cations and 15-crown-5 ether has an affinity for sodium cations. Therefore 18-crown-6 ether was used in conjunction with potassium cyanide and 15-crown-5 ether with sodium cyanide. Disappointingly, there was no reaction progression observed in any of the trial reactions.

It was postulated that neither the tosylate or mesylate group was sufficiently reactive to efficiently facilitate the desired substitution reaction with this hindered substrate. Thus the triflate group was considered as an alternative. The conjugate acid of the triflate group, trifluoromethansulonic acid, is highly acidic and is a therefore a very good leaving group. It was hoped that this increased reactivity would enable the difficulties with the displacement reaction to be overcome. This approach was favoured versus attempting further changes to reaction parameters, e,g, solvent.

The reaction of 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39** with trifluoromethane sulfonic anhydride in the presence of trimethylamine in DCM (Scheme 17) resulted in the successful formation of the triflate **60**. The reaction was swift at 0°C producing the desired product in good purity (>95%). A 70% yield was achieved. Analysis of the product of the reaction by ^{19}F NMR confirmed the presence of three fluorine nuclei.

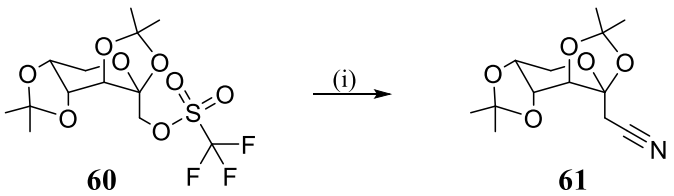


39 **60**

Reagents and conditions: (i) Tf_2O, Et_3N, DCM, 0^0C.

Scheme 17: Triflation of 2,3:4,5-di-*O*-isopropylidene-*D*-fructopyranose **39**

The subsequent nucleophilic displacement reaction of triflate **60** was initially carried out using sodium cyanide in DMSO as solvent (Scheme 18). Heating the reaction to 80°C resulted in complete consumption of the starting material **60** in 12 hours. The desired nitrile **61** was obtained in a 54% yield following purification by column chromatography. The successful outcome of the reaction does suggest that the more reactive triflate group was necessary to facilitate the nucleophilic displacement of the nitrile group of **60** when results are compared to the lack of observed reaction with the analogous mesylate and tosylate substrates, despite the use of catalysts and harsher conditions.



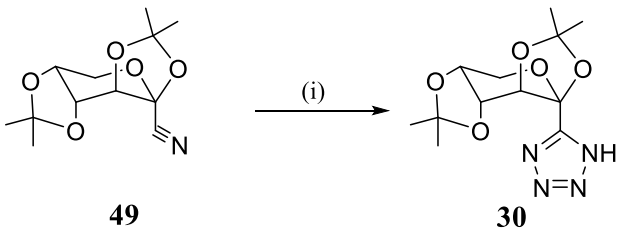
60 **61**

Reagents and conditions: (i) NaCN, DMSO, 80^0C.

Scheme 18: Displacement of the triflate group of **60** to produce nitrile **61**

The formation of tetrazole **35** was the next key step in the overall scheme. The proposed methodology was to replicate the conditions that were used to successfully prepare the related compound, tetrazole **30**, from nitrile **49** as depicted in Scheme 19. It was postulated that a 1,3-dipolar cycloaddition of sodium azide and **61** would proceed in a similar manner to that of sodium azide and nitrile **49** due to the structural similarity of the two nitrile substrates. Thus, the chosen reaction conditions for the preparation of nitrile **35** mirrored those that had been

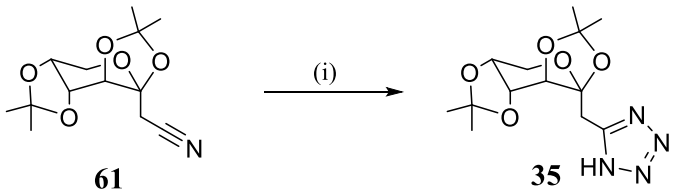
successfully employed previously. DMF was selected as the aprotic reaction solvent using sodium azide as the inorganic azide source in combination with ammonium chloride to form the required additive.



49 **30**

Reagents and conditions: (i) NaN_3, NH_4Cl, DMF, RT, 72h.

Scheme 19: Formation of tetrazole **30**



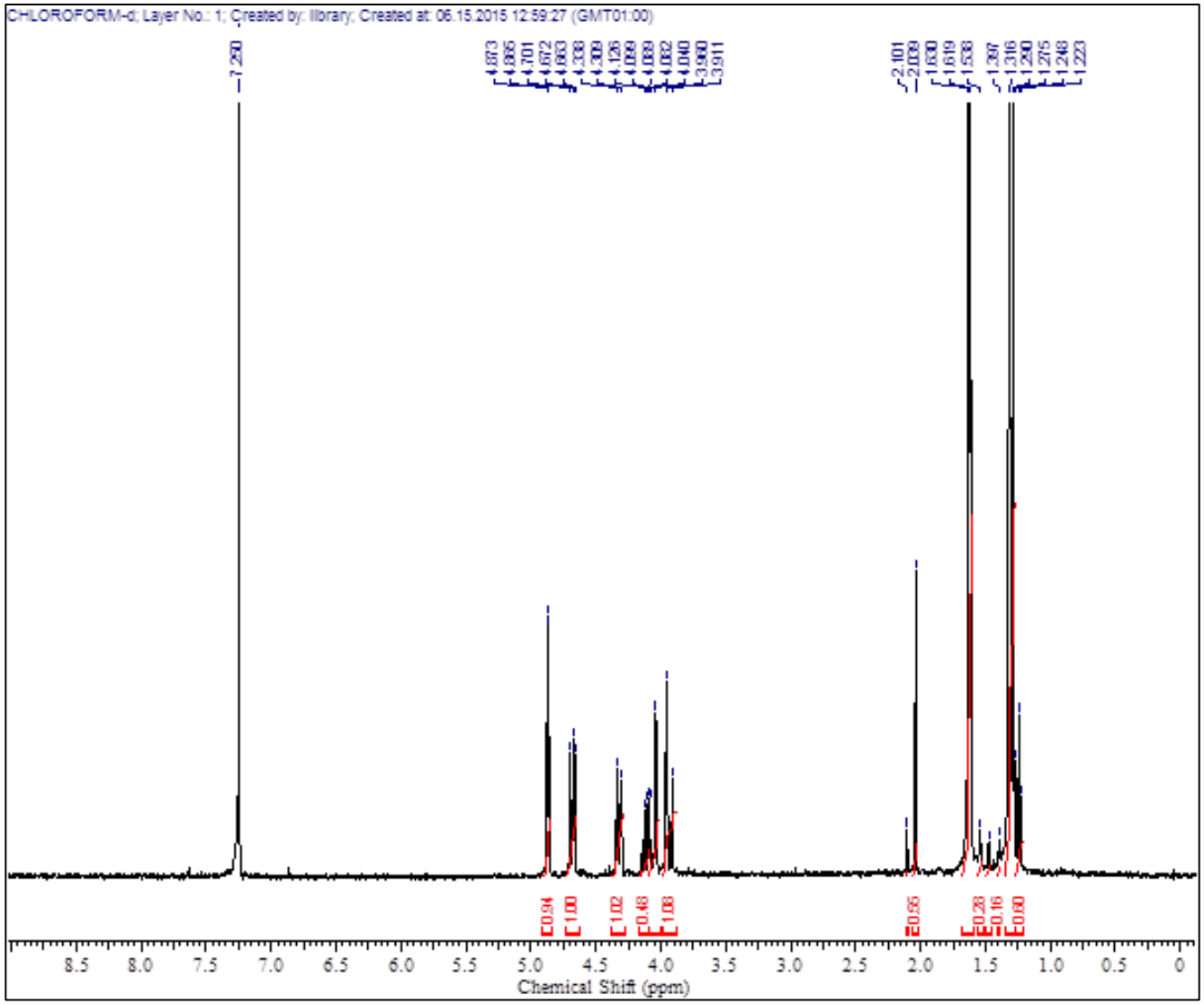
61 **35**

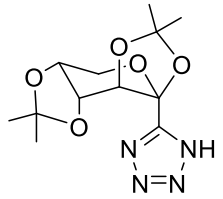
Reagents and conditions: (i) NaN_3, NH_4Cl, DMF, 100^0C.

Scheme 20: Formation of tetrazole **35**

Disappointingly, the cycloaddition reaction showed no appreciable progression after 16 hours at 100°C as was observed in the reaction to form tetrazole **30**. However, as a review of the literature has demonstrated, often process methods require long reaction times and high reaction temperatures to afford the desired azide-nitrile addition process.[61] The literature methodology reported for the preparation of tetrazole **59** from the corresponding nitrile of *D*-galactose suggested that the 1,3-dipolar cycloaddition reaction in this case could only be stimulated by heating the reaction to 105°C for five days coupled with the addition of a very large excess, 14 equivalents, of sodium azide and ammonium chloride to the reaction.[60] Adopting these conditions for the cycloaddition of nitrile **61** and sodium azide produced a successful outcome and after five days of heating the reaction to 105°C, after which time, TLC

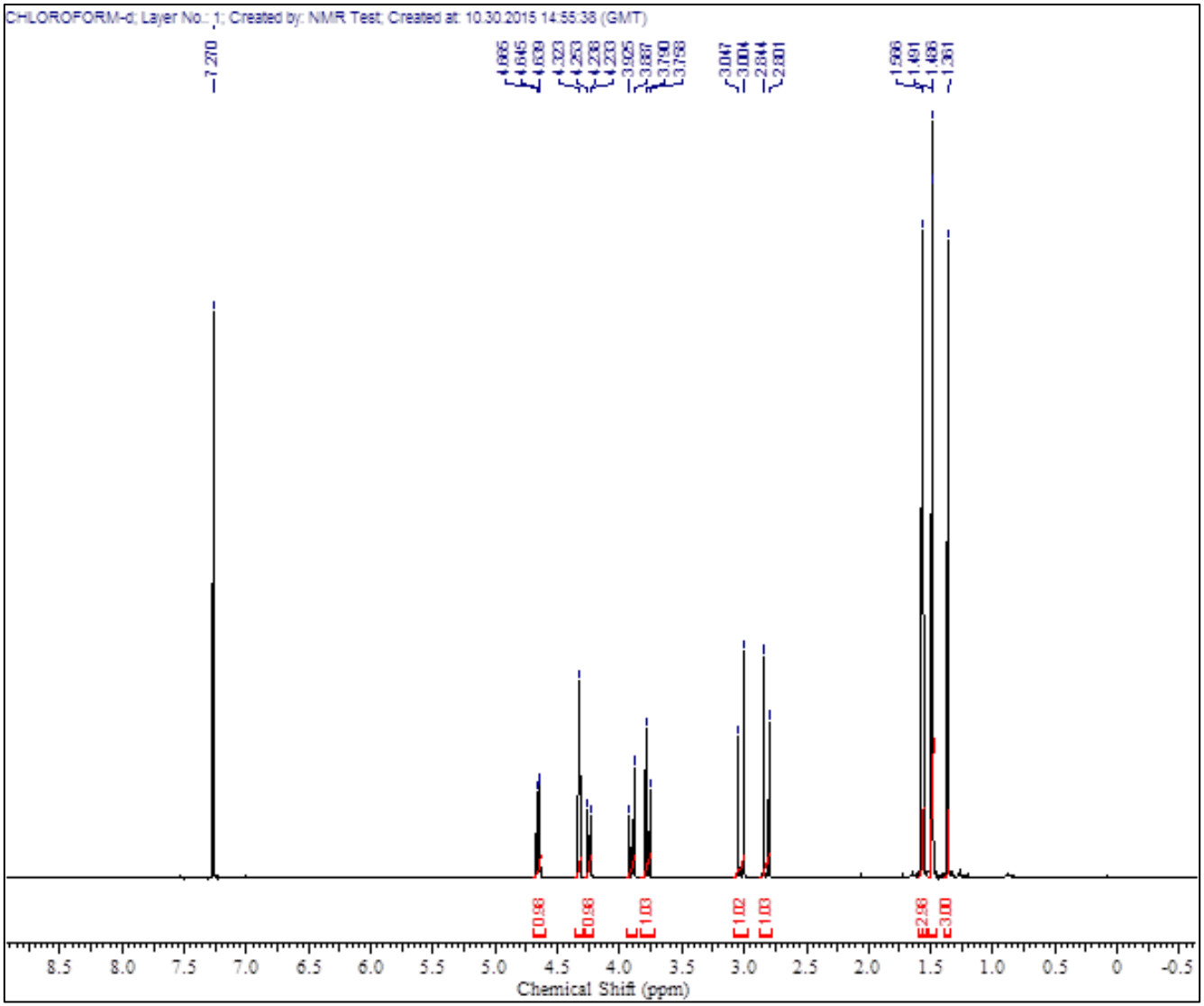
analysis indicated complete consumption of the starting material. Tetrazole **35** was isolated as a white crystalline solid in a reasonable yield of 54% following purification by column chromatography. It is likely that the harsh conditions required for the reaction to go to completion results in degradation of both starting material and product, particularly as the reaction duration increases, which will contribute to the relatively low yield. Comparison of the proton NMR spectra for tetrazole **30** and tetrazole **35**, Figures 33 and 34 respectively, clearly shows the presence of the two additional diasteriomeric protons of the extra methylene group in tetrazole **35**.

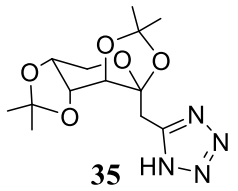




30

Figure 34: ^{1}H NMR spectrum of tetrazole **30**

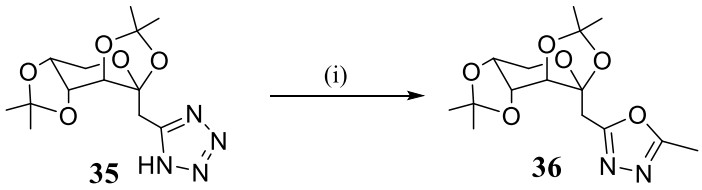




35

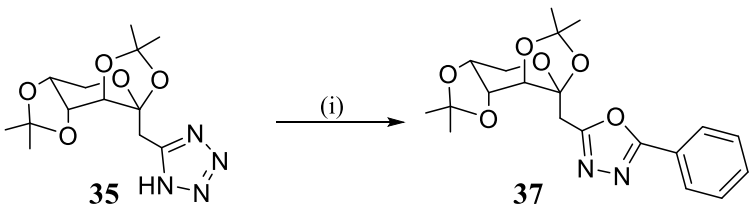
Figure 35: ^{1}H NMR spectrum of tetrazole **35**

The successful preparation of tetrazole **35** provided access to two further heterocyclic derivatives of topiramate, methyl oxadiazole **36** and phenyl oxadiazole **37**. Using the same principles that were successfully used to prepare methyl oxadiazole **31** and phenyl oxadiazole **32** from tetrazole **30** appeared a reasonable starting point. Tetrazole **35** was dissolved in acetic anhydride and heated to reflux, *ca* 140°C. TLC analysis confirmed reaction completion within 3 hours. Following reaction work-up, the crude product of the reaction was successfully purified by column chromatography to give a yellow solid in a 48% yield. Analysis of the material by ^{1}H, ^{13}C NMR and LCMS confirmed that the desired methyl oxadiazole **36** had been successfully obtained in accordance with Scheme 21.



Reagents and conditions: (i) Ac_2O, 120^0C.

Scheme 21: Formation of methyl oxadiazole **36**



Reagents and conditions: (i) BzCl, pyridine, RT.

Scheme 22: Formation of phenyl oxadiazole **37**

The synthesis of phenyl oxadiazole **37** was attempted by reacting tetrazole **35** with benzoyl chloride in the presence of pyridine. The reaction proceeded well, with the formation of two highly UV active compounds clearly visible by TLC. LCMS analysis of the crude reaction mixture confirmed the formation of the desired product with a peak at a retention time of 2.3 minutes that displayed the correct mass of 389 (MH^+) in the corresponding mass spectrum. Isolation of phenyl oxadiazole **37** by column chromatography proved very challenging.

Initially, it was suspected that the product of the reaction was unstable to silica. However, 2D TLC assessment of the crude product did not suggest that this was the case. Alternative solvent systems were trialled to assess if better separation of the product and by-products could be achieved. A three component solvent system of ethyl acetate, heptane and acetone coupled with increased equivalents of silica used in the column enabled adequate separation. ^{1}H NMR of the product showed that residual benzoic acid remained. The solid was dissolved in ethyl acetate and washed twice with saturated sodium bicarbonate which was successful in removing the benzoic acid. A yield of 94% was achieved. A modified work-up would facilitate an easier purification on silica.

The biological testing of the novel tetrazole **35** and the two novel oxadiazoles **36** and **37** is supported and the compounds will be submitted to assess their CA inhibitory profiles in the first instance once additional funding is secured.

2.6. Conclusions and future work

The *in silico* evaluation study identified three metabolites of topiramate whose synthesis was considered as an important initial focus of the project. It was anticipated that the compounds would be used as probes to compliment the *in silico* models and provide positive, or just as valuable negative, indicators that could be further developed to design selective CAIs. The successful preparation and characterisation of the three known metabolites led to their submission for biological testing for evaluation of carbonic anhydrase inhibitory activity. Each of the compounds were tested against human isoforms of CAII, CAVA, CAVB and CAIX. The hydroxylated metabolite of topiramate **4** was shown to be a potent inhibitor of CAIX and CAVA and demonstrated positive selectivity for these two isozymes. The metabolite of zonisamide, *N*-acetyl zonisamide **24** showed excellent selectivity for CAVA whilst having very little inhibitory effect on CAII. This inhibitory profile is of particular interest as it is hoped that it will result in a specific and desired biological response. Both **4** and **24** have been selected for further *in vivo* evaluation with particular emphasis on their mitochondrial and anti-tumour activity. Both compounds have been subjected to stability assessments in DMSO and phosphate buffered saline to support the next stage of the screening process and pharmacokinetic evaluations. These compounds will already have been evaluated for toxicology when clinical studies were performed on the parent compounds. For example, human exposure has been completed and although this will require further evaluation, this is very positive.

A second family of compounds related to topiramate but in which the sulfamate group is replaced by a five-membered heterocycle were considered as interesting alternative compounds. Although known compounds chemically, there is no published data (at least in the literature review undertaken here), on their biological activity either as carbonic anhydrase inhibitors or in other therapeutic areas. This may lead to interesting opportunities for the development of novel APIs if the compounds demonstrate biological activity. A tetrazole **30**, two oxadiazole compounds **31** and **32** and two thiodiazoles **33** and **34** were successfully prepared and characterised. All were submitted for biological testing against the panel of human carbonic anhydrases used for testing the topiramate metabolites.

The biological testing for CA inhibition of these heterocyclic compounds revealed that the replacement of the sulfamate group of topiramate with various heterocycles does not impede the inhibition of CA isozymes but was shown to elicit a different CA inhibitory profile in certain instances. An interesting pattern that was observed was that compounds **31**, **32**, **33** and **34** showed very positive selectivity for CAVA over CAII and all other CA isozymes. This desired selectivity for only certain CA isozymes, namely CAVA and to some extent, CAVB, presents the potential for the development of selective CAIs that will only negatively influence the activity of mitochondrial variants of CA. Three heterocyclic compounds have been selected for further biological evaluation. Further work is required to establish the likely binding modes of this series of compounds within the CA isozymes of interest. Another feature that may have potential for exploitation is the removal or addition of functionalisation around the pyranose ring. The excellent inhibitory response identified for compound **4** in which the acetal group at the four and five position around the pyranose ring is hydroxylated suggests that this feature of the molecule may be significant to improving its affinity with the active sites of CA isozymes. Also comparing the inhibitory profiles of the two thiodiazoles **33** and **34** shows that deprotection of the acetal group at position four and five does improve the inhibitory effect. For example, thiodiazole **33** with two unprotected hydroxyl groups the 4,5 position had a K_I value for CAVA of 149 whereas for **34** with the acetal group present, the K_I value for CAVA was 178. Thus developing an understanding of the effect of combining a heterocycle with modifications to the functionality around the pyranose ring would be worthwhile. To date, with the exception of the two thiodiazole compounds discussed previously, all other heterocyclic compounds that were tested were in the fully acetal protected forms. The chemical synthesis and biological testing of the compounds **52** to **58** (Figure 31) could pose the next challenge of the project.

The positive CA inhibitory profiles of several of the heterocyclic topiramate analogues led to the consideration of alternative variations that could incorporate a heterocycle. Earlier structure-activity studies conducted on different topiramate variants had shown that altering the length of the linker between the sulfamate group and the pyranose ring did have a marked effect on the biological activity of the molecule.[59] Compounds **35**, **36**, **37** and **38** were therefore identified as the next tranche of synthetic targets. The compounds are novel and thus of particular interest. Tetrazole **35** and the two oxadiazoles **36** and **37** have been successfully prepared and characterised and their biological testing is supported.

Ultimately, the project goals have been achieved and with the heterocyclic compounds, exceeded. Three compounds are moving forwards into *in vivo* and pharmacokinetic evaluation. For efficacy evaluation the validation of biological targets is required, in this instance, CAVA for anti-obesity and CAIX for anti-cancer.

In terms of chemistry, significant development is still required and will be supported once validation is achieved. However, in the first instance, methodology is in place for gram scale production with the potential to expand and produce further analogue series. This in turn will lead to second generation and supporting candidates for the current lead.

2.7. Experimental details

2.7.1. General methods

All commercial reagents and solvents were used as supplied without prior purification. Thin layer chromatography was performed on aluminium sheets coated with silica gel 60 with fluorescent indicator. TLC plates were visualised using a 10% w/v phosphomolybdic acid in ethanol or by UV. Flash chromatography was performed on Silicagel 40-63 micron silica. Melting points were recorded on a Kofler hot block and are uncorrected. Infrared spectra were recorded on a PerkinElmer Spectrum Two spectrometer using a diamond universal ATR (attenuated total reflection) sample stage under compression. Characteristic peaks are quoted in units of cm^{-1}. Low resolution mass spectra (LRMS) were recorded on Agilent1100 series liquid chromatograph. The technique used was electrospray ionisation. Nuclear magnetic resonance spectra were recorded on a JEOL ECX 270/400MHz spectrometer and the data was acquired using Delta NMR Processing and Control Software version 4.3. Samples were

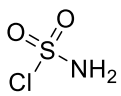
dissolved in a suitable deuterated solvent as stated. All chemical shifts (δ) are quoted in ppm and coupling constants (*J*) in Hz.

2.7.2. General methods used to assess CA activity

A generalised methodology for the measurement of CA inhibition by the potential inhibitory compounds was provided by Professor Supuran and is provided here for completeness. An Applied Photophysics stopped-flow instrument was used for assaying the CA-catalysed CO_2 hydration activity.[62] Phenol red (at a concentration of 0.2mM) was used as indicator, working at the absorbance maximum of 557nm, with 20mM Hepes buffer (pH 7.5) and 20mM $NaClO_4$ for maintaining constant ionic strength, following the initial rates of the CA-catalysed CO_2 hydration reaction for a period of 10-100 seconds at 20°C. The CO_2 concentrations ranged from 1.7 to 17mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5-10% of the reaction have been used to determine the initial velocity. The uncatalysed rates were measured in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.01nM) were prepared in distilled-deionised water and dilutions down to 0.01nM were made thereafter with the assay buffer. Inhibitor and enzyme solutions were pre-incubated together for 15 minutes at room temperature prior to assay, in order to allow for the formation of the enzyme-inhibitor complex. The inhibition constants were obtained by non-linear least squares methods using PRISM 3 and the Cheng-Prusoff equation and represent the mean from at least three different determinations. All CA isoforms were recombinant ones and their concentrations in the assay system were 9.2nM (CAII), 15.8nM (CAVA), 14.7nM (CAVB) and 8.6nM (CAIX) determined spectrophotometrically at 280nm. [58]

2.7.3. Synthesis of topiramate metabolites

Preparation of sulfamoyl chloride **43**

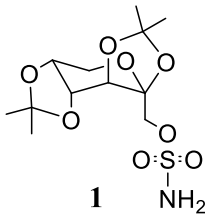


43

Chlorosulfonyl isocyanate (100ml/ 1.149mol) was charged to a 500ml flask fitted with an overhead stirrer under N_2 and cooled to 0°C. Formic acid (43.35ml/ 1.149mol) was added dropwise over 45 minutes maintaining the temperature at 0-5°C during the addition.

Anhydrous toluene (100ml) was added during the addition of the formic acid in 20ml portions to ensure mobility and homogeneity of the reaction. A dense off-white precipitate formed. Additional anhydrous toluene (150ml) was added and the reaction was warmed and stirred at ambient temperature overnight. The beige suspension was vacuum transferred into a flask and the solvent removed *in vacuo* below 25°C. A yellow solid (123.40g/ 92%) was obtained. The solid was stored under N_2 at <0°C. No further purification was undertaken due to the reactivity of the material and it was used directly its efficacy being assessed by use tests.

Preparation of 2,3:4,5-di-O-isopropylidene-β-D-fructopyranose sulfamate **1**

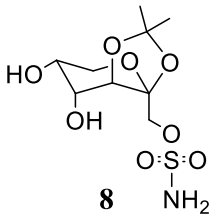


1

2,3:4,5-di-*O*-isopropylidene-β-*D*-fructopyranose **39** (30g/115.26mmol) was dissolved in anhydrous THF (500ml) under N_2 and cooled to 0°C. Et_3N (64.23ml/461.04mmol) was added and the reaction stirred for *ca.*30 minutes. Sulfamoyl chloride **43** (26.6g/ 230.25mmol) was added portionwise to the reaction, maintaining the reaction temperature at *ca.* 0°C. A white suspension was obtained. Reaction progression was monitored by TLC and ^{1}H NMR. Further additions of Et_3N (22.12ml/ 230.52mmol) and sulfamoyl chloride (26.61/ 230.52mmol) were made during the next three hours. TLC and ^{1}H NMR indicated consumption of the starting material. The reaction temperature was allowed to stabilise to room temperature. A dense white paste-like solid formed in the flask impeding stirring. An overhead stirrer was fitted to improve stirring. The suspension was decanted into water (200ml). Water (300ml) was added to the white solid remaining in the reaction flask. The white solid dissolved and the solution was added to the reaction mixture. The aqueous solution was extracted with ethyl acetate (350ml). The organic layer was washed with water (3x200ml) and brine (150ml), dried over magnesium sulfate and vacuum filtered. The solvent was removed *in vacuo* to give a pale yellow sticky solid. The crude yellow solid was purified by column chromatography on silica eluted with 60% heptane/ 40% acetone. The relevant fractions were combined and the solvent removed *in vacuo*. A colourless crystalline solid (11.84g/ 30%) was obtained. LCMS showed a m/z of 361.9 (MNa^+). The melting point range of the solid was recorded as 120-122°C (literature value: 125-126°C).[12] ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.33, 1.41, 1.47, 1.54 (4 s, 12H,

C*H*$_3$), δ 3.77 (d, 1H, *J* = 13.0Hz, *H*$_6$), δ 3.91 (d, 1H, J = 13.0Hz, *H*$_6$), δ 4.19 – 4.33 (dd, 2H, J = 11.6Hz, 3.8Hz, *H*$_1$/H$_{1'}$), δ 4.26 – 4.28 (m, 2H, *H*$_3$/*H*$_5$), δ 4.58 – 4.62 (dd, 1H, *J* = 2.7, 10.3Hz, *H*$_4$), δ 5.04 (bs, 2H, N*H*$_2$).

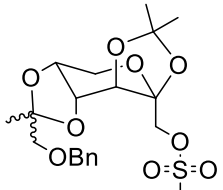
Preparation of 2,3-O-isopropylidene-β-D-fructopyranose sulfamate **8**



8

2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose sulfamate **1** (11.48g/ 33.8mmol) was dissolved in THF (220ml). 3N HCl (116ml) was added and the reaction was stirred and heated to 40°C for 3.5 hours. Reaction progress was monitored by TLC and ^{1}H NMR. The reaction was cooled to 5°C and anhydrous sodium carbonate (30.10g) was added to raise the pH to 7/8. The reaction was filtered and ethyl acetate (300ml) was added to the filtrate. The organic layer was separated and the aqueous was further extracted with ethyl acetate (2x100ml). The organics were combined, dried over magnesium sulfate and vacuum filtered. The reaction was concentrated *in vacuo* to give a yellow oil which crystallised immediately on standing. The solid was dissolved in acetone (100ml) and adsorbed onto silica (20g) by removing the solvent *in vacuo*. The crude material was purified by column chromatography on silica eluted with 40-70% acetone/heptane. Relevant fractions were combined and the solvent removed *in vacuo* to yield the product as a white solid (2.91g/ 29%). The melting point range of the solid was recorded as 140-142°C (literature value: 130-132°C).[3] LCMS showed a m/z of 322 (MNa^+). ^{1}H NMR (CD_3OD, 270MHz): δ 1.37, 1.49 (2 s, 6H, C*H*$_3$), δ 3.65 – 3.78 (m, 2H, *H*$_6$/ *H*$_{6'}$), δ 4.06 – 4.20 (dd, 2H, J = 11.6Hz, 3.8Hz, *H*$_1$/H$_{1'}$), δ 3.93 – 3.98 (m, 1H, *H*$_5$), δ 4.13 – 4.17 (m, 1H, *H*$_4$), δ 4.18 (d, 1H, J = 3.5Hz, *H*$_3$), δ 4.25 (bs, 2H, N*H*$_2$).

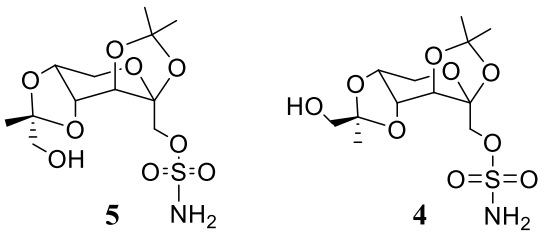
Preparation of 4,5-O-(1-benzyloxymethylethylidene)-2,3-O-isopropylidene-β-D-fructopyranose sulfamate **40**



40

Benzyloxyacetone (5.05ml/29.07mmol), ethanol (5ml), triethylorthoformate (3.71ml/22.29mmol) and concentrated H_2SO_4 (0.14ml) were charged to a flask under N_2. An orange solution was obtained. The reaction was stirred at room temperature for 3 hours. 2,3-*O*-isopropylidene-β-*D*-fructopyranose sulfamate **8** (2.91g/9.69mmol) was dissolved in THF (18ml) and added to the reaction. The reaction was stirred at room temperature for 4 hours. HPLC and TLC confirmed that the desired products were forming. The reaction was stirred at room temperature for a total of 21 hours. Anhydrous sodium carbonate (11.10g) was added to the reaction to raise the pH to 7. Following filtering, the reaction was concentrated *in vacuo* to give a yellow oil. The crude material was purified by column chromatography on silica eluting with 2:1 heptane/ethyl acetate. Relevant fractions were combined and the solvent removed *in vacuo*. Unreacted benzyloxyacetone (2.37g) was recovered with the products obtained as a pale yellow sticky syrup (2.83g). The sample was dissolved in ethanol (50ml) and the reaction concentrated *in vacuo* to give a yellow syrup (2.70g/56%). LCMS showed two peaks at very close retention times in the chromatogram each showing m/z of 467.9 (MNa^+) and 446 (MH^+). ^{1}H NMR ($CDCl_3$, 270MHz) (data for major diastereoisomer): δ 1.34, 1.40, 1.53 (3 s, 9H, CH_3), δ 3.51 – 3.60 (dd, 2H, *J* = 3.2, 13.8Hz, OCH_2), δ 3.78 (d, 1H, J = 13.0Hz, H_6), δ 3.90 – 3.94 (dd, 1H, J = 13.0, 2.2Hz, H_6) δ 4.20 – 4.30 (m, 4H, H_1/H_1'/ H_3/H_5), δ 4.56 – 4.75 (m, 3H, CH_2Ph/H_4), δ 4.90 (bs, 2H, NH_2), δ 7.26 – 7.36 (m, 5H, aromatic).

Preparation of 4,5-O-(1-hydroxymethylethylidene)-2,3-O-isopropylidene-β-D-fructopyranose sulfamate (R and S diastereomers) **4/5**

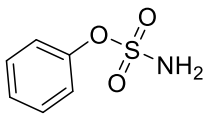


5 **4**

4,5-*O*-(1-benzyloxymethylethylidene)-2,3-*O*-isopropylidene-β-*D*-fructopyranose sulfamate **40** (2.43g/5.46mmol) was dissolved in ethanol (40ml). 10% Pd/C catalyst (50% wet/1.95g)

was added and the reaction was stirred at room temperature under nitrogen for 15 minutes. Hydrogen was sparged through the reaction for 3 hours and the reaction kept under a hydrogen atmosphere overnight. TLC showed consumption of the starting material. The reaction was placed under nitrogen and then filtered through a pad of Celite. The Celite pad was washed with ethanol (250ml) and the filtrate concentrated *in vacuo* to give a white foam. The white foam was dissolved in DCM (50ml) and dried over sodium sulfate. Following vacuum filtration, the solvent was removed *in vacuo* and a white crystalline solid was obtained. The crude material was purified by column chromatography on silica eluted with 2:1 ethyl acetate/ heptane. The relevant fractions were combined and the solvent removed *in vacuo*. Mixed fractions were repeatedly repurified by column chromatography to provide complete separation. Both diastereomers were obtained as white solids and were dried *in vacuo* at 40°C overnight resulting in a mass of 762mg of **5** (39% yield) and a mass of 109mg of **4** (6% yield). MS data for each solid was positive and both showed peaks at m/z 378 (MNa^+) and 356 (MH^+). Full NMR data presented at Section 2.1.

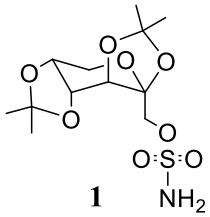
Preparation of phenyl sulfamate **46**



46

Chlorosulfonyl isocyanate (9.25ml/106.25mmol) was stirred in toluene (60ml). Phenol (10g/106.25mmol) in toluene (60ml) was added at room temperature. The reaction was heated to 100°C and stirred for 22 hours after which TLC confirmed consumption of phenol. The reaction was cooled to room temperature and the solvent removed *in vacuo* using a PTFE pump. A yellow oil (9ml) was obtained which was added dropwise to water (150ml) with stirring. A white precipitate formed immediately. The reaction was stirred at room temperature overnight. The reaction mixture was filtered *in vacuo* and the white solid collected. The solid was washed with water (50ml) and dissolved in ethyl acetate (300ml). The pale yellow solution was washed with brine (100ml), dried over magnesium sulfate and filtered. The solvent was removed *in vacuo* to give an off-white solid. The solid was dried *in vacuo* at 40°C overnight resulting in 10.4g of white solid. LCMS showed a peak with m/z 196 (MNa^+). ^1H NMR (DMSO, 270MHz): δ 7.22 (br, 2H, NH_2), δ 6.92 – 7.02 (m, 5H, aromatic).

Preparation of 2,3:4,5-di-O-isopropylidene-β-D-fructopyranose sulfamate

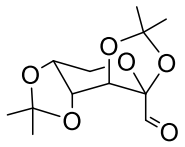


1

2,3:4,5-di-*O*-isopropylidene-β-*D*-fructopyranose **39** (1.5g/5.77mmol) was dissolved in toluene (30ml) under nitrogen. Et_3N (12ml/86.4mmol) and phenyl sulfamate **46** (5g/28.8mmol) were added to the reaction. The reaction was heated to 80°C and stirred for 5 hours. TLC showed the production of phenol, product and starting material but no remaining phenyl sulfamate. The reaction was cooled to room temperature and Et_3N (2.4ml/17.31mmol) and phenyl sulfamate (1g/5.77mmol) were added to the reaction and stirred overnight at 80°C. The TLC remained unchanged, so the reaction was cooled to room temperature and Et_3N (2.4ml/17.31mmol) and phenyl sulfamate (1g/5.77mmol) were added. The temperature of the reaction was raised to 100°C and stirred for 1 hour. A sample of the reaction was removed and a ^1H NMR obtained which confirmed that the desired product had formed but it was difficult to quantify the extent of the reaction. The reaction was cooled back to room temperature. A brown paste formed in the bottom of the flask and as this turned more solid like on addition of ethyl acetate (100ml), the reaction was filtered. Analysis of the brown solid confirmed it contained no product. The filtrate was washed with $NaHCO_3$ (aq)(3x100ml), water (150ml) and brine (100ml), dried over magnesium sulfate and filtered. The solvent was removed *in vacuo* to give a yellow oil (3.71g). The oil was purified by column chromatography on silica, eluted with 2:1 heptane/ethyl acetate. Relevant fractions were combined and the solvent removed *in vacuo* to give unreacted starting material (0.696g) and a colourless sticky syrup as product (0.165g/8.5%). Data was as for **1** above.

2.7.4. Synthesis of five-membered heterocyclic analogues of topiramate

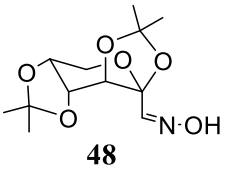
Preparation of 2,3:4,5-Di-*O*-isopropylidene-*β*-D-*arabino*-hexos-2-ulo-2,6-pyranose "diacetone fructose aldehyde" **47**



47

N-chlorosuccinimide (7.69g/ 57.6mmol) was dissolved in DCM (315ml) in a 500ml three-necked flask and was cooled to 0°C under N_2. Dimethyl sulphide (8.64ml/ 115.2mmol) was added and the reaction cooled to -25°C with an acetone/ CO_2 bath. 2,3:4,5-di-*O*-isopropylidene-β-*D*-fructopyranose **39** (10.0g/ 38.4mmol) was added dropwise as a solution in DCM (35ml) maintaining the reaction temperature at *ca*. -25°C. The reaction was stirred at -25°C for 2 hours. Triethylamine (5.88ml/ 42.2mmol) was added. The reaction was stirred for a further 10 minutes and then poured over water (100ml) and the organic layer separated. The aqueous was further extracted with DCM (2x150ml). The organic layers were combined and dried over sodium sulfate, filtered and concentrated *in vacuo* to give a colourless opaque syrup. The crude material was purified by column chromatography on silica eluted with 70% diethyl ether/heptane. The product fractions were combined and the solvent evaporated to give a colourless syrup (8.93g/90%). MS: 281 (MNa^+). ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.32, 1.38, 1.41, 1.54 (4s, 12H, CH_3), δ 3.90 (d, 1H, *J*=13.0Hz, H_6), δ 3.92 (dd, 1H, *J*=1.8Hz, 13.0Hz, $H_{6'}$), δ 4.26 (d, 1H, *J*=8.1Hz, H_5), δ 4.47 (d, 1H, *J*=2.7Hz, H_3), δ 4.60 (dd, 1H, *J*=2.7Hz,8.1Hz, H_4), δ 9.51 (s, 1H, aldehyde).

Preparation of 2,3:4,5-Di-*O*-isopropylidene-*β*-D-*arabino*-hexos-2-ulo-2,6-pyranose oxime **48**

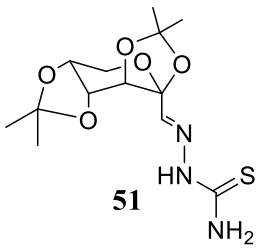


48

"Diacetone fructose aldehyde" **47** (5.0g/ 19.4mmol) was dissolved in ethanol (100ml) in a 250ml three-necked flask. Hydroxylamine hydrochloride (1.75g/ 25.2mmol) and DIPEA (6.64ml/ 38.7mmol) were added. The reaction was stirred at room temperature overnight. TLC

indicated consumption of starting material. The reaction was transferred into an evaporating flask and was concentrated *in vacuo* to give a colourless syrup (9.23g). The crude material was purified by column chromatography on silica eluted with 10-20% acetone/ heptane. The product fractions were combined and the solvent evaporated to give a white amorphous solid (4.1g/ 77%). The melting point range of the solid was recorded as 138-140°C (literature value: 137-138°C).[51] MS: 296 (MNa^+). ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.34, 1.37, 1.45, 1.54 (4s, 12H, C*H*$_3$), δ 3.78 (dd, 1H, *J*=1.0Hz, 13.0Hz, *H*$_6$), δ 3.92 (dd, 1H, *J*=1.8Hz, 13.0Hz, *H*$_{6'}$), δ 4.25 (dd, 1H, *J*=1.8Hz, 8.1Hz, *H*$_5$), δ 4.60 – 4.66 (m, 2H, *H*$_{3,4}$), δ 7.80 (s, 1H, O*H*). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ24.30, 24.67, 25.90, 26.03, 61.15, 70.13, 70.50, 71.64, 100.39, 109.02, 109.30, 149.62.

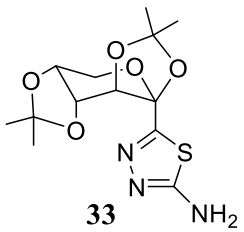
Preparation of 2,3:4,5-Di-*O*-isopropylidene-*β*-D-*arabino*-hexos-2-ulo-2,6-pyranose thiosemicarbazone **51**



51

"Diacetone fructose aldehyde" **47** (2.0g/7.74mmol) was dissolved in ethanol (50ml). Thiosemicarbazide (0.87g/11.61mmol) was added. The reaction was heated to reflux. Reaction progression was monitored by TLC and LCMS. On full consumption of starting material (2 hours), the reaction was cooled to ambient temperature and transferred to an evaporating flask and concentrated *in vacuo* to give a white solid. The crude material was purified by column chromatography on silica eluted with 40% acetone/heptane. Product fractions were combined and the solvent evaporated. Product was obtained as a white amorphous solid (2.53g/98%). The melting point range of the solid was recorded as 100-102°C (literature value: 98-100°C).[51] MS showed a peak with m/z 354 (MNa^+). ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.33, 1.37, 1.41, 1.55 (4s, 12H, C*H*$_3$), δ 3.76 (d, 1H, *J*=13.0Hz, *H*$_6$), δ 3.81 (d, 1H, *J*=13.0Hz, *H*$_{6'}$), δ 4.06 – 4.20 (dd, 2H, J = 11.6Hz, 3.8Hz, *H*$_1$/H$_{1'}$), δ 4.28 (d, 1H, *J*=7.6Hz, *H*$_5$), δ 4.57 – 4.63 (m, 2H, *H*$_{3,4}$), δ 6.32 (br, 1H, N*H*$_2$), δ 7.12 (br, 1H, N*H*$_2$), δ 9.12 (s, 1H, -N*H*). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ24.30, 25.90, 26.03, 26.91, 61.15, 70.13, 70.50, 72.24, 100.39, 109.02, 109.30, 143.62, 180.23.

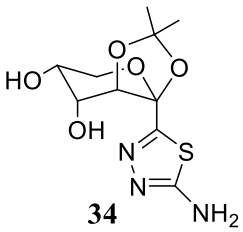
Preparation of 2,3:4,5-Di-*O*-isopropylidene-*β*-D-*arabino*-hexos-2-ulo-2,6-pyranose thiosemicarbazone **33**



33

Thiosemicarbazone **51** (2.0g/7.54mmol) was dissolved in pyridine (200ml). $FeCl_3.6H_2O$ (28ml of a 2M solution in ethanol) was added and the reaction was heated to 50°C for 1 hour. TLC/LCMS showed that no remaining starting material was present. The reaction was cooled and concentrated *in vacuo*. The resulting brown solid was taken up in ethyl acetate (150ml), filtered and washed with water (100ml). The aqueous was further extracted with ethyl acetate (3x50ml). The organic layers were combined and washed with water (150ml) and brine (150ml). The solvent was removed *in vacuo* to give a brown paste and this crude material was purified by column chromatography on silica. Fractions containing product were combined and the solvent evaporated. The product was obtained as an off-white solid (1.36g/55%). The melting point range of the solid was recorded as 179-181°C (literature value: 185-187°C).[51] LCMS showed product in 98% purity with m/z 330.2 (MH^+) in the corresponding mass spectrum. ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.31, 1.37, 1.57 (3s, 12H, CH_3), δ 3.84 (d, 1H, *J*=13.0Hz, H_6), δ 4.00 (dd, 1H, *J*=2.1Hz, 13.0Hz, $H_{6'}$), δ 4.27 (dd, 1H, *J*=1.3Hz, 7.6Hz, H_5), δ 4.66 (dd, 1H, *J*=2.7Hz, 8.1Hz, H_4), δ 4.92 (br, 2H, NH_2), δ 5.00 (d, 1H, *J*=2.7Hz, H_3). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ24.1, 24.79, 25.90, 26.24, 61.15, 70.13, 70.40, 73.64, 100.39, 109.02, 110.30, 161.62, 169.50.

Preparation of 2,3-*O*-isopropylidene-*β*-D-*arabino*-hexos-2-ulo-2,6-pyranose thiosemicarbazone **34**

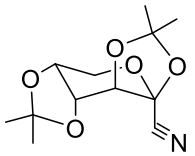


34

Thiosemicarbazone **33** (500mg/1.53mmol) was dissolved in THF (10ml). 5N HCl (1ml) was added. The reaction was stirred overnight at room temperature. LCMS and TLC showed

remaining starting material was present so the reaction was heated to 50°C for 5 hours. The reaction was neutralised to pH 7 by the addition of sodium carbonate (325mg) and partitioned between ethyl acetate (10ml) and brine (10ml). The organics were separated and concentrated *in vacuo*. The crude yellow solid was purified by column chromatography on silica eluted with 10-25% acetone/heptane. Relevant fractions were collected and the solvent evaporated. Product was obtained as a white solid (88mg/20%). LCMS showed product in 94% purity with m/z 290 (MH^+) in the corresponding mass spectrum. The melting point range of the solid was recorded as 153-155°C. ^{1}H NMR (DMSO, 270MHz): δ 1.34, 1.45 (2s, 3H, CH_3), δ 3.56 – 3.67 (m, 2H, $H_{6,6'}$), δ 3.80 – 3.88 (m, 1H, H_5), δ 3.99 – 4.07 (m, 1H, H_4), δ 4.66 (d, 1H, J=3.24Hz, H_3), δ 4.77 (d, 1H, J=6.75Hz, OH), δ 5.07 (d, 1H, J=4.59Hz, OH), δ 7.29 (ds, 2H, NH_2).

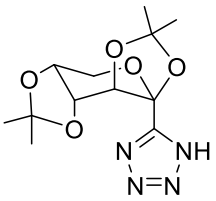
Preparation of 2,3:4,5-Di-*O*-isopropylidene-*β*-D-*arabino*-hex-2-ulosonitrile **49**



49

Oxime **48** (100mg/0.37mmol) was dissolved in THF (2ml) under N_2. Thionyl chloride (54µl as a solution in 0.2ml THF/0.74mmol) was added dropwise. The reaction was heated to reflux overnight. TLC showed full consumption of starting material. 3M sodium hydroxide (aq) (0.1ml) was added to the reaction raising the pH to *ca*. 11. The reaction was then concentrated *in vacuo* and the resulting orange syrup was purified by column chromatography on silica (2g) eluted with 10% acetone/ heptane (35ml). Product containing fractions were combined and the solvent evaporated. Product was obtained as a yellow sticky syrup (85mg/ 90%). LCMS showed product with m/z 256 (MH+) and 278 (MNa^+) in the corresponding mass spectrum. ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.35, 1.48, 1.53, 1.55 (4s, 12H, CH_3), δ 3.75 (dd, 1H, J=1.6Hz, 13.0Hz, H_6), δ 3.82 (dd, 1H, J=1.6Hz, 13.0Hz, $H_{6'}$), δ 4.24 (d, 1H, J=8.1Hz, H_4), δ 4.59 – 4.64 (m, 2H, $H_{3,5}$). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ23.86, 24.41, 25.69, 25.89, 61.39, 69.39, 69.51, 93.87, 106.87, 109.93, 111.50, 116.58.

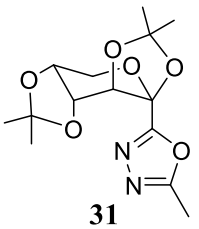
Preparation of 2,3:4,5-Di-*O*-isopropylidene-1-(tetrazol-5'-yl)-*β*-D-arabinopyranose **30**



30

Nitrile **49** (100mg/ 0.39mmol) was dissolved in DMF (2ml) under N_2. Sodium azide (51mg/ 0.78mmol) and ammonium chloride (42mg/ 0.78mmol) were added and the reaction was stirred at 100°C for 16 hours. TLC confirmed consumption of the starting material. The reaction was concentrated *in vacuo* to give an orange residue which was dissolved in DCM (10ml), filtered through a syringe frit and concentrated *in vacuo*. An orange syrup was obtained which was purified by column chromatography on silica eluted with 10-20% acetone/ heptane. Fractions containing product were combined and the solvent evaporated. Product was obtained as a white solid (76mg/65%). LCMS showed product with m/z 299 (MH^+) and 321 (MNa^+) in the corresponding mass spectrum. The melting point range of the solid was recorded as 137-139°C (literature value: 144-146°C).[51] ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.29, 1.32, 1.62, 1.63 (4s, 12H, CH_3), δ 3.94 (d *obs*, 1H, *J*=13.0Hz, H_6), δ 4.06 (dd, 1H, *J*=1.6Hz, 13.0Hz, $H_{6'}$), δ 4.33 (d, 1H, *J*=7.8Hz, H_5), δ 4.68 (dd, 1H, *J*=2.4Hz, 7.8Hz, H_4), δ 4.88 (d, 1H, *J*=2.4Hz, H_3). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ23.70, 24.73, 25.80, 26.07, 61.39, 69.63, 69.97, 74.48, 97.62, 109.29, 111.34, 156.49.

Preparation of 1,2:3,4-Di-*O*-isopropylidene-1-(2′-methyl-1′,3′,4′-oxadiazol)-5′-yl-β-D-arabinopyranose **31**

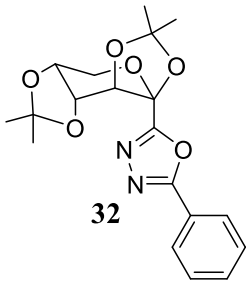


31

Tetrazole **30** (400mg/1.34mmol) was dissolved in acetic anhydride (10ml) and the reaction was heated to reflux under N_2 for 5 hours. TLC confirmed reaction completion. The reaction was cooled to room temperature and ethanol (5ml) was added to quench the reaction. The reaction was concentrated *in vacuo* to give a yellow syrup which was purified by column chromatography on silica eluted with 10% acetone/heptane. Product containing fractions were

combined and the solvent evaporated. Product was obtained as a white solid (223mg/53%). LCMS showed product in 95% purity with m/z 313 (MH^+) in the corresponding mass spectrum. The melting point range of the solid was recorded as 104-106°C (literature value: 100-102°C).[51] ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.33, 1.36, 1.45, 1.59 (s(x4), 12H, CH_3), δ 2.55 (s, 3H, CH_3) δ 3.91 (d, 1H, *J*=13.0Hz, $H_{6'}$), δ 4.01 (dd, 1H, *J*=1.6Hz, 13.0Hz, $H_{6''}$), δ 4.30 (d, 1H, *J*=7.8Hz, H_5), δ 4.68 (dd, 1H, *J*=2.2Hz, 7.8Hz, H_4), δ 5.02 (d, 1H, *J*=2.3Hz, H_3). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 11.12, 24.27, 24.59, 25.81, 26.11, 61.63, 69.96, 70.16, 73.13, 97.83, 109.55, 110.57, 164.53, 164.62.

Preparation of 1,2:3,4-Di-*O*-isopropylidene-1-(2′-phenyl-1′,3′,4′-oxadiazol)-5′-yl-β-D-arabinopyranose **32**

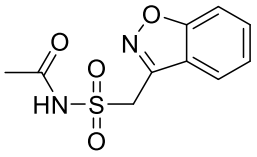


32

Tetrazole **30** (400mg/1.34mmol) was dissolved in pyridine (4ml) under N_2. Benzoyl chloride (0.66ml/1.61mmol) was added and the reaction heated to 50°C. TLC confirmed reaction completion after 3 hours. Water (4ml) was added to the reaction which was then concentrated *in vacuo* and the residue taken up in DCM (20ml) and washed with $NaHCO_3$ (aq.)(20ml). The organic layer was separated, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting orange syrup was purified by column chromatography on silica eluted with 5% acetone/heptane. Product containing fractions were combined and the solvent evaporated. The product was obtained as a white solid (539mg/95%). LCMS showed product in 97% purity with m/z 375 (MH^+) in the corresponding mass spectrum. ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.33, 1.38, 1.38, 1.51 (4s, 12H, CH_3), δ 3.95 (d, 1H, *J*=13.0Hz, H_6), δ 4.04 (d, 1H, 13.0Hz, $H_{6'}$), δ 4.33 (d, 1H, *J*=7.8Hz, H_5), δ 4.71 (dd, 1H, *J*=2.2Hz, 7.8Hz, H_4), δ 5.10 (d, 1H, *J*=2.2Hz, H_3), δ 7.50 (m, 3H, aromatic), δ 8.09 (m, 2H, aromatic). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 24.37, 24.64, 25.95, 26.14, 61.69, 70.06, 70.22, 73.25, 98.08, 109.63, 110.67, 123.70, 127.28, 128.31, 128.97, 129.08, 132.02, 164.40, 165.49.

2.7.5. Synthesis of N-acetyl zonisamide

Preparation of N-acetyl zonisamide **24**

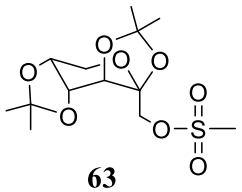


24

Zonisamide **17** (200mg/0.94mmol) was added to acetic anhydride (5ml) under N_2 and DMAP (11mg/0.094mmol) was added. The reaction was heated to 90°C for 24 hours and completion was confirmed by TLC and LCMS. The reaction was cooled to room temperature, ethanol (5ml) was added and the reaction concentrated *in vacuo*. The resulting residue was azeotroped with toluene (3 x 5ml) to remove any residual acetic acid. An off-white solid (254mg) was obtained which was purified using a silica pad eluted with 10-20% ethyl acetate/heptane. Product was obtained as a white solid (197mg/82%) after drying *in vacuo* at 45°C for 16 hours. LCMS showed product in 94% purity with m/z 255 (MH^+) and 277 (MNa^+) in the corresponding mass spectrum. ^{1}H NMR (DMSO, 270MHz): δ 1.99 (s, 3H, C*H*$_3$), δ 5.30 (s, 1H, N*H*), δ 7.48 (t, 1H, *J*=7.29Hz, Ar), δ 7.71 (t, 1H, *J*=7.29Hz, Ar), δ 7.81 (d, 1H, *J*=7.83Hz, Ar), δ 7.97 (d, 1H, *J*=7.83Hz, Ar).

2.7.6. Synthesis of additional five-membered heterocyclic analogues of topiramate

Preparation of mesylate **63**

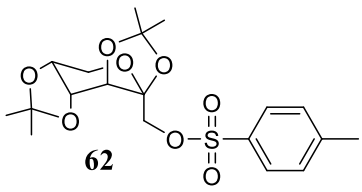


63

2,3:4,5-Di-*O*-isopropylidene-α-*D*-fructopyranose **39** (5.00g/19.2mmol) was dissolved in DCM (60ml) and cooled to 0°C under N_2. Et_3N (5.35ml/38.4mmol) was added maintaining the reaction temperature at <5°C. Methane sulfonyl chloride (1.8ml/23.04mmol) was added dropwise maintaining reaction temperature <5°C throughout addition. The reaction was

warmed gradually to room temperature. After one hour, TLC confirmed reaction completion. The reaction was poured over saturated $NaHCO_3$ (aq.). The organic layer was separated and the aqueous further extracted with DCM (2x50ml). The organic layers were combined, washed with water (200ml), brine (200ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a white solid which was dried *in vacuo* at 40°C for 16 hours (6.22g/96%). MS data revealed a peak with m/z of 339 (MH^+). ^1H NMR ($CDCl_3$, 400MHz): δ 1.34, 1.41, 1.47, 1.54 (4s, 12H, CH_3), δ 3.05 (s, 3H, S-CH_3), δ 3.78 (d, 1H, *J*=13.6Hz, H_6), δ 3.91 (d, 1H, *J*=13.6Hz, $H_{6'}$), δ 4.20 – 4.26 (m, 2H, $H_1/H_{1'}$), δ 4.31 – 4.35 (m, 2H, $H_{3,5}$), δ 4.62 (dd, 1H, *J*=2.8Hz, 8.0Hz, H_4).

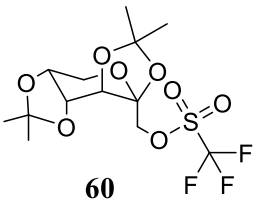
Preparation of tosylate **62**



62

2,3:4,5-Di-*O*-isopropylidene-α-*D*-fructopyranose **39** (1.00g/3.84mmol) was dissolved in pyridine (15ml) under N_2. *p*-Toluene sulfonyl chloride (880mg/4.61mmol) was added and the reaction was stirred at room temperature for 16 hours. The reaction was concentrated *in vacuo* and the residue taken up in ethyl acetate (20ml). The solution was washed with water (20ml), saturated $NaHCO_3$(aq.)(20ml), brine (20ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a clear, colourless syrup. The crude material was purified by column chromatography on silica eluted with 10-20% ethyl acetate/heptane. Product containing fractions were combined and the solvent evaporated. The product was obtained as a white solid which was dried *in vacuo* at 40°C for 16 hours (915mg/57%). LCMS showed product in 96% purity with m/z 415 (MH^+) and 437 (MNa^+) in the corresponding mass spectrum. ^1H NMR ($CDCl_3$, 270MHz): δ 1.30, 1.35, 1.54 (s(x3), 12H, CH_3), δ 2.43 (s, 3H, CH_3), δ 3.69 (d, 1H, *J*=13.0Hz, H_6), δ 3.85 (d, 1H, *J*=13.0Hz, $H_{6'}$), δ 4.02 (dd *obs*, 2H, *J*=12.2Hz, 1.9Hz, $H_{1,1'}$), δ 4.18 (d, 1H, *J*=7.8Hz, H_5), δ 4.29 (d, 1H, *J*=2.4Hz, H_4), δ 4.64 (dd, 1H, *J*=2.4Hz, 7.8Hz, H_3), δ 7.33 (d, 2H, *J*=8.1Hz, aromatic), δ 7.78 (d, 2H, *J*=8.1Hz, aromatic).

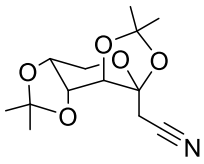
Preparation of triflate **60**



60

2,3:4,5-Di-*O*-isopropylidene-α-*D*-fructopyranose **39** (3.84g/ 14.77mmol) was dissolved in DCM (50ml) and cooled to 0°C under N_2. Et_3N (4.09ml/29.54mmol) was added and the reaction temperature was maintained at 0°C. Trifluoromethane sulfonic anhydride (5.0g/17.72mmol) was added dropwise maintaining reaction temperature <5°C throughout the addition. The reaction was stirred at <5°C for 1 hour after which time TLC indicated remaining starting material. Et_3N (2.0ml/14.8mmol) was added followed by trifluoromethane sulfonic anhydride (1.6g/5.9mmol) which was added dropwise maintaining reaction temperature <5°C throughout addition. TLC indicated that no starting material was remaining. The reaction was warmed to room temperature and poured over water (150ml). The organic layer was separated and the aqueous was further extracted with DCM (2x50ml). The organic layers were combined and washed with water (100ml), brine (100ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo*. A brown syrup was obtained which was purified by column chromatography on silica eluted with 10-20% ethyl acetate/heptane. Relevant fractions were combined and the solvent evaporated. A yellow oil (3.69g/64%) was obtained. The mass spectrum contained peak with m/z 393 (MH+) and 415 (MNa^+). ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.36, 1.41, 1.48, 1.57 (s(x4), 12H, CH_3), δ 3.80 (d, 1H, *J*=12.0Hz, H_6), δ 3.92 (d, 1H, *J*=12.0Hz, $H_{6'}$), δ 4.26 (d, 1H, *J*=7.2Hz, H_3), δ 4.29 (d, 1H, *J*=3.2Hz, H_5), δ 4.41 (d, 1H, *J*=10.4Hz, H_1), δ 4.53 (d, 1H, *J*=10.4Hz, $H_{1'}$), δ 4.64 (dd, 1H, *J*=3.2Hz, 7.2Hz, H_4). ^{13}C NMR ($CDCl_3$, 100MHz): δ 24.02, 25.12, 25.82, 26.58, 61.71, 69.80, 70.23, 70.55, 74.14, 99.93, 118.20, 120.27, 123.30.

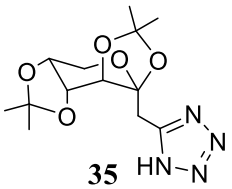
Preparation of nitrile **61**



61

Triflate **60** (3.5g/8.92mmol) was dissolved in DMSO (65ml) and NaCN (874mg/17.84mmol) was charged. The reaction was heated to 80°C for 12 hours after which time TLC confirmed that the reaction was complete. The reaction was partitioned between water (100ml) and ethyl acetate (100ml). The organic layer was separated and the aqueous further extracted with ethyl acetate (2x50ml). The organic layers were combined and washed with water (150ml), brine (150ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a dark orange syrup which was purified by column chromatography on silica eluted with 10% ethyl acetate/heptane. Relevant fractions were combined and the solvent evaporated to give a colourless syrup (1.3g/54%). MS showed peaks with m/z 270 (MH^+) and 292 (MNa^+). ^1H NMR ($CDCl_3$, 400MHz): δ 1.36, 1.49, 1.56 (3s, 12H, C*H*$_3$), δ 2.82 (d, 1H, *J*=12.6Hz, C*H*), δ 3.02 (d, 1H, *J*=12.6Hz, $H_{1'}$), δ 3.78 (d, 1H, *J*=13.6Hz, H_6), δ 3.90 (d, 1H, *J*=13.6Hz, $H_{6'}$), δ 4.24 (d, 1H, *J*=8.0Hz, H_3), δ 4.33 (d, 1H, *J*=3.2Hz, H_5), δ 4.65 (dd, 1H, *J*=3.2Hz, 8.0Hz, H_4). ^{13}C NMR ($CDCl_3$, 100MHz): δ 24.14, 25.22, 25.99, 26.60, 28.94, 62.12, 70.08, 70.54, 72.19, 100.17, 109.43, 109.62, 116.01.

Preparation of tetrazole **35**

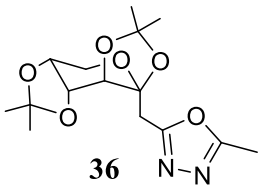


35

Nitrile **61** (330mg/1.23mmol) was dissolved in DMF (10ml) under nitrogen and NaN_3 (1.1mg/17.22mmol) and NH_4Cl (921mg/17.22mmol) were added. The reaction was heated to 105°C and after 120 hours, TLC indicated reaction completion. The reaction was cooled to room temperature and was partitioned between DCM (50ml) and water (50ml). The organic layer was separated and the aqueous further extracted with DCM (3x25ml). The combined organics were washed with water (5x100ml), brine (100ml) and dried over $MgSO_4$. After filtering, the solution was concentrated *in vacuo* to give a yellow syrup. The crude material was purified by column chromatography on silica eluted with 10-50% ethyl acetate/heptane. Product containing fractions were combined and the solvent evaporated to give a white solid (207mg/54%). MS showed peaks with m/z 313 (MH^+) and 335 (MNa^+). Melting point was recorded as 156-158°C. ^1H NMR ($CDCl_3$, 400MHz): δ 1.36, 1.49, 1.57 (s(x3), 12H, C*H*$_3$), δ 2.82 (d, 1H, *J*=17.2Hz, H_1), δ 3.03 (d, 1H, *J*=17.2Hz, $H_{1'}$), δ 3.77 (d, 1H, *J*=12.8Hz, H_6), δ

3.84 (d, 1H, *J*=12.8Hz, $H_{6'}$), δ 4.24 (d, 1H, *J*=7.2Hz, H_3), δ 4.33 (d, 1H, *J*=2.4Hz, H_5), δ 4.65 (dd, 1H, *J*=2.4Hz, 7.2Hz, H_4). ^{13}C NMR ($CDCl_3$, 100MHz): δ 24.01, 24.79, 25.96, 26.28, 35.67, 53.51, 61.79, 70.21, 70.44, 73.70, 109.37, 109.40, 156.84.

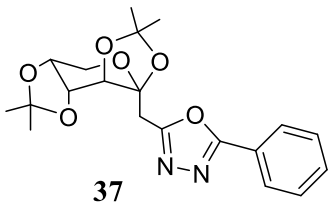
Preparation of methyl oxadiazole **36**



36

Tetrazole **35** (50mg/0.16mmol) was dissolved in acetic anhydride (1.25ml). The reaction was stirred at 130°C for 12 hours. TLC confirmed reaction completion. The reaction was cooled to room temperature and quenched with ethanol (3ml). The reaction was concentrated *in vacuo*. The residue was azeotroped with toluene (3x3ml) to remove residual acetic acid. A brown syrup was obtained which was purified by column chromatography on silica eluted with 50% ethyl acetate/heptane. Product containing fractions were combined and the solvent removed *in vacuo*. Product was obtained as a pale yellow solid (25mg/48%). MS showed a peak with m/z 327 (MH^+). Melting point was recorded as 134-136°C. ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.09, 1.35, 1.47, 1.51 (s(x4), 12H, CH_3), δ 2.53 (s, 3H, CH_3), δ 3.35 (d, 1H, *J*=14.8Hz, H_1), δ 3.44 (d, 1H, *J*=14.8Hz, $H_{1'}$), δ 3.76 (d, 1H, *J*=12.8Hz, H_6), δ 3.89 (d, 1H, *J*=12.8Hz, $H_{6'}$), δ 4.23 (d, 1H, *J*=7.6Hz, H_3), δ 4.48 (d, 1H, *J*=2.4Hz, H_5), δ 4.64 (dd, 1H, *J*=2.4Hz, 7.2Hz, H_4). ^{13}C NMR ($CDCl_3$, 100MHz): δ 11.38, 24.39, 24.87, 26.25, 26.67, 34.12, 62.12, 70.59, 70.84, 72.29, 101.73, 109.15, 109.49, 162.80, 164.61.

Preparation of phenyl oxadiazole **37**

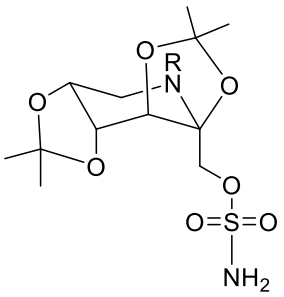


37

Tetrazole **35** (250mg/0.8mmol) was dissolved in pyridine (5.0ml) and benzoyl chloride (110µl/1.1mmol) was added. The reaction was stirred at 50°C for 16 hours after which time TLC confirmed reaction completion. The reaction was cooled to ambient temperature and concentrated *in vacuo* to give a pale brown syrup. This residue was taken up in ethyl acetate (20ml) and the solution washed with saturated sodium hydrogen carbonate (aq) (25ml), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by column chromatography on silica eluted with 10-20% ethyl acetate/heptane. Product containing fractions were combined and the solvent removed *in vacuo*. Product was obtained as a white solid (194mg/63%). LCMS showed a peak with m/z 389 (MH^+). ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.03, 1.33, 1.44, 1.49 (4xs, 12H, 4xCH_3), δ 3.51 (2xd, 2H, *J*=6.8, 14.8Hz, $H_{1,1'}$), δ 3.77 (d, 1H, *J*=12.8Hz, H_6), δ 3.91 (d, 1H, *J*=12.8Hz, $H_{6'}$), δ 4.23 (d, 1H, *J*=8.0Hz, H_3), δ 4.57 (d, 1H, *J*=2.4Hz, H_5), δ 4.65 (dd, 1H, *J*=2.4, 8.0Hz, H_4), δ 7.47 – 7.52 (m, 3H, Ar), δ 8.05 (d, 2H, *J*=8.0Hz, Ar). ^{13}C NMR ($CDCl_3$, 100MHz): δ 24.20, 24.71, 26.07, 26.46, 35.83, 62.02, 70.46, 70.68, 71.95, 100.00, 101.65, 109.03, 109.33, 124.06, 127.03, 128.63, 129.10, 131.71, 162.43, 165.41.

CHAPTER 3. Attempted Synthesis of Iminosugar Analogues of Topiramate

A key objective of the project was to consider and investigate structural modifications to topiramate that might influence its pharmacological effects. The *in silico* modelling work identified the topiramate analogue **25** as a target compound. **25** was shown to have positive affinity with the active sites of the CA isozymes of particular interest to the project. The analogues **25**, **26**, **27** and **28** represent an interesting class of compounds called iminosugars that are well documented within the scientific literature.



25 R=H, **26** R=Me, **27** R=CF_3, **28** R=OH, **29** R=OMe

Figure 36: Iminosugar analogues of topiramate

3.1. Iminosugars as natural products and therapeutic agents

Iminosugars are analogues of monosaccharides in which the endocyclic oxygen is replaced by a nitrogen atom. The iminosugar class of compounds has been subject to significant research and development during the last 25 years. Iminosugars were recognised as having potential biological and therapeutic applications following the discovery during the 1980s that several naturally occurring iminosugars were potent inhibitors of the glycosidase family of enzymes.[63] This stimulated, not only research into the biomedical application of iminosugars, but also the synthesis of novel and modified iminosugars.[2]

Iminosugars are naturally occurring sugar mimics and are widespread in plants and microorganisms. To date over 100 iminosugars have been isolated from natural sources.[64] Iminosugars can be classified into five key structural classes and representative structures are shown in Figure 37.[65]

- Polyhydroxylated piperidines – for example, 5-amino-5-deoxy-glucopyranose, trivially named nojirimycin, **64** was discovered in 1966 as the first natural glucose related iminosugar. Nojirimycin was first identified as an antibiotic produced by *Streptomyces roseochromogenes* and was shown to be a potent inhibitor of α- and β-glucosidases.[66] [67] 1-Deoxynojirimycin (DNJ) **65** was isolated from the roots of the mulberry trees and the species has been shown to produce many other iminosugars. Several other plant species and microorganisms have been shown to naturally produce a range of epimers and functional relations of nojirimycin and DNJ. [65]
- Pyrrolidines – for example, 2,5-Dideoxy-2,5-imino-d-mannitol (DMDP) **66** was found in leaves of the legume *Derris elliptica* in 1976 and was later shown to be present, along with several other derivatives, in many disparate species of plants and microorganisms.[68]
- Indolizidines – for example, swainsonine **67** and castanospermine **68** were isolated from the legumes *Swainsona canescens* and *Castanospermum australe* in Australia after finding that the plants from which they derive were toxic to livestock.[65] Although this class of iminosugars have a less obvious structural relationship to monosaccharides, the configuration of the hydroxyl substituents on the ring can be compared to those of sugars. Castanospermine **68** may be regarded as a bicyclic derivative of DNJ **65** with an ethylene bridge between the hydroxymethyl group and the ring nitrogen. The stereogenic centres of its 6-membered ring were shown by X-ray crystallography to correspond to *gluco*-configuration.[65]
- Pyrrolizidines – for example, alexine **69** was the first compound of this group to be isolated from natural sources from the legume *Alexa leiopetala*. [65]
- Nortropanes – for example, calystegine **70** which were first discovered as secondary metabolites of plants and are abundant in the underground organs and roots of *Calystegia sepium*.[65] This group of iminosugars all contain a nortropane ring system, two to four hydroxyl groups varying in position and stereochemistry and a novel aminoketal functionality which results in a tertiary hydroxyl group at the bicyclic ring bridgehead.

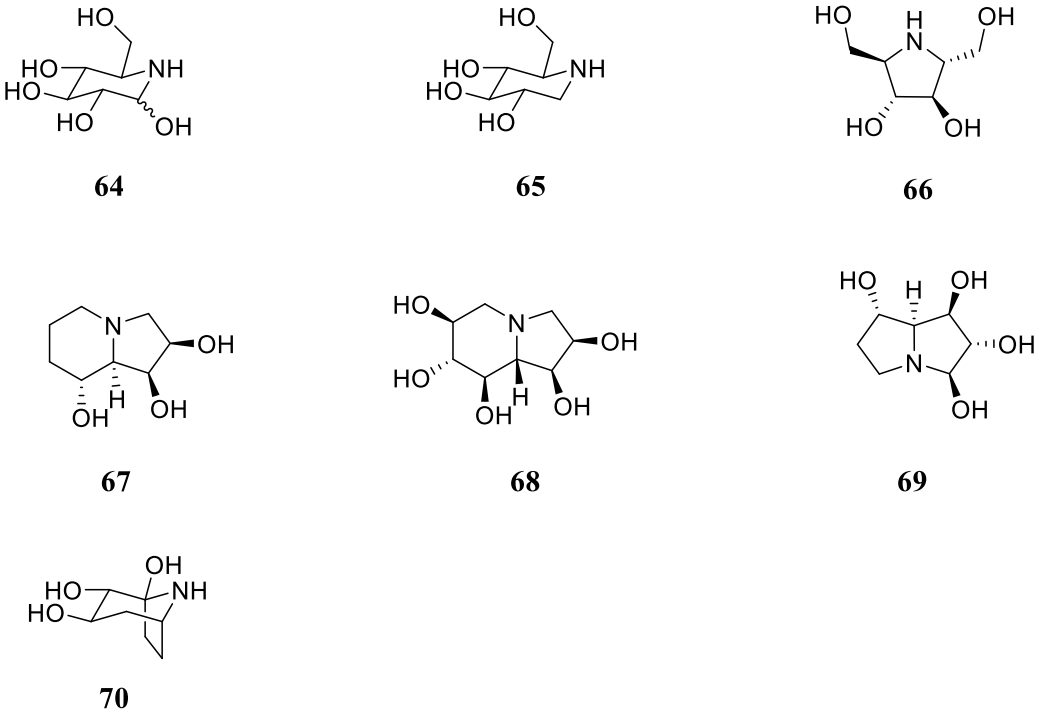


Figure 37: Representative structures of naturally occurring iminosugars

Iminosugars have been shown to inhibit or moderate the activity of a wide range of enzymes that act on carbohydrates.[2] Glycosidases are involved in the degradation of complex carbohydrates and glycoconjugates, for example glycoproteins, by catalysing the hydrolysis of glycosidic linkages. The enzymes are typically named after the substrates upon which they act, for example, lactase, amylase and sucrase. Glycotransferases are enzymes that establish natural glycosidic linkages involved in the synthesis of various glycoconjugates. It is thought that iminosugars are potent inhibitors of these carbohydrate processing enzymes as they resemble the cationic transition states formed during glycoside catalysis.[69] Iminosugars can be considered to be in their protonated state at physiological pH and the resulting ammonium ions mimic the oxocarbenium character of the hydrolysis transition states of carbohydrate substrates (Figure 38) and block the enzyme active sites.[64]

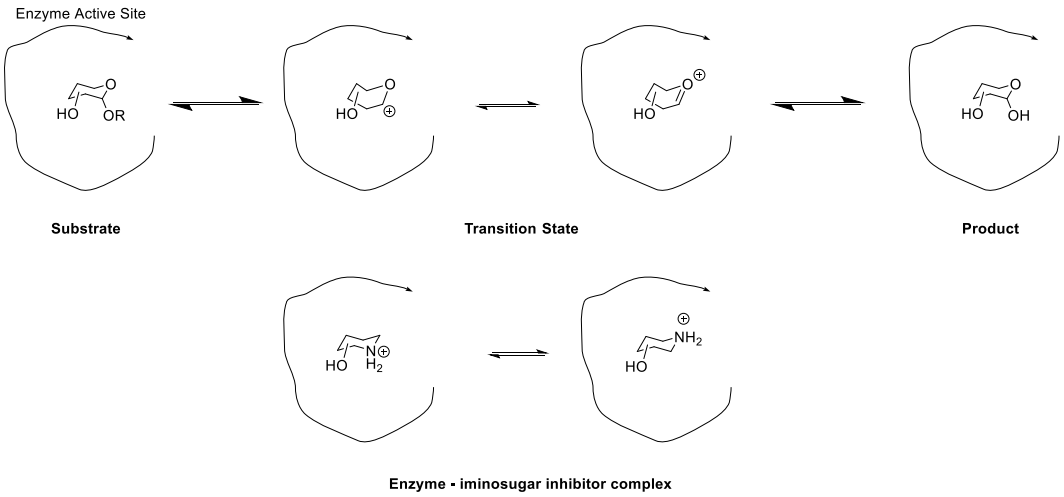
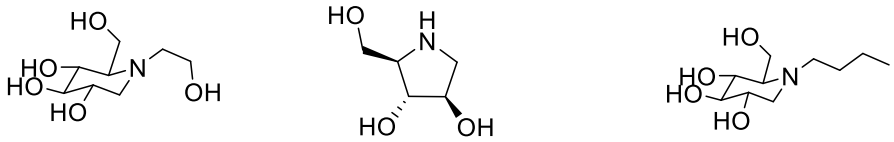


Figure 38: Diagrammatic representation of the resemblance of iminosugars to hydrolysis transition states of carbohydrate substrates in glycosidases [64]

The glycosidase and glycotransferase enzymatic groups are involved in a wide range of important biological processes and their inhibition can affect the synthesis, metabolism and degradation of carbohydrates.[70] The ability to modulate the activity of these carbohydrate processing enzymes has many potential medical applications as the biological processes they control are so widespread. Defects in the activity of glycosidases and glycotransferases are implicated in many diseases and medical conditions related to metabolic disorders, for example, diabetes, cancer and viral infections.[64] Hence, the discovery of new drugs and therapeutic strategies to influence enzymatic activity is of great interest. Certain iminosugars offer interesting candidates in this regard as they have a number of positive characteristics; they are chemically and metabolically stable, they have high aqueous solubility, oral bioavailablilty is good and their ability to cross the blood-brain barrier enables penetration of the central nervous system. Also, manipulation of the stereochemistry of the iminosugar scaffold enables a greater degree of opportunity for selective and specific inhibitors.

At present there are 25 known natural iminosugar glycosidase inhibitors.[69] DNJ **64** and castanospermine **68** have been shown to inhibit intestinal disaccharidases and delay post prandial hyperglycemia making them potential antidiabetic and obesity drugs. A synthetic derivative of DNJ, *N*-hydroxyethyl DNJ (Glyset/Miglitol) **71** was commercialised in 1996 as a treatment for type II diabetes.[71] DMDP **66** is best known for its ability to inhibit glucosidase I and swainsonine **67** competitively inhibits α-mannosidase II.[69] 1,4-dideoxy-1,4-imino-*D*-

arabinitol, also known as DAB-1 **72**, is a potent inhibitor of glycogen phosphorylase which is the enzyme responsible for the degradation of glycogen into glucose monomers.[69] This makes it, and its synthetic derivatives, potential drugs for the treatment of type II diabetes as it can modulate glucose levels. Another iminosugar clinically used as a drug is miglustat **73** used for the treatment of Type I Gaucher disease, a genetic disorder resulting in an excessive accumulation of glucosylceramide, a membrane lipid in lysosomes, resulting in liver and bone marrow problems.[69] The disease is caused by the dysfunctional activity of glucocerebrosidase, the enzyme responsible for the destruction of glucosylceramide. Miglustat offers an effective substrate reduction therapy by inhibiting the enzyme responsible for glucosylceramide production, glucosylceramide synthase. This reduces the amount of glucosylceramide rather than acting upon the dysfunctional glucocerebrosidase.[72]

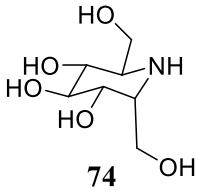


71 **72** **73**

Figure 39: Structure of iminosugars that are glycosidase inhibitors or in clinical use

However, the activity of iminosugars is not restricted to interactions with carbohydrate processing enzymes or in their role as glycomimetics. The utility of iminosugars to a range of other targets such as matrix metalloproteinase with potential for oncology treatment and the local treatment of skin disorders such as psoriasis is also under investigation.[73]

The exploitation of iminosugars as therapeutics has been modest to date having resulted in only eight clinical candidates and the two marketed drugs noted earlier.[64] It is costly and time consuming to isolate iminosugars in the pure form from natural sources in sufficient quantities to enable full toxicological and clinical evaluation.[74] Thus, the development of synthetic analogues of natural iminosugars has received much research since the first synthetic iminosugar, nojirimycin **64**, was reported by Paulsen in 1967.[75] Homonojirimycin (HNJ) **74** is an iminosugar which was made synthetically by Bayer's chemists almost 10 years before natural α-homonojirimycin was isolated for the first time from *Omphalea diandra*.[71]



74

Figure 40: Structure of homonojirymicin (HNJ)

3.2. Synthesis of iminosugars

Given the enormous therapeutic potential of iminosugars, the development of efficient and general synthetic methodologies for their synthesis and the generation of structural diversity has become the objective of many synthetic chemistry research groups. The structure of iminosugars presents a number of synthetic challenges. Firstly, iminosugars are generally stereochemically complex molecules containing at least four contiguous stereogenic centres. These must be obtained with high stereochemical control as the stereochemistry of one particular hydroxyl group within the molecule can dramatically change the biological activity of a compound. Secondly, the piperidine ring, pyrrolidine ring or bicyclic scaffold must be generated efficiently. The molecules are highly polar which can complicate isolation and purification methodologies. In addition, the presence of a high number of functional groups, particularly hydroxyl groups, requires cautious protection and deprotection strategies to ensure chemoselectivity during syntheses.

With five key structural classes of iminosugars and numerous synthetic ring forms and derivations, there is a vast literature on the synthesis of a wide range of iminosugars employing a variety of chemistries to achieve the final targets. The approach of using carbohydrates as starting materials for iminosugar syntheses is well represented in the literature. However, other inexpensive chiral-pool starting materials including amino acids and tartaric acids have also been successfully used in the synthesis of iminosugars. Asymmetric methodologies and chemoenzymatic approaches also feature in the literature.[71] Rather than attempt to provide an exhaustive list of iminosugar syntheses, several key synthetic strategies which have been used to prepare iminosugars in the piperidine class will be discussed and some interesting examples provided. Synthetic methodologies are grouped in respect of whether carbohydrate or non-carbohydrate starting materials are used and then classified according to the key step to form the requisite C-N bond or bonds.

3.2.1. Carbohydrates as starting materials

Due to their commercial availability and structural relationship with iminosugars, carbohydrates have been used as starting materials in the preparation of a range of iminosugars.[71] A structurally related carbohydrate as a starting material provides a means of introducing the correct stereochemistry at the relevant stereogenic centres into the final iminosugar. Figure 41 shows the stereochemical relationship between *D*-glucose and nojirimycin.

***D*-glucose** **nojirimycin**

Figure 41: Stereochemical relationship between *D*-glucose and the iminosugar, nojirimycin

A wide range of carbohydrates is available providing an excellent variety of chirons that will facilitate the introduction of stereochemical characteristics into the target iminosugar. Strategic, stereoselective chemical transformations to either retain or alter the chirality of a particular stereogenic centre can be used to increase the diversity of iminosugar targets by providing access to epimeric or anomeric relations.[70] Depending upon the target, the main challenge in employing carbohydrates as starting materials is the differentiation of the hydroxyl groups often necessitating the inclusion of several protection and deprotection steps into syntheses.

To prepare an iminosugar from a carbohydrate requires the introduction of two carbon-nitrogen bonds and is a crucial step in the total synthesis of iminosugars in both the piperidine and pyrrolidine forms. Figure 42 illustrates the key bond disconnections in the formation of the piperdine ring of an iminosugar.

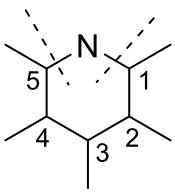


Figure 42: Key C5-N and/or C1-N disconnections in the formation of a piperidine ring

Figure 43 provides a generic outline of the key steps involved in preparing an iminosugar from a general carbohydrate chiron. The first step is the introduction of an amino group or precursor into the carbohydrate generally by the conversion of one of the hydroxyl groups. A favoured method for doing this is via nucleophilic displacement with common nitrogen nucleophiles including hydrazine, ammonia, benzylamine, phthalimide and azide. Of these, the azide group offers benefits in that it is a powerful nucleophile, once installed it is unreactive under a broad range of reaction conditions and is readily reduced to the corresponding amine functionality when required. Tosylates and mesylates are often used as leaving groups but a range of other leaving groups have been reported.[71] The activation of the amino function and an appropriate functional group in a suitable corresponding position within the molecule are generally required for the cyclisation that generates the C-N bond or bonds. Various methods have been used to promote cyclisation including intramolecular S_N2 reactions, electrophilic induced cyclisation, reductive amination and ring closing metathesis.[71] Further modification to the carbon backbone is often required to afford the iminosugar the appropriate functionalisation.

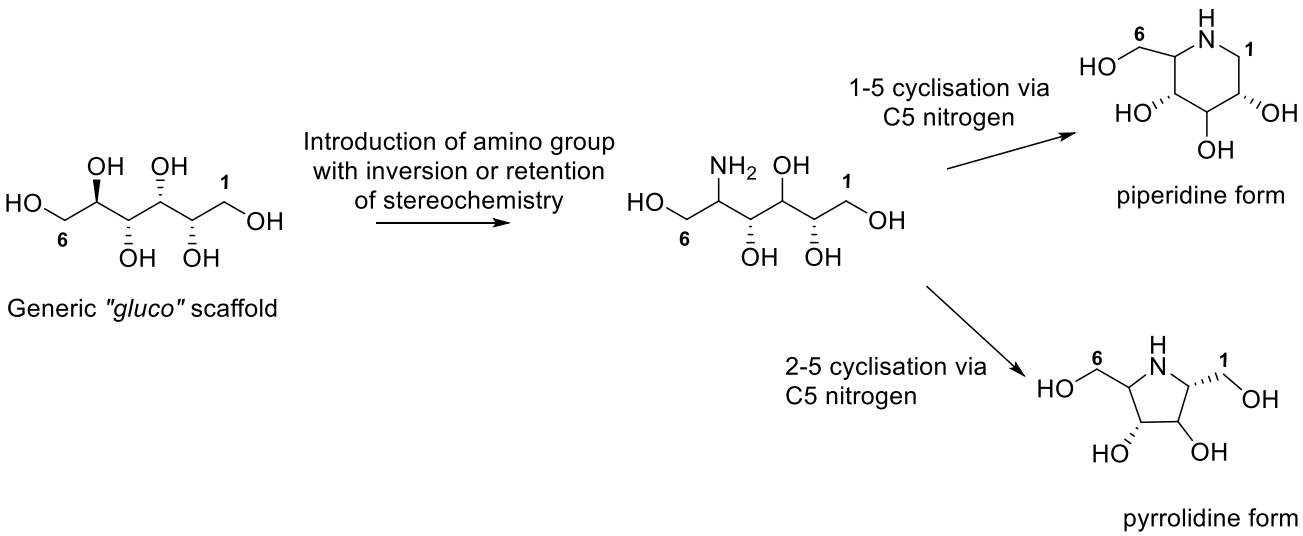
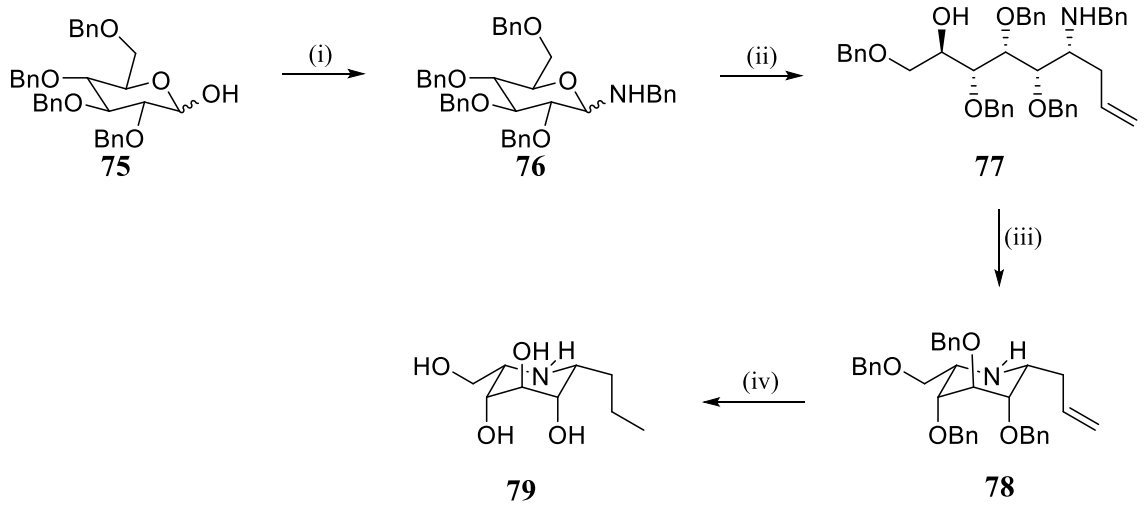


Figure 43: Generic route to iminosugars in the piperidine and pyrrolidine forms from a general carbohydrate starting material.[76]

3.2.1.1. C-N bond formation by intramolecular S_N2 reactions

Intramolecular cyclisation to form the piperidine ring can be accessed via the displacement of a leaving group by an amino group or masked amino group. The ring opening of an epoxide by an amino group is another strategy regularly used to induce cyclisation. An advantage of an S_N2 intramolecular reaction to form the C5-N bond of an iminosugar is that it leads to inversion of configuration at the C5 position due to the displacement of a leaving group. This allows potential access to iminosugars in the *L*-series.[71]

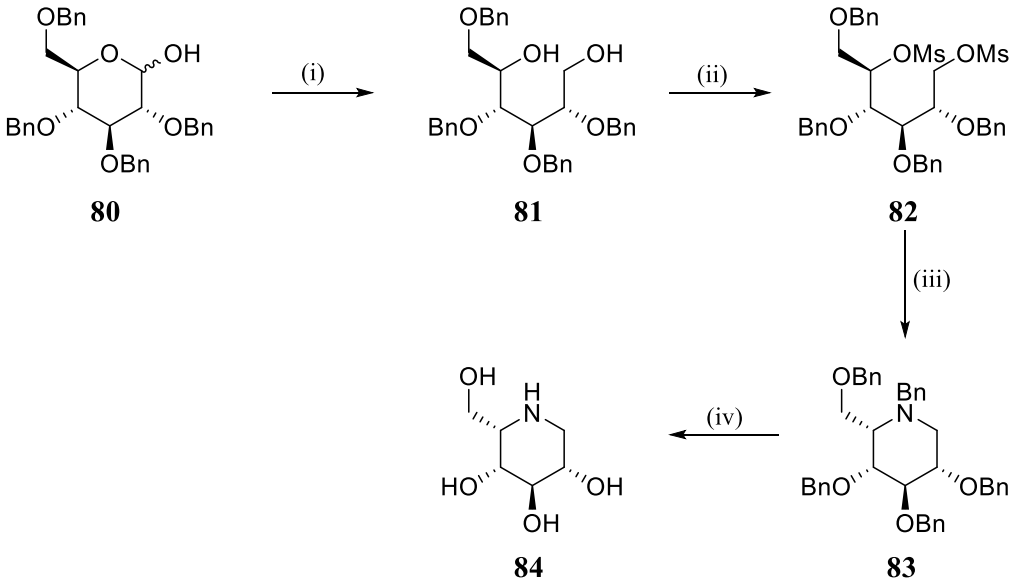
Cipolla et al [77] developed a new synthetic procedure whereby a variety of C-glycosides could be synthesised from different carbohydrates. The general scheme involves the reaction of a tetra-*O*-benzyl protected monosaccharide with a primary amine to introduce the amino group followed by the stereoselective addition of a Grignard reagent to the glycosamine. The subsequent cyclisation of the resulting amino alcohol is mediated by an intramolecular S_N2 displacement of a triflate group. Altering the primary amine and the Grignard reagent affords the flexibility to synthesise a great variety of piperidine iminosugars. Scheme 23 provides an example of the steps involved in preparing an iminosugar in the *L*-series, *(2S,3R,4R,5S,6R)*-2-hydroxymethyl-6-propyl-3,4,5-trihydroxypiperidine **79** from 2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranose **75**.



Reagents and conditions: (i) DCM, $BnNH_2$, 4A MS, 4 days; (ii) THF, vinyl magnesium bromide, 24h; (iii) Pyridine, Tf_2O, 1.5h; (iv) H_2, 10%Pd/C, MeOH, H^+.

Scheme 23: Example of ring formation by S_N2 displacement. Synthesis of *(2S,3R,4R,5S,6R)*-2-hydroxymethyl-6-propyl-3,4,5-trihydroxypiperidine.

An efficient synthesis of the C5 epimer of 1-deoxynojirimycin **65**, l,5-dideoxy-1,5-imino-*L*-iditol (1-deoxy-*L*-idonojirimycin) **84**, via an S_N2 displacement reaction was reported by Fowler *et al* (Scheme 24). [78] In this instance, 2,3,4,6-tetra-*O*-benzyl-*D*-glucitol **81** was prepared by the reduction of commercially available 2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranose **80**. The two resulting hydroxyl groups were mesylated to give the methanesulfonyl derivative **82** that was reacted with benzylamine to afford **83**. Subsequent hydrogenolysis gave the desired compound **84**. An alternative, more time efficient method of preparing **84** has been described. [79] In this case, the C1 mesylate group is substituted with an amino group by treating **82** with sodium azide followed by an *in situ* Staudinger reaction with triphenylphosphine. A spontaneous, intramolecular S_N2 cyclisation resulted providing the desired *O*-benzyl protected piperidine iminosugar **83**.



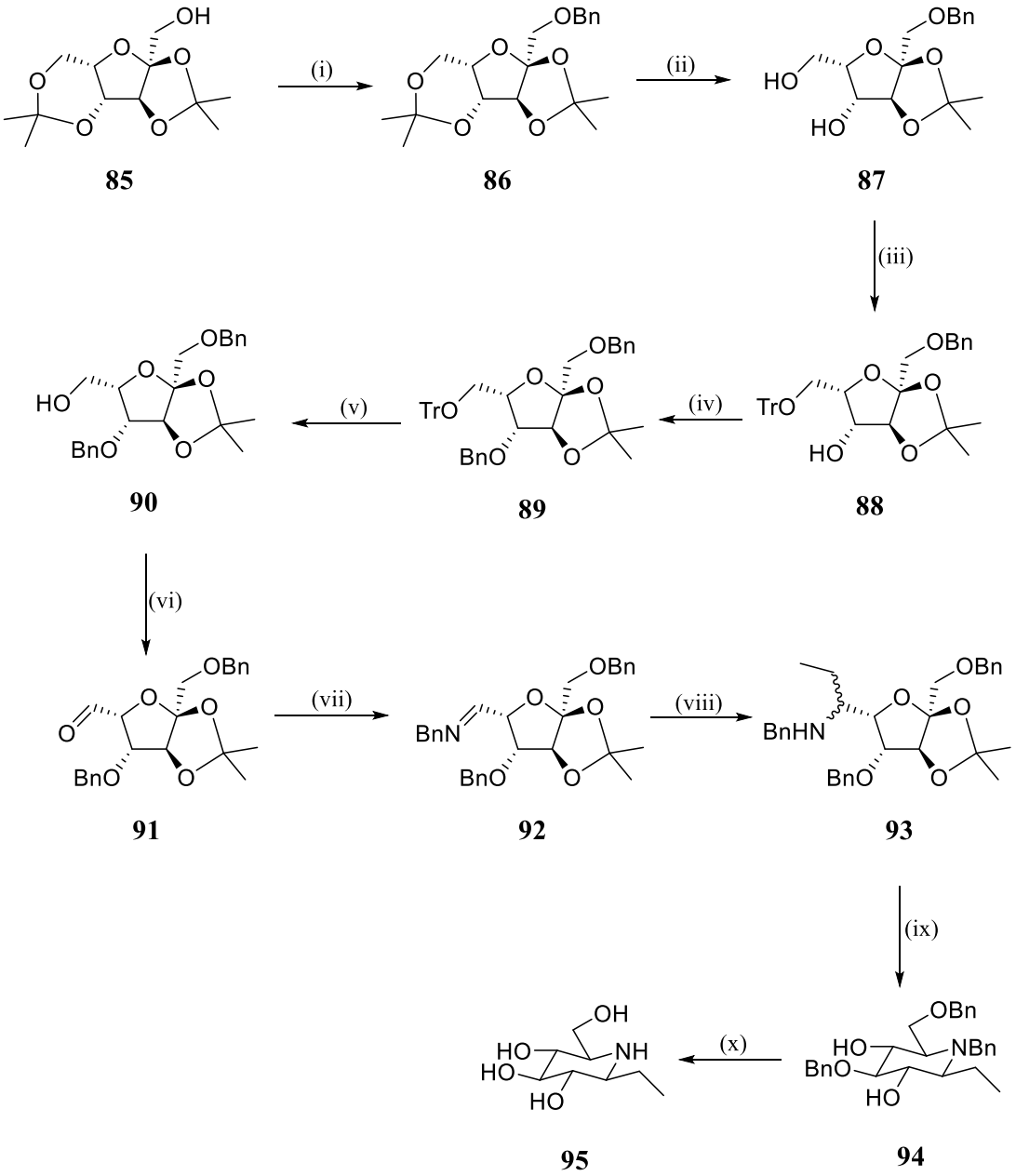
Reagents and conditions: (i) $NaBH_4$, THF/H_2O, room temperature, 24h; (ii) MsCl, Py., 0^0C, 72h; (iii) $BnNH_2$, 55^0C, 5 days; (iv) H_2, Pd/C, EtOH, 6 days.

Scheme 24: Example of ring formation by S_N2 displacement. Synthesis of l,5-dideoxy-1,5-imino-*L*-iditol.

3.2.1.2. Examples of C-N bond formation by reductive amination

A reductive amination reaction to form the C5-N and/or C1-N bond offers several advantages to an iminosugar synthetic strategy. The reaction fully utilises the intrinsic reactivity of the amino group already introduced into the molecule. One or two stereogenic centres are concomitantly generated as a result of the reaction. Reductive amination is compatible with many other functional groups that may be present in the molecule.

Scheme 25 illustrates the synthetic strategy adopted by Godin *et al* [80] for the preparation of various α and β-1-C substituted-1 deoxynojirimycins. *L*-sorbose **85** is a readily available carbohydrate and was selected as the starting material for the synthesis to take advantage of its three stereogenic centres at C2, C3 and C4 which are present in the desired structure. An orthogonal protecting group strategy was designed to ensure the discrimination of hydroxyl groups during various key synthetic steps. The key intermediate in the synthesis is **92**, an imine. In the particular instance shown in Scheme 25, the imine is employed as a means to introduce diversity into the molecule by the diastereoselective addition of organometallic nucleophiles. Following the extension of the chain, intramolecular reductive amination of the latent keto function of the aminosorbose derivative **93** was achieved with sodium cyanoborohydride. Removal of the benzyl groups via catalytic hydrogenation afforded the desired product **95**.

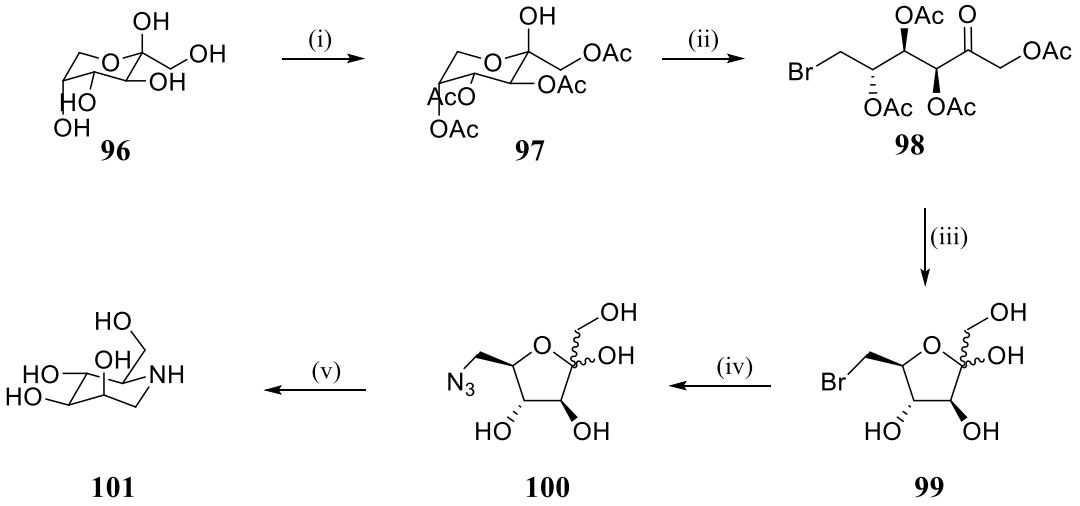


Reagents and conditions: (i) NaH, BnBr,nBu_4NI, THF, 3h; (ii) H_2SO_4, H_2O, acetone, 3h; (iii) TrCl, pyridine, 55^0C, 48h; (iv) NaH, BnBr, THF, 4h; (v) HBr, AcOH, 5^0C; (vi) Dess-Martin periodinane, DCM, 0.5hr; (vii) $BnNH_2$, MS 4A, DCM, 4^0C,3h; (viii) EtMgBr, Et_2O, 0^0C, 12h; (ix) a. TFA/H_2O (9:1), 30h; b. $NaBH_3CN$, MeOH, AcOH, 24h; (x) H_2, Pd/C, H^+, MeOH, 48h.

Scheme 25: Synthesis of nojirimycin C-ethyl glycoside. Example of ring formation by reductive amination strategy.

Another example of where intramolecular reductive amination has been used to successfully prepare a piperidine type iminosugar is shown in Scheme 26.[81] The synthesis of 1-deoxymannojirimycin **101** includes five steps from the carbohydrate *D*-fructose. Here, the

reductive amination step occurs concurrently with the reduction of the azide to an amino group by catalytic hydrogenation. The unmasked amine then reacts with the hemiketal of the furanose ring to form the iminosugar.

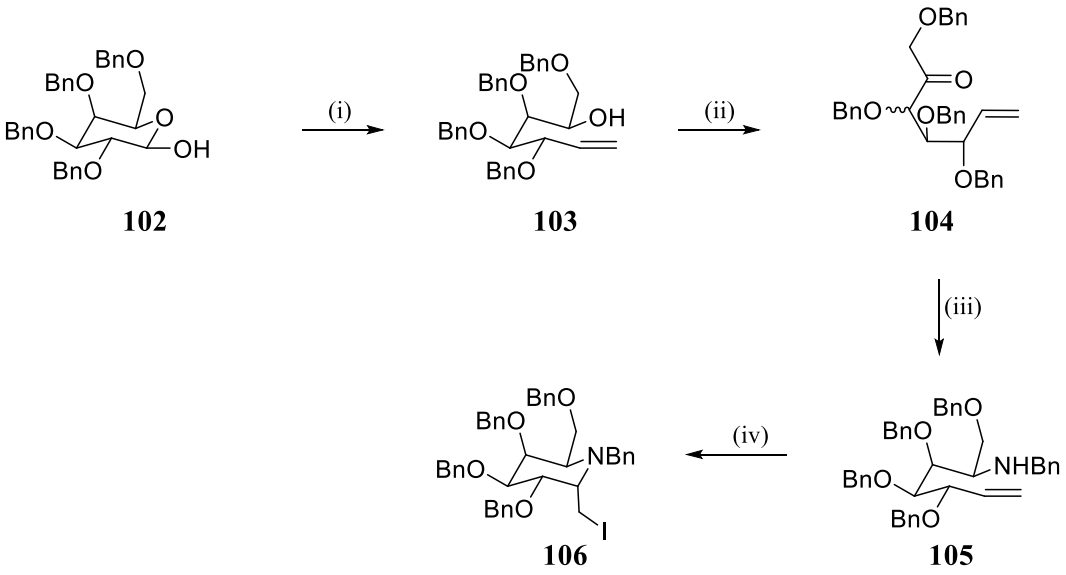


Reagents and conditions: (i) AcCl, H_2SO_4; (ii) PPh_3Br_2, DCM, Py. 3h; (iii) NaOMe, MeOH, 0^0C, 5h; (iv) NaN_3, DMF; (v) H_2, Pd/C, MeOH, 4h.

Scheme 26: Example of ring formation by reductive amination strategy. Synthesis of 1-deoxymannojirimycin.

3.2.1.3. Electrophile induced C-N bond formation

A further strategy is to use an electrophile to induce the intramolecular cyclisation step. Cyclisation of unsaturated amino alkenes has usually been promoted with mercury (II) salts or N-iodosuccinimide (NIS). [82] [83] [84] An advantage of this method is that the piperidine ring is formed and an organomercurial or iodo derivative also results that constitute versatile precursors for further functionalisation. Scheme 27 [83] provides an example of a synthetic strategy in which NIS is used to mediate the cyclisation of amino alkene **105**. The iodo product **106** is a key precursor of imino-C-glycosides compounds and the overall scheme offers a highly efficient route from tetra-*O*-benzyl-*D*-galactopyranose.



Reagents and conditions: (i) $Ph_3P{=}CH_2$, toluene, 0^0C, 48h ; (ii) DMSO-TFAA, DCM, -78^0C. 4h; (iii) Benzylamine, AcOH, MeOH, room temperature, 2h then $NaBH_3CN$, reflux, 4h; (iv) NIS, DCM, room temperature, 3h.

Scheme 27: Example of ring formation by electrophile induced cyclisation. Synthesis of a 1,2,6-trideoxy-2-6-imino-1-iodoheptitol derivative.

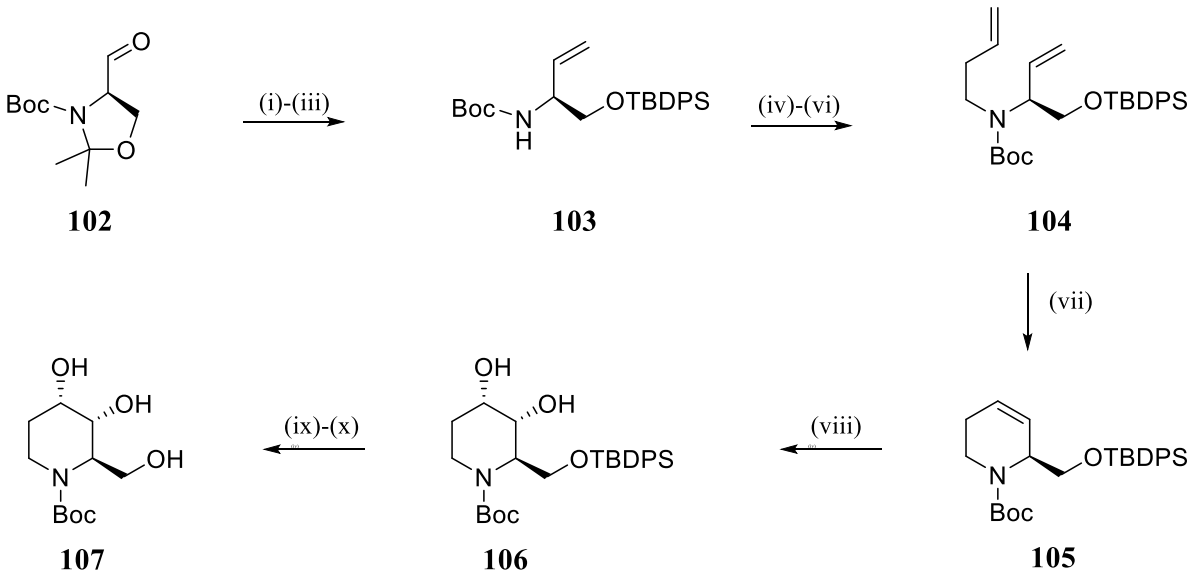
3.2.2. Non-carbohydrates as starting materials

A disadvantage of using carbohydrates as starting materials in the synthesis of iminosugars is that they can necessitate the need for long synthetic sequences with low overall yields; this can limit the commercial potential of an iminosugar. Attempts to overcome this perceived barrier have generated interesting recent advances in the use of non-carbohydrates as precursors to iminosugars with approaches employing amino acids and their derivatives, nitrogen containing heterocycles and nitrones reported.[64] In most cases, the use of alternative starting materials simplifies the key chemical steps involved in preparing an iminosugar which improves convenience and increases commercial viability.

3.2.2.1. Amino acids as starting materials

Commercially available Garner aldehyde **102** which is derived from *D*-serine has found successful application in providing a chiral building block in the syntheses of iminosugars. [70] Fagomine **107** was successfully synthesised from Garner aldehyde as illustrated in Scheme 28.[85] The synthesis begins with a Wittig reaction of Garner aldehyde with

methyltriphenylphosphonium iodide in the presence of sodium bis(trimethylsilyl)amide to give an olefin. This compound then undergoes hydrolysis with *p*-toluene sulphonic acid, followed by *O*-silylation to afford **103**. Then follows a three step sequence, N-Boc deprotection, alkylation and subsequent N-protection, to give the butenylated product **104**. The ring closing metathesis step, (vii) in Scheme 28, is a key step in the overall reaction scheme. Grubbs catalyst, bis(tricyclohexylphosphine)benzylidineruthenium(IV) dichloride, was used to effect the ring closure. Stereoselective dihydroxylation of the double bond of **105** introduced the two hydroxyl groups. Deprotection of the silyl ether and N-Boc group gave the desired product.



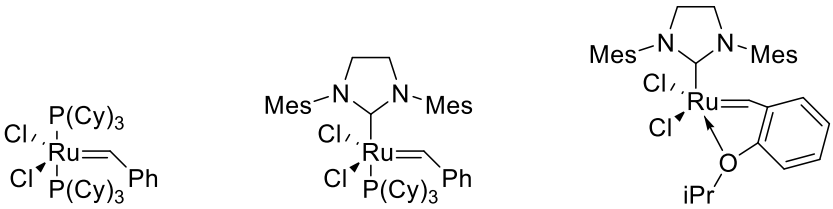
102 **103** **104** **105** **106** **107**

Reagent and conditions: (i) $Ph_3P^+CH_3I^-$, NaHMDS, THF; (ii) TsOH, MeOH; (iii) TBDPSCl, DMAP, imidazole, DCM; (iv) TFA, DCM; (v) 4-bromo-1-butene, K_2CO_3, CH_3CN; (vi) $(Boc)_2O$, Et_3N, DCM; (vii) [Ru]-129, DCM; (viii) $K_2OsO_4.2H_2O$, NMO, H_2O, acetone; (ix) 10% HCl, dioxane; (x) Dowex (OH^-)

Scheme 28: Example of the use of an amino acid starting material. Synthesis of 3-*epi*-fagomine from Garner aldehyde.

Scheme 28 provides an example of an important and well utilised iminosugar construction methodology when non-carbohydrate starting materials are used in which a dual strategy of ring closing metathesis and cis-hydroxylation is employed. Several iminosugars have been prepared using this method with a typical procedure involving the ring closing metathesis of a suitably functionalised nitrogen containing diene to yield a dehydropiperidine that is subsequently dihydroxylated, often using OsO_4.[69] The scope of ring closing metathesis as a

tool in the synthetic strategies of iminosugars has widened due to the ongoing development of stable, reactive and functional group tolerant olefin metathesis catalysts.[86] Of the several catalysts described in the literature, three of the most popular are the ruthenium derived catalysts developed by Grubbs, **108**, **109** and **110**.



108 Grubbs I

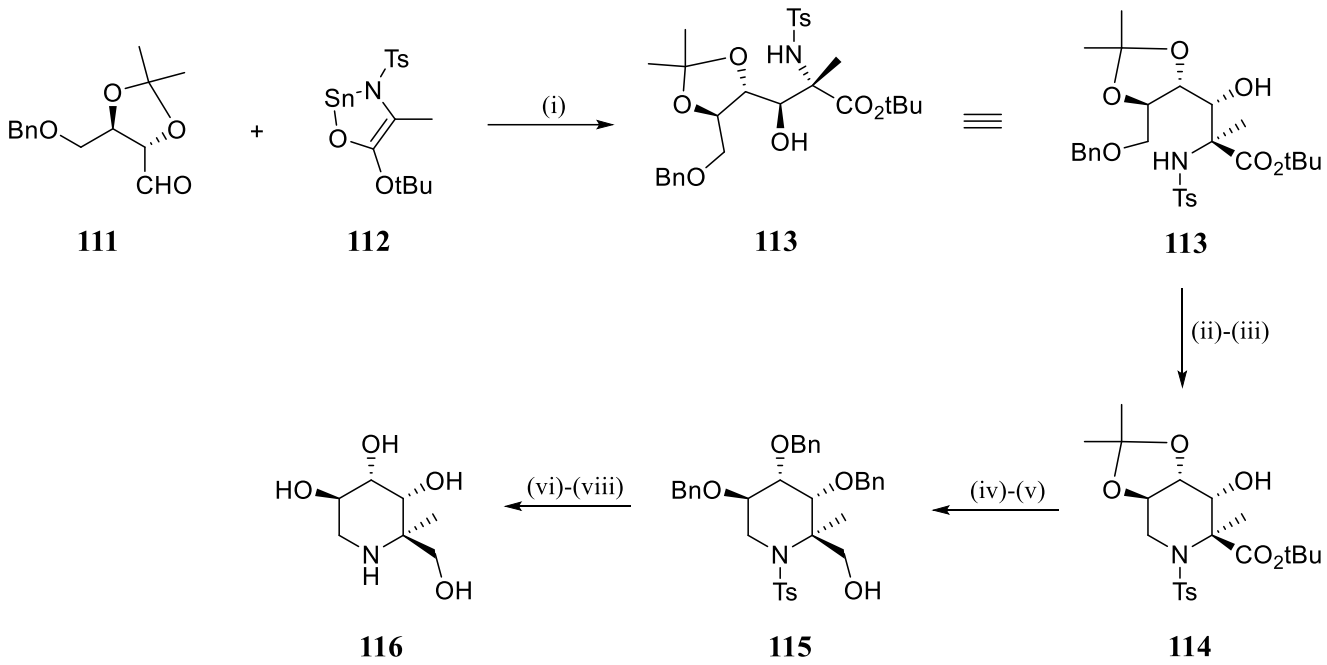
109 Grubbs II

110 Hoveyda-Grubbs

Figure 44: Example of catalysts employed in ring closing metathesis

3.2.2.2. Tartaric acids as starting materials

Some syntheses of iminosugars have been reported from tartaric acid, its diasteroisomers and derivatives. Tartaric acid can be considered as a four carbon skeleton moiety that has a *threo*-type configuration and is a useful chiral building block.[70] Scheme 29 details the synthesis of the iminosugar **116** from an aldol reaction between the protected threose derivative **111** and the nucleophilic metal chelated N-protected analine *tert*-butyl ester **112** as the key step.[87] After removal of the benzyl group of **113**, a cyclisation under Mitsonobu conditions yielded compound **114**. The labile isopropylidene ketal was cleaved and the resulting diol along with the other unprotected hydroxyl group were protected with benzyl groups. The iminosugar **116** was accessed by reduction of the *tert*-butyl ester with lithium aluminium hydride, deprotection of the tosyl amide by titration with sodium napthalide and cleavage of the benzyl ethers by catalytic hydrogenation.



Reagent and conditions: (i) THF,78 ^{0}C; (ii) H_2 (3 bar), Pd/C, MeOH, room temperature; (iii) DEAD, PPh_3, THF, room temperature; (iv) Dowex 50WX8, MeOH/H_2O, room temperature, 10h; (v) NaH, BnBr, DMF, 0^{0}C; (vi) $LiAlH_4$, THF,room temperature, 2h; (vii) Sodium naphthalide, DME, -60^{0}C; (viii) H_2 (1 bar), Pd/C, THF, room temperature, 3d.

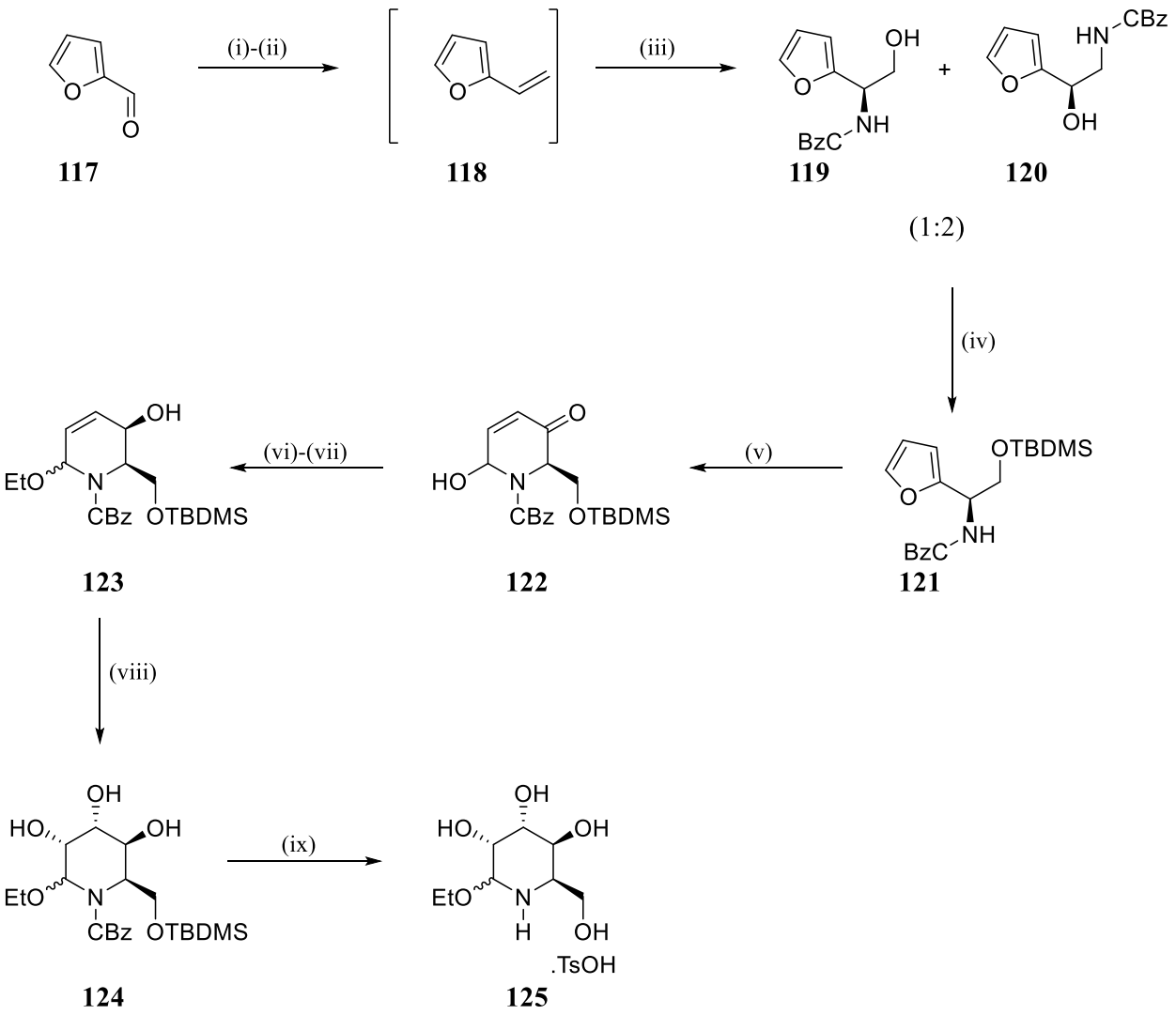
Scheme 29: Synthesis of an iminosugar from a tartaric acid derivative

3.2.2.3. Asymmetric methodologies

The asymmetric epoxidation of allylic alcohols, the asymmetric dihydroxylation of olefins and the asymmetric aminohydroxylation of alkenes are the three most prominent catalytic oxidations and their efficiency has been fully demonstrated in iminosugar synthesis. [70] An elegant example of the successful application of the methodology is shown in Scheme 30, the synthesis of 1-deoxygulonojorimycin **125** from furfural and is analogous to the methodology in which furfural was successfully converted to *D*- and *L*-carbohydrates. [70] [88] Vinyl furan **118** was prepared by the addition of furfural **117** to an ethereal solution of TMSMgCl followed by acidic treatment of the resulting alcohol. Enantiomerically enriched N-CBz-protected amino alcohols **119** and **120** were obtained by applying the Sharpless asymmetric aminohydroxylation chemistry to vinyl furan using an ether solution of vinyl furan with the sodium salt of N-chlorobenzylcarbamate and an OsO_4/$(DHQ)_2$PHAL admixture.

The regioisomers **119** and **120** were separated by chromatography and the primary alcohol group of **119** was protected as its silyl ether. Next, an aza-Achmatowicz rearrangement with

m-CPBA led to the formation of the hemiaminal **122** which was treated with ethyl orthoformate to give the corresponding ethylaminal. The allylic alcohol **123** was obtained by reduction and diastereoselective catalytic OsO_4 dihydroxylation of **123** gave **124**. Deoxygulonojirimycin **125** was produced by hydrogenolysis which was purified by isolation as the *p*-toluene sulphonate salt.



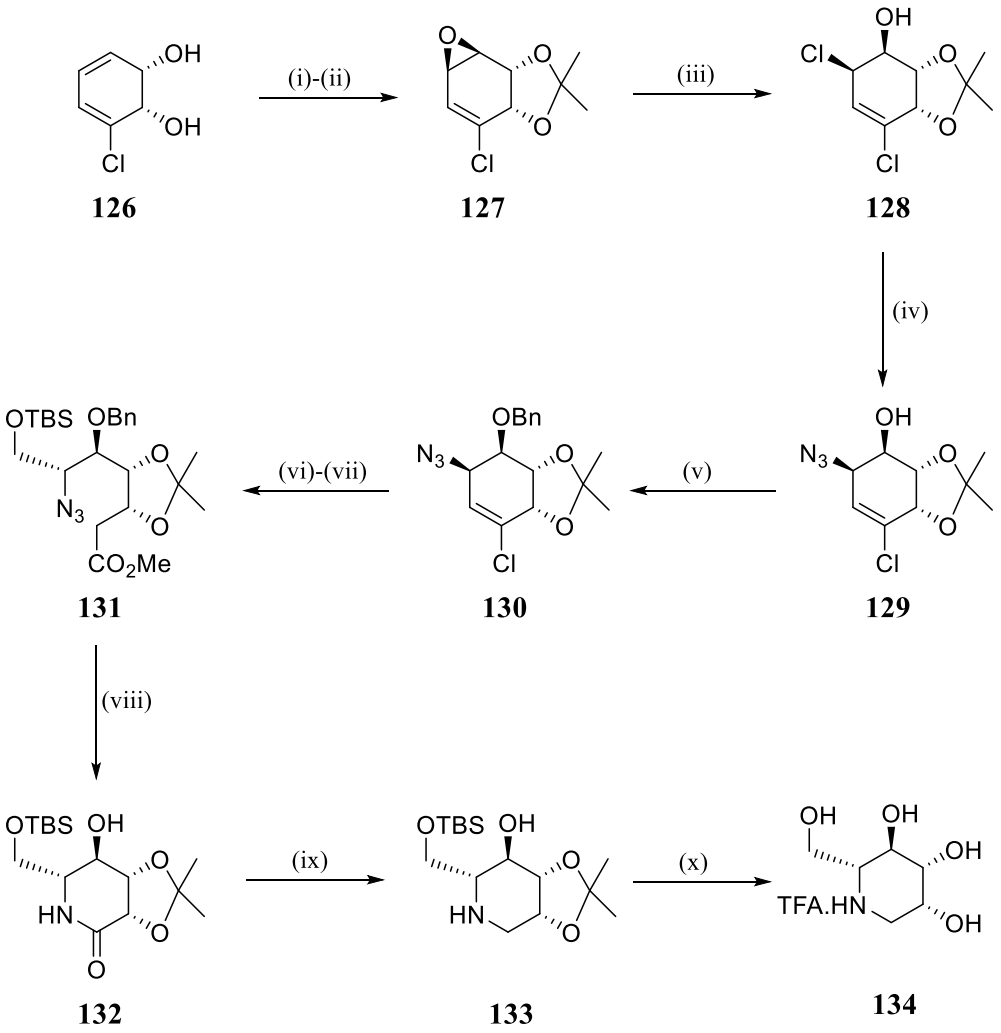
Reagent and conditions: (i) Mg, $TMSCH_2Cl$, Et_2O, reflux, 12h then fufural, Et_2O, 0^0C, 12h; (ii) aq. HCl, Et_2O, 1h; (iii) $CbzNH_2$, tBuOH,tBuOCl, aq. NaOH then $(DHQ)_2PHAL$, XX, OsO_4, room temperature, 1h; (iv) TBDMSCl, Et_3N, DMAP, DCM, room temperature, 3h; (v) m-CPBA, DCM, 0^0C, 3h; (vi) $(EtO)_3CH$, TsOH (5 mol%), DCM, room temperature, 24h; (vii) $NaBH_4$, $CeCl_3$, DCM, -78^0C, 2h; (viii) OsO_4, NMO/H_2O, DCM, 0^0C, 12h; (ix) H_2, Pd/C, MeOH, room temperature, 12h then TsOH, room temperature, 3h.

Scheme 30: An example of an asymmetric methodology. Synthesis of 1-deoxynojirimycin from furfural.

It is worthy of note that of all the examples quoted thus far, **125** is one of a few reported compounds containing the hemiaminal function within the pyranose ring. This functionality is a requisite of the target compounds of this project (see Figure 36, page 102) and therefore synthetic examples of compounds which include a hemiaminal are of particular interest. Iminosugars that contain the hemi aminal chemotype have been shown to have an intrinsic instability[75] and need almost to be trapped, as with the CBz protected nitrogen atom and the ethereal oxygen in this example, prior to the iminosugar's isolation as a *p*-toluene sulphonate salt. This would form a key consideration when determining the synthetic viability of the target compounds.

3.2.2.4. Chemoenzymatic approaches

Several groups have employed chemical or enzymatic resolution as a key aspect in the synthesis of iminosugars. Banwell *et al* [89] successfully employed microbial oxidation of chlorobenzene to provide a versatile and readily elaborated starting material for the syntheses of several iminosugars. Scheme 31 shows the synthesis of 1-deoxymannojirimycin (DMJ) from **126** which is derived from chlorobenzene.



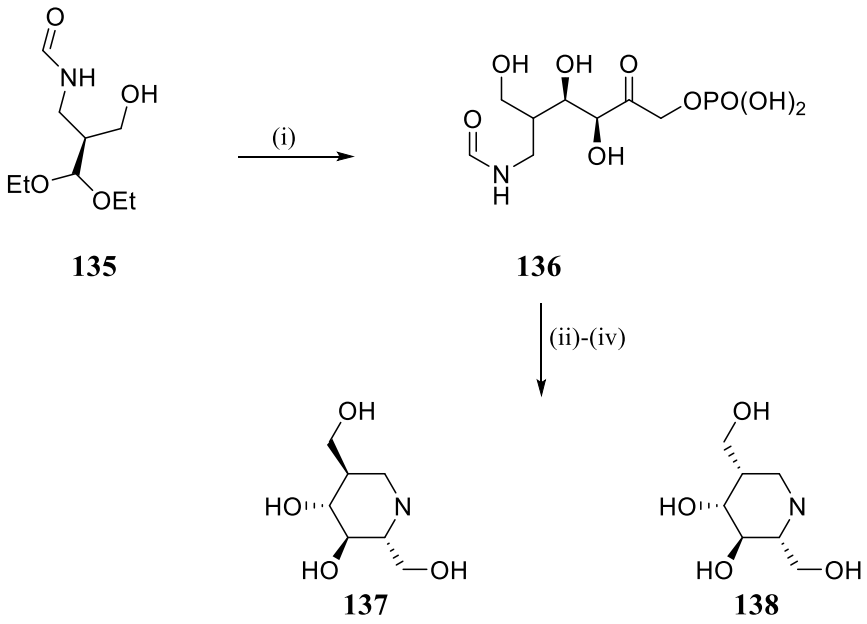
Reagents and conditions: (i) 2,2-DMP, *p*-TsOH.H_2O (cat), room temperature, 1h; (ii) *m*-CPBA, DCM, 0-18^{0}C. 11h; (iii) LiCl, AcOH, THF, room temperature, 17h; (iv) LiN_3, DMF, room temperature, 72h; (v) BnBr, KI, NaH, THF, 0^{0}C-room temperature, 72h; (vi) O_3, pyridine, MeOH, -78^{0}C, 1h then $NaBH_4$, -10^{0}C, 3h; (vii) TBSCl, imidazole, DCM, room temperature, 2h; (viii) H_2, Pd/C, EtOAc, room temperature, 36h; (ix) BH_3.DMS, THF, room temperature, 4.5h then Pd/C, MeOH, room temperature, 38h; (x) 80% v/v TFA.H_2O, room temperature, 20h.

Scheme 31: An example of the inclusion of a chemoenzymatic step. Synthesis of 1-deoxymannojirimycin from a chlorobenzene derivative.

The readily prepared acetonide derivative of **126** was subject to epoxidation with *m*-CPBA and the resulting epoxide **127** converted to the chlorohydrin **128** upon reaction with lithium chloride. An S_N2 type displacement reaction with lithium azide followed to give **129** which was *O*-benzylated to give **130**. Ozonolytic cleavage of this compound followed by a sodium borohydride reductive work up and subsequent protection of the ensuing primary alcohol using TBSCl afforded the azido-ether **131**. Two-fold hydrogenolysis of **131** resulted in the formation of lactam **132** which was converted to the corresponding piperidine with a borane sulphide

complex. The resulting borane-amine complex was cleaved with methanol and palladium on carbon. The desired compound DMJ **134** was obtained as the TFA salt.

Another example of where chemoenzymatic methods play a key role in the preparation of an iminosugar is shown in Scheme 32.[90] In this instance a homoisofagomine type iminosugars **137** and **138** were prepared without relying on the chiral pool with the key stereoselective carbon-carbon bond forming step being catalysed by aldolase. The acetal of the starting material **135** is cleaved under mild acidic conditions. Dihydroxyacetonephosphate (DHAP) was added followed by rabbit muscle aldolase (RAMA) to catalyse the formation of the carbon-carbon bond. The resulting phosphate was hydrolysed using acid phosphatase. The N-formyl group was removed by treatment with hydrochloric acid and subsequent catalytic hydrogenation of the crude imine gave a mixture of **137** and its epimer **138** which were separated by column chromatography.

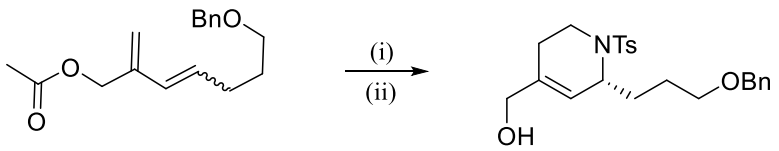


Reagents and conditions: (i) HCl, room temperature, 2h then DHAP/RAMA, room temperature,12h; (ii) Acid phosphotase, room temperature, 3d; (iii) HCl, 35^{0}C, 36h; (iv) H_2, Pd/C, room temperature, 12h.

Scheme 32: Example of the use chemoenzymatic methodology. Synthesis of a homoisofagomine type iminosugar

3.2.2.5. Miscellaneous starting materials

An aza-Diels Alder type reaction was used by Schurer[91] as a key step to providing polysubstituted piperidines from non-carbohydrate starting precursors. A versatile and very mild methodology was demonstrated in which various 1,3-disubstitued butadienes, for example **139**, were reacted with an electron deficient dienophile, in this instance, N-trichloro-ethylidene-p-toluenesulfonamide, to form compounds similar to **140** which can be regarded as potential precursors of iminosugar derivatives (Scheme 33).



139 **140**

Reagents and conditions: (i) N-trichloro-ethylidene-p-toluenesulfonamide, DCM, 70^{0}C, 40h; (ii) NaOMe, room temperature.

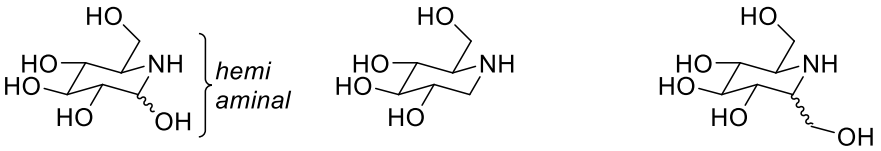
Scheme 33: Example of aza-Diels-Alder reaction used to construct piperidine ring. Synthesis of a polysubstituted piperidine as an iminosugar precursor.

The potential of iminosugars as therapeutic agents has led to the development of many new methodologies for the synthesis of both known and novel iminosugars. Since 2005, methodologies for piperidine type iminosugar synethesis have extended well precedented procedures such as reductive amination and metathesis and have led to the development of strategies that include *de novo* methods and the use of bioorgano-catalysis.[69] The range of starting materials and precursors for iminosugar syntheses have also expanded to fully exploit not only the wide range of available carbohydrates but to incorporate the use of other chirons and compounds beyond the chiral pool. Future studies in the field of iminosugars are likely to focus upon developing syntheses that are more efficient, enable the introduction of increased structural diversity and deliver higher stereocontrol.

3.3. Iminosugar analogues of topiramate

The ultimate synthetic targets of the project were novel iminosugar analogues of topiramate (**25**–**29**) (see page 101). The compounds were identified as "hits" by the *in silico* modelling work as results demonstrated that they were promising candidates as interactions with active sites demonstrated the potential for selectivity and binding within the target enzymes. Iminosugar **25** and its derivatives are novel compounds; thus their attempted synthesis was supported. A review of the literature identified that iminosugars that are structurally similar to the monosaccharide *D*-fructose in its pyranose form both from natural sources and produced synthetically are extremely rare. Therefore, it was anticipated that the synthesis of iminosugars containing the *D*-fructose scaffold would not be facile. Consideration of the various methodologies and chemistries that have been employed to synthesise iminosugars from a range of starting materials suggested that the preparation of iminosugar analogues of topiramate would pose several synthetic challenges. A key issue was how to form the piperidine ring whilst retaining and stabilising the anomeric hydroxyl group at the C2 position during cyclisation. Two widely used methods to effect the cyclisation step to form an iminosugar, either via an S_N2 displacement reaction or by reductive amination, result in the loss of the oxygen atom from the carbon atom that forms the new C-N bond. This oxygen atom within the topiramate iminosugar analogues ultimately forms part of the 2,3-acetal group with the adjacent hydroxyl group at the C3 position being an important structural feature of the compound with respect to binding.

The presence of an oxygen atom at the C2 position in the desired iminosugar results in a hemiaminal structure that would be predicted to result in an inherent instability within the molecule. For example, nojirimycin **141** has been reported as being highly unstable and can only be isolated and stored as its bisulfite adduct.[75] The majority of stable iminosugars that have been successfully synthesised, isolated and have shelf lives of sufficient length to make them viable compounds are either iminosugar C-glycosides, in which the oxygen atom of the hemiaminal function is replaced by a methylene group or "deoxy" variants of iminosugars (Figure 45). [71, 92] A comparison of the generic structures of each of these iminosugar variants is shown in Figure 45; nojirimycin **141**, contains the unstable hemiaminal functionality and homonojirimycin **143** has a C-C bond at the C2 position to form a C-glycoside. Contrastingly, 1-deoxynojirmycin **142**, has no adjoining atoms to the C2 carbon. Both **143** and **142** are stable compounds.[70]



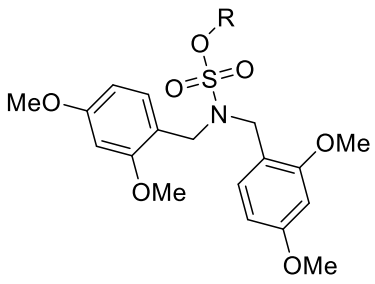
141 nojirimycin

142 1-deoxynojirimycin

143 homonojirimycin

Figure 45: General structure of "deoxy" iminosugars **142** and C-glycoside iminosugars 143

Another potential synthetic challenge was the timing of the introduction of the sulfamate group into the compound. The early introduction of the sulfamate functional group would necessitate its stability to multi-step syntheses in which a variety of chemical conditions would be employed. Conflictingly, the introduction of the sulfamate group towards the latter stages of any preparative route would require the sugar moiety to be able to withstand the relatively harsh sulfamoylation conditions. Development of the syntheses of metabolites of topiramate previously undertaken had provided some evidence of the robustness of the sulfamate group to selected reaction conditions, for example, hydrogenolysis. However, it has been noted that the *O*-sulfamate group is particularly labile to basic conditions and its reactivity/polarity can compromise its successful passage through several steps of chemical synthesis and result in challenging separations.[93] The development of sulfamates in which *N*-protection was achieved with either 4-methoxy- or 2,4-dimethoxybenzyl groups (Figure 46) have been shown to be stable to many common reaction conditions.[93] Such sulfamate protection strategies might offer synthetic opportunities should orthogonality with other protecting groups be favourable.

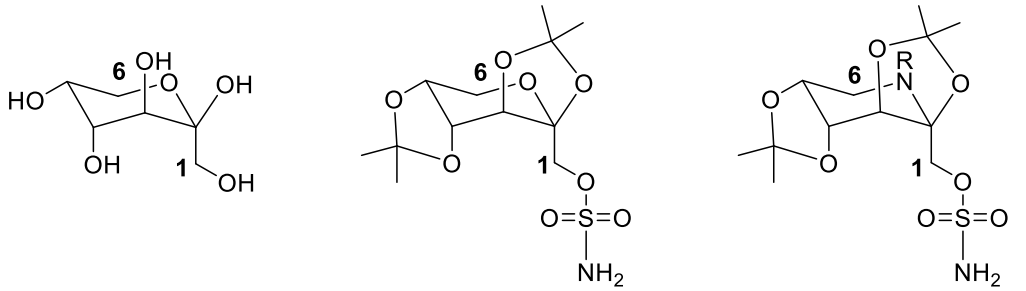


2,4-dimethoxybenzyl N-protected sulfamate

Figure 46: Generic structure of a 2,4-dimethoxybenzyl *N*-protected sulfamate

Accounting for the evident challenges of synthesising an iminosugar based upon topiramate and the general lack of corresponding literature, the focus of the developmental chemistry was to derive a direct route and address the primary objective which was to establish proof in principle for the synthetic viability and stability of this class of compound. It was hoped that this would facilitate the development of alternative efficient and versatile synthetic routes to prepare a range of iminosugars analogous of topiramate.

As *D*-fructose is structurally similar to topiramate (Figure 47) and contains the same stereogenic centres as those desired in the iminosugar analogues, it was considered an appropriate starting material, being both commercially available and cheap. However, very few syntheses of iminosugars utilising *D*-fructose as a starting material have been reported.[94] Whilst it was successfully used in the relatively facile synthesis of 1-deoxymannonojirimycin (DNJ) **65** (see page 104), this compound is less complex as it lacks the key hemiaminal functionality.[95] To date there appears to be no direct precedent for the preparation of a piperidene type iminosugar containing the fructose configuration from either the carbohydrate starting material or alternatives.[96] A review of more general literature on carbohydrate chemistry also identified that the use of *D*-fructose as a chiral building block is not as developed as other monosaccharides such as *D*-glucose. [97] In addition, *D*-fructose was regularly noted as being problematical to work with. Thus, as the key starting material, it was important initially to gain a familiarisation in working, characterising and isolating relatively simple *D*-fructose analogues that would also develop the proposed synthetic route.

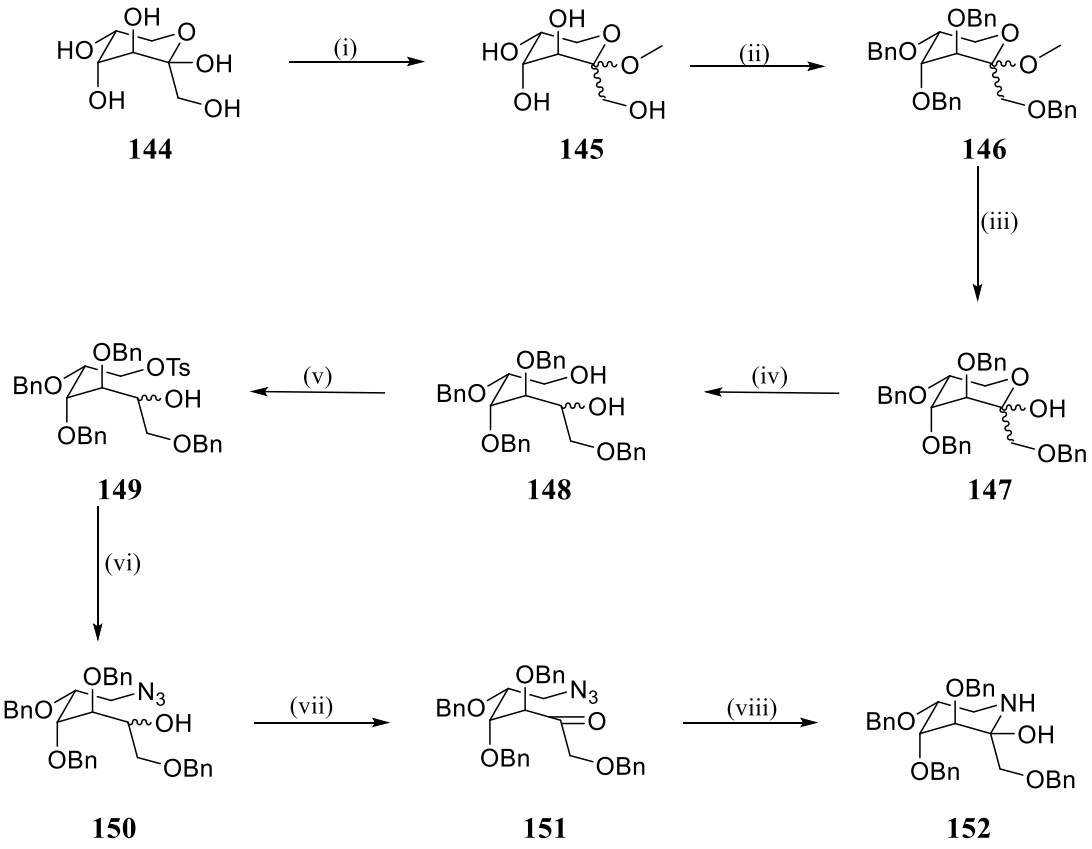


***D*-fructose** **Topiramate** **Iminosugar analogue**

Figure 47: Stereochemical relationship between *D*-fructose, topiramate and iminosugar analogues

3.3.1. Early synthetic stages

With these aims in mind, the initial synthetic plan shown in Scheme 34 was devised to attempt to prepare an *O*-benzylated iminosugar **151** as a simpler model compound to assess the stability of the key hemiaminal subunit present in the ultimate synthetic targets. It was intended to afford a relatively direct route to an iminosugar from *D*-fructose **144** and will provide confirmation of the general ease of preparation and stability of such compounds. The synthetic route incorporates several reactions that are very common in iminosugar syntheses and for which there is much literature precedent, albeit with different carbohydrate substrates. The proposed methodology also enables late stage modification of the final cyclisation steps and the ability to introduce structural variation at the nitrogen atom should the "naked" hemiaminal prove to be very labile and predominating in its open chain form. A further advantage of the proposed synthetic scheme is that it does not include a single step that would be predicted to lead to epimerisation.



Reagents and conditions: (i) NH_4Cl, MeOH, 64°C, o/n; (ii) BnBr, NaH, DMF, 0°C to RT, o/n; (iii) 1M H_2SO_4, AcOH, 50°C, 1.5h; (iv) $NaBH_4$, THF/ H_2O, RT, o/n; (v) TsCl, Et_3N, DMAP, DCM, RT, 48h; (vi) NaN_3, DMF, 50°C, 24h; (vii) Dess-Martin periodinane, DCM, 0°C to RT, 0.5h; (viii) PPh_3, THF, H_2O.

Scheme 34: Proposed synthetic route to *O*-benzyl protected iminosugar **152**

The first stage of Scheme 34 is a glycosidation reaction of *D*-fructose to obtain the methyl-*D*-pyranoside **145**. The aim of the reaction was to differentiate the anomeric hydroxyl group in order to selectively functionalise at remaining positions within the compound in subsequent stages. Glycosidation is an extremely common reaction in carbohydrate chemistry and is often achieved using the Fischer glysosidation methodology. During the Fischer glycosidation, the formation of a glycoside is mediated by the reaction of an unprotected aldose or ketose with an alcohol in the presence of an acid catalyst. The Fischer glycosidation is an equilibrium process and as such can lead to a mixture of ring size isomers, anomers and, in some cases, small amounts of acyclic forms of the carbohydrate. The relative proportion of products obtained can theoretically be controlled by manipulating the duration of the reaction. Shorter reaction times tend to lead to furanose ring forms and longer reaction times to the more

thermodynamically stable pyranose forms. The methyl glycosidation reaction of *D*-fructose can typically result in the formation of several products, the most likely of which are methyl-*α*-*D*-fructopyranoside **153**, methyl-*β*-*D*-fructopyranoside **154**, methyl-*α*-*D*-fructofuranoside **155** and methyl-*β*-*D*-fructofuranoside **156**.

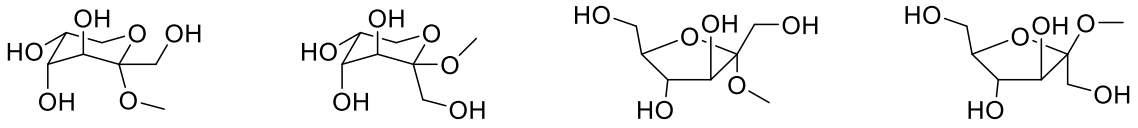
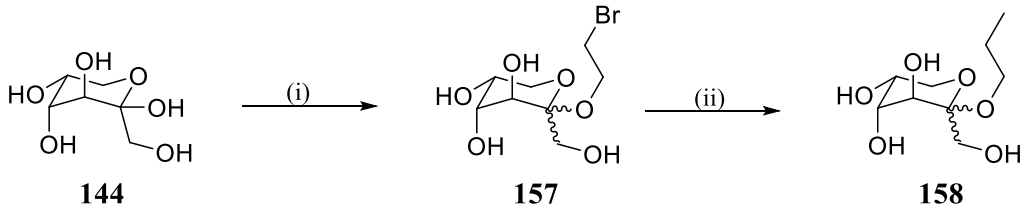


Figure 48: Structures of potential products of methyl glycosidation reaction of *D*-fructose

A standard Fischer glycosidation procedure was attempted with *D*-fructose following a literature procedure successfully employed in the formation of *L*-sorbose methyl glycosides in which hydrochloric acid is generated *in situ* from acetyl chloride in methanol.[98] The progress of the reaction was found to be extremely slow under the ambient conditions used to prepare the methyl glycosides of *L*-sorbose and vigorous heating was required to induce the reaction. The work up of the reaction also proved challenging with the removal of inorganic salts from the quenched reaction mixture proving surprisingly difficult. A further review of the literature indicated that a Fischer glycosidation of *D*-fructose was commonly found to produce unsatisfactory results when attempting to prepare methyl fructosides. Two alternative glycosidation procedures were considered. Ramstadius *et al* [99] reported a method of preparing ethyl glycosides of *D*-fructose with a Fischer type glycosidation using bromoethanol (Scheme 35). An advantage of the reaction was that the crystalline bromide derivative of the α- and β-ethyl pyranosides precipitated from the reaction mixture making isolation straightforward. However, the bromide intermediates must be reduced prior to any subsequent chemistry which adds a further stage of chemistry to the overall synthetic scheme which is less favourable.



Reagents and conditions: (i) 2-bromoethanol; (ii) Pd/C, H_2, $NaHCO_3$, H_2O.

Scheme 35: Preparation of ethyl fructoglycoside with bromoethanol[99]

Ultimately, the glycosidation reaction to obtain **145** was carried out in refluxing methanol in the presence of ammonium chloride and was adapted from a literature procedure.[100] Reaction progression was found to be relatively swift and was monitored by TLC. The formation of two distinct compounds was visible by TLC and reaction completion was indicated by TLC following 16 hours at reflux temperature. Although at least four products of the reaction were anticipated only two spots were visible by TLC. However, TLC investigations did not identify a solvent system that would successfully elucidate the two spots further suggesting that two or more of the products from the reaction co-eluted. The separation of the products from the reaction proved to be challenging. An extractive separation of the products of the reaction using boiling acetone was initially attempted as this had proven successful for the isolation of the α- and β methyl glycosides of *L*-sorbose.[98] However, despite exhaustive attempts, it was not a viable methodology to separate the products of the methyl glycosidation reaction of *D*-fructose. Separation was attempted by column chromatography on silica. Further TLC investigations identified a suitable three component solvent system that adequately separated the two spots visible by TLC. Using 25 equivalents of silica was found to be satisfactory. It was necessary to adsorb the crude reaction mixture onto silica and dry load it onto the column due to the relative insolubility of the crude products in the chosen eluent. Chromatography proved challenging and resulted in only partial separation of the crude reaction mixture. The more hydrophobic of the two compounds that appeared as the top spot by TLC was readily separable although it was necessary to recolumn the material obtained from several mixed fractions to maximise yields. Analysis of the product by ^{1}H NMR confirmed that it was a single compound, not a mixture, and that it had been isolated cleanly. The product was obtained as a yellow oil in a 38% yield. The correct mass of 195 (MH^+) for an isomer of methyl fructoside was confirmed by mass spectrometry. Comparison of ^{1}H and ^{13}C NMR spectra of this compound with the limited published data available suggested that it was one of the

undesired five membered methyl fructofuranosides.[98, 100, 101] Further support that this compound was a fructofuranoside was provided by analysis of the splitting patterns of the four hydroxyl groups in the ^{1}H NMR spectrum in DMSO-d_6 which is shown in Figure 49. The hydroxyl groups were initially identified by D_2O shake. Two hydroxyl groups were clear doublets at *ca*. 5ppm and the other two hydroxyls were represented as observed triplets. Examination of the structure of fructofuranosides **155** and **156** shows that both molecules contain two primary alcohol groups and two secondary alcohol groups which would conform to the observed splitting patterns in the ^{1}H NMR spectrum. In contrast, the two fructopyranosides **153** and **154** each contain only one primary hydroxyl group and would thus result in a different ^{1}H NMR splitting pattern. The lower spot on the TLC was also isolated and was shown to contain a mixture of products by NMR. It was anticipated that three compounds were present, the anomer of the methyl fructofuranoside successfully isolated and two methyl fructopyranosides.

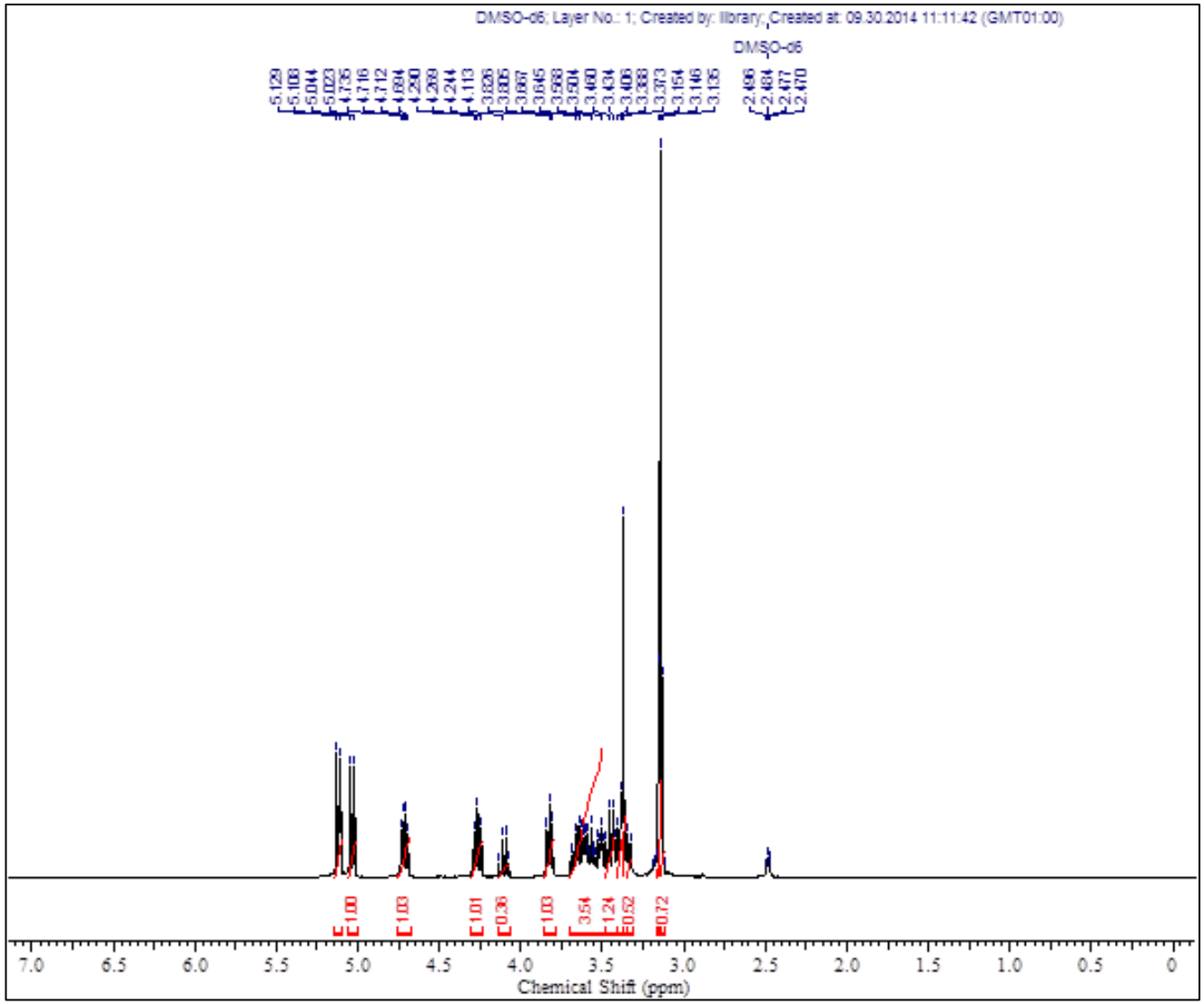
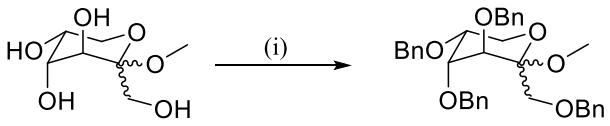


Figure 49: ^{1}H NMR spectrum of the product isolated from the methyl glycosidation reaction of *D*-fructose

The next step in the reaction sequence to prepare **146** was fast tracked with the mixture of products obtained from the successful first stage reaction to trial whether further separation of the anomers would be possible at this stage. The data collected from the *O*-benzyl protection reaction was positive but a greater amount of material was required. Therefore, the glycosidation reaction was scaled up significantly to allow development and characterisation and to yield enough material to fully investigate the downstream chemistry. The reaction was successfully scaled up to process one kilogram of *D*-fructose. Column chromatography was made feasible on this larger scale by investigating the effect of reducing the amount of silica used for chromatography. It was found that reducing the equivalents of silica used to 10 times that of the mass of the crude products resulted in the successful separation of the faster running methyl furanoside in a yield comparable to that obtained on the small scale.

A number of small scale reaction trials were carried out to determine whether the ratio of products could be influenced by increasing the reaction duration. It was hoped that leaving the reaction for longer than 16 hours would favour the formation of the more thermodynamically stable and desired fructopyranosides. Reactions were allowed to proceed for 24, 36, 48 and 72 hours and were monitored by TLC. There was no discernible differences in the reaction profiles by TLC. Each reaction was worked up and the crude mixture of compounds subjected to column chromatography. In each case, a methyl fructofuranoside was cleanly isolated in a similar yield to that obtained in the initial 16 hour reaction. It was not possible to resolve the other three isomers readily so it was difficult to assess whether there was any improvement to the ratio of fructopyranosides present in the mixture.

On the basis that the three other isomers could not be separated, the subsequent stage of chemistry, an *O*-benzylation reaction, was initially trialled on the crude mixture of products to determine the outcomes and assess if further efforts in purifying the crude materials was required at this stage or more successful on the *O*-benzyl protected materials.



145 **146**

Reagents and conditions: (i) BnBr, NaH, DMF, 0^0C to RT, o/n.

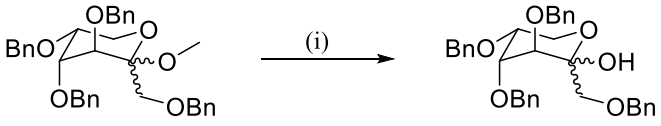
Scheme 36: *O*-Benzylation of methyl pyranosides

O-benzyl protection of the remaining four hydroxyl groups in each of the compounds present within the mixture of anomeric pyranosides and remaining furanoside was readily achieved by adding sodium hydride to a solution of benzyl bromide and the three substrates in DMF. The transformation is widely used in carbohydrate chemistry and the methodology adopted is reported numerously. A modification to the order of addition of reagents was made when carrying out the reaction on a larger scale to prevent significant exotherming and foaming of the reaction. It was found that controlling the rate of addition of benzyl bromide to a cooled solution of the substrates and sodium hydride in DMF gave a more controllable reaction profile on a larger scale. Benzyl ethers were chosen as a suitable protecting group following consideration of the planned stages of chemistry shown in Scheme 34. Benzyl ethers are relatively stable to a range of reaction conditions and show good orthogonality to several other typical hydroxyl protecting groups. Their inclusion here was useful; they were compatible with the proposed chemistry and there was precedent of their use in related synthetic schemes. [71] [69]

As anticipated, three products were obtained from the reaction. The major product, which was obtained in a 60% yield, was easily separated by column chromatography. Data from mass spectrometry confirmed that it was an isomer of the desired product with the compound giving the anticipated mass. The isomeric identity of the material was determined in various ways. An initial review of the ^{1}H NMR spectrum of the material contained certain distinct signals which were indicative of a methyl fructofuranoside. Within the ^{1}H NMR spectrum two distinctive sets of diastereotopic protons were visible which supported the view that the second furanose anomer had been isolated. To support the identification further a readily identifiable marker within the ^{1}H NMR spectrum was required. The splitting patterns of the hydroxyl groups present within the methyl fructofuranoside isolated in the stage 1 reaction proved very useful to the identification process. However, in this compound, all of the hydroxyl groups

were protected with benzyl ethers. It was considered pertinent to remove the benzyl ethers to expose the hydroxyl groups and undertake a further NMR assessment. A catalytic atmospheric hydrogenation was thus carried out to afford the unprotected methyl fructoglycoside. Analysis of the resulting product by ^{1}H NMR in DMSO-d_6 revealed four hydroxyl groups, two of which were doublets and two of which were represented by an observed doublet of doublets. This would be indicative of the compound containing two primary and two secondary alcohol groups which fitted for the anticipated structure of the fructofuranoside. This provided further support of the compound's identity. One additional investigation was undertaken in which a small amount of the discarded methyl fructofuranoside from the stage 1 reaction was *O*-benzylated employing the same reaction conditions. The NMR of the resulting product contained the same distinctive proton shifts considered indicative of a furan based compound. It was now felt appropriate to confirm the identity of the isolated compound as the second undesired methyl fructofuranoside.

The other two compounds were therefore assumed to be the desired *O*-benzyl protected α and β fructopyranosides. The two desired fructopyranoside products were obtained in a combined yield of 24%. As the separation of the two anomers was very challenging and ultimately not necessary to the subsequent chemistry, no additional efforts were made in this regard and the mixture of products was retained and processed through to the next stage within the synthetic scheme.

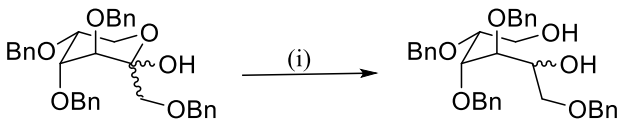


146 **147**

Reagents and conditions: (i) 1M H_2SO_4, AcOH, 50^0C, 1.5h.

Scheme 37: Acid mediated hydrolysis

The acid mediated hydrolysis reaction to obtain **147** was relatively straightforward. The two methyl pyranosides were dissolved in acetic acid and 1M aqueous sulfuric acid was added. The reaction was heated to 50°C and swift reaction completion was indicated by HPLC and TLC in 1.5 hours. LCMS and ^{1}H NMR analysis confirmed the presence of two compounds in an approximate 13:1 ratio with a combined yield of 83%. LCMS confirmed the desired products had been obtained with both peaks in the chromatogram showing the correct mass in the corresponding mass spectrum. Following neutralisation and reaction work up the crude material was purified by a silica pad to remove residual impurities. Attempts to separate the two anomers proved unsuccessful here due to the very challenging nature of the separation. However, as with previous steps, it was deemed unnecessary to fully separate the two anomers at this stage and the mixture of products was carried forward to the next stage with the aim of fast tracking to the key acyclic compounds **148**.

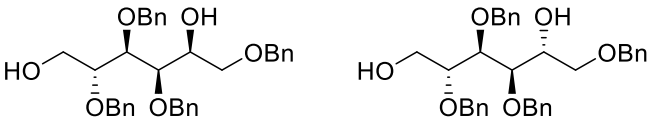


147 **148**

Reagents and conditions: (i) $NaBH_4$, THF/ H_2O, RT, o/n.

Scheme 38: Ring opening reduction

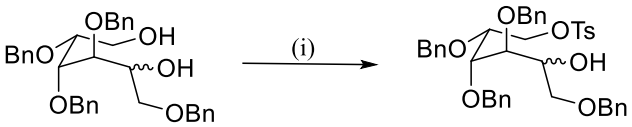
A standard methodology to achieve the reduction of carbohydrates into the corresponding alditols is to reduce the aldehyde or ketone present within the open chain form of the sugar with a hydride reagent. Although the open chain form of the ketone is usually present in very low concentration, reactions involving the ketone take place very rapidly. Sodium borohydride is often used to effect the reduction as would be the case for any other ketone or aldehyde. The ring opening reduction of **147** was achieved with sodium borohydride in THF and water and the reaction was observed to proceed rapidly at ambient temperature. An epimeric mixture of products was expected and the reaction resulted in the formation of the two corresponding alditols, 1,3,4,5-tetra-*O*-benzyl-*D*-glucitol **157** and 1,3,4,5-tetra-*O*-benzyl-*D*-mannitol **158**. The two epimers were produced in a roughly 5:1 ratio by ^{1}H NMR.



159 **160**

Figure 50: Structures of alditol products from ring opening reduction reaction

The ^{1}H NMR of the mixture of products in deuterated chloroform clearly shows the presence of two hydroxyl groups with different coupling patterns confirming that there was a primary and secondary hydroxyl group was present in both of the reduced sugars. This was the expected outcome as examination of the structures of **159** and **160** confirms. The two products were again not easily separable by chromatography but it was again not deemed necessary to separate the products here.



148 **149**

Reagents and conditions: (i) TsCl, Et_3N, DMAP, DCM, RT, 24h.

Scheme 39: Activation of primary alcohol group with *p*-toluenesulfonyl chloride

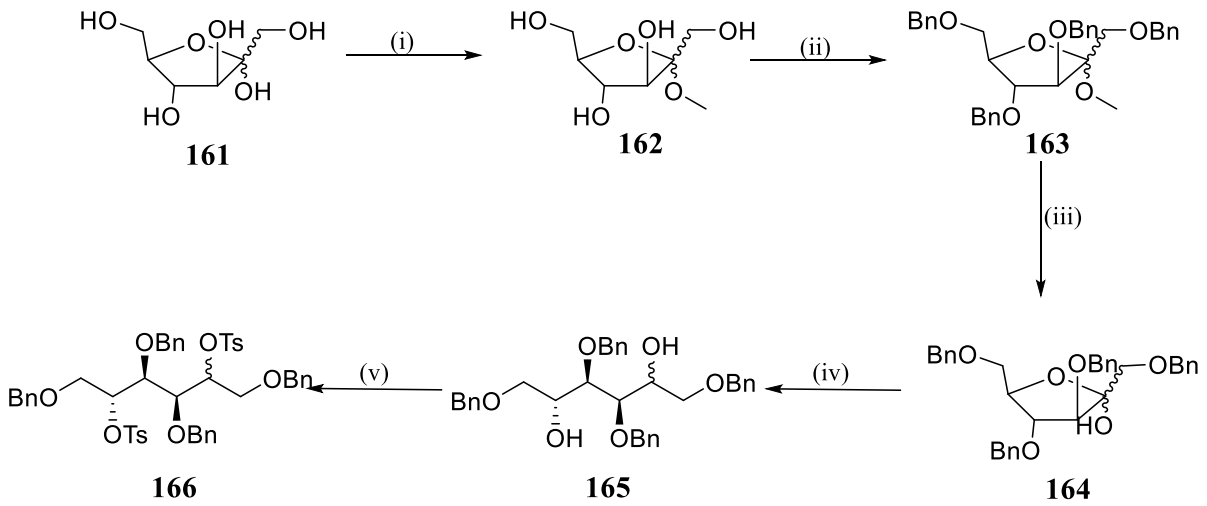
The next stage in the preparative route was to activate the primary alcohol to a good leaving group in anticipation of the subsequent nucleophilic displacement step. This was successfully achieved using *p*-toluenesulfonyl chloride to give tosylate **149** in accordance with Scheme 39. By controlling the reaction conditions with respect to temperature and duration, selective tosylation of the primary alcohol was possible. The reaction was conducted at room temperature over a 24 hour period in the presence of a 10% mole loading of catalytic DMAP. The successful preparation of the mono tosylated product was confirmed by ^{1}H NMR and LCMS. As anticipated, two epimeric products were obtained. Both products were visible by ^{1}H NMR and LCMS in roughly a 5:1 ratio. In the ^{1}H NMR spectrum, only one hydroxyl group was present in each compound along with corresponding aromatic signals that indicated the

presence of only one tosyl group. The achievement of the correct chemoselectivity of the tosyl group was confirmed by the splitting pattern of the single hydroxyl group signal which was a doublet suggesting coupling to only one other proton, as would be the case for the remaining secondary alcohol. There was also a marked chemical shift of the diastereotopic protons adjacent to the tosylate group with no evidence of OH coupling. A yield of 85% was achieved for the epimeric mixture. A small amount of a bis-tosylated alditol was obtained as a by-product of the reaction. Isolation and purification of this product, although undesired, enabled NMR and LCMS data to be obtained. The ^{1}H NMR spectrum of the material contained no signals that related to hydroxyl groups and the correct number of aromatic protons for the bis-tosylated product were observed. LCMS showed the correct mass for the bis-tosylated product. The positive identification of the bis-tosylated product was useful in that it provided further support to the identity of the input and output materials of the reaction. The separation of the two epimers of **149** was not required and the epimeric mixture was progressed to the next synthetic step.

The formation of the corresponding mesylate was also attempted using similar reaction conditions as those employed for the tosylation reaction. It proved more difficult to successfully achieve the desired mono mesylation at the primary alcohol position only when using methane sulfonyl chloride. Thus, tosylate **149** was selected as the preferred compound for onward processing.

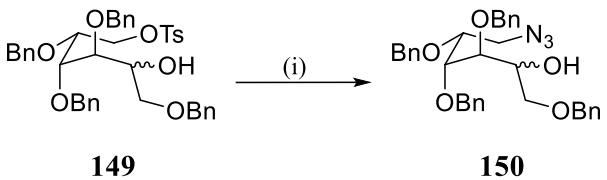
To further confirm that the correct pyranose isomers of fructose had been isolated, additional chemistry was performed on the two furanose isomers isolated during the earlier stages of the synthetic scheme in accordance with Scheme 40. At each stage TLC and ^{1}H NMR data for the products was compared to that of the products obtained for the same reactions with the pyranose isomers. In all cases, differences in the data confirmed that the products of the reactions were not equivalent. The key results were generated from the ring opening reduction and the subsequent tosylation reactions. On ring opening, the furanose and pyranose isomers form differently constituted alditols. For the pyranoses, the two epimeric alditols contain one secondary alcohol attached to the carbon at the C2 position and one primary alcohol at the C6 position as in alditols **159** and **160**. For the furanoses, the resulting alditols contain two secondary alcohols in the C2 and C5 positions as in alditol **165**. The differences in the two alcohol groups in alditols **159** and **160** enabled this chemoselectivity to be exploited in the subsequent tosylation step. It was shown that under certain conditions, the primary hydroxyl

group would selectively react with *p*-toluene sulfonyl chloride whilst the secondary hydroxyl group did not undergo any reaction. Employing similar conditions for the tosylation reaction of alditol **165** resulted in bis-tosylation which could not be avoided. This does suggest that both of the hydroxyl groups where chemically similar and selectivity was therefore not possible and alditol **166** was obtained.



Reagents and conditions: (i) NH_4Cl, MeOH, 64^0C, o/n; (ii) BnBr, NaH, DMF, 0^0C to RT, o/n; (iii) 1M H_2SO_4, AcOH, 50^0, 1.5h; (iv) $NaBH_4$, THF/ H_2O, RT, o/n; (v) TsCl, Et_3N, DMAP, DCM, RT, 48h.

Scheme 40: Earlier stages of synthetic scheme with furanose isomers



Reagents and conditions: (i) NaN_3, DMF, 50^0C, 21h.

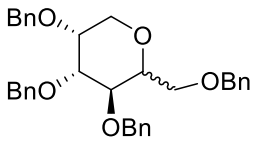
Scheme 41: Nucleophilic displacement of tosyl group with an azide group

The successful formation of tosylate **149** generated the opportunity for the introduction of a precursor to an amino group into the carbohydrate by converting the activated hydroxyl group via a nucleophilic displacement reaction. This was considered a critical stage in the overall synthetic scheme. Several nitrogen containing nucleophiles were considered and the azide group was selected based upon the propensity of its use reported in reviewed literature. Initially the reaction to displace the tosyl group with an azide group to prepare **150** produced disappointing results. The use of one equivalent of sodium azide at room temperature in DMF as solvent resulted in very slow reaction progression. To ascertain whether altering key reaction parameters would improve reaction outcomes several small scale trial reactions were run in parallel. The effect of the number of equivalents of sodium azide and temperature were initially considered. The results, as shown in Table 7, indicated that both temperature and the stoichiometric amount of sodium azide present in the reaction affected both the time taken for the reaction to go to completion and the product yield. As expected, the rate of reaction increased with increased temperature. However, the reactions carried out at 100° C showed lower yields than the reactions conducted at lower temperatures. This suggested that higher temperatures resulted in the degradation of products and/or starting materials which limited the yield. The amount of sodium azide used was found to have little effect at 100° C but a greater stoichiometric amount at 50° C gave a shorter reaction time. The most favourable conditions with respect to reaction duration and product yield were considered to be the addition of two stoichiometric equivalents of sodium azide at 50° C. The reaction was scaled up using these conditions generating positive results.

Number of equivalents of NaN_3	Solvent	Reaction Temperature	Time taken for full consumption of starting material	Product Yield
1	DMF	20° C	Starting material remaining after 96 hours	-
1.5	DMF	50° C	32 hours	53%
2	DMF	50° C	21 hours	55%
1.5	DMF	100° C	16 hours	43%
2	DMF	100° C	16 hours	41%

Table 7: Results of trial nucleophilic displacement reactions

It was anticipated that the products of the reaction would be two epimeric alcohols. However, in-reaction monitoring by HPLC indicated the presence of three products of the reaction. Following reaction work up, it was possible to cleanly isolate one of the products by column chromatography which provided sufficient material to undertake analysis. ^{1}H NMR and LCMS confirmed that the compound was not one of the desired alditols which suggested that it was an unwanted by-product of the reaction. It was postulated that the contaminant arises from the intramolecular displacement of the tosylate or azido by the alcohol forming a cyclic tetrahydropyran-like product. A proposed structure is shown in Figure 51. This structure would correspond with the mass spectrometry data obtained for the identified The ^{1}H NMR spectrum of the by-product has no visible hydroxyl group which does provide a further indication that it may be a cyclic species.



167

Figure 51: Proposed structure of the by-product of the nucleophilic displacement reaction

The two other products of the reaction were not adequately separable by column chromatography and were isolated as a mixture in a 12:1 ratio. A 58% yield overall was achieved. Analysis of these two compounds by ^{1}H NMR and LCMS provided data to support that they were the two desired epimeric azido alcohols. Only one hydroxyl group per compound was visible in the ^{1}H NMR spectrum and no aromatic signals were present indicating the removal of the tosyl group. LCMS confirmed the correct mass for each product. As the azide group has a characteristic and strong absorption by FTIR, the material was also analysed by FTIR. The spectrum is shown in Figure 53 and the characteristic peak at a frequency of 2100cm^{-1} is indicative of a typical azide stretching absorption. A mixture of the two products was progressed to the next stage of chemistry.

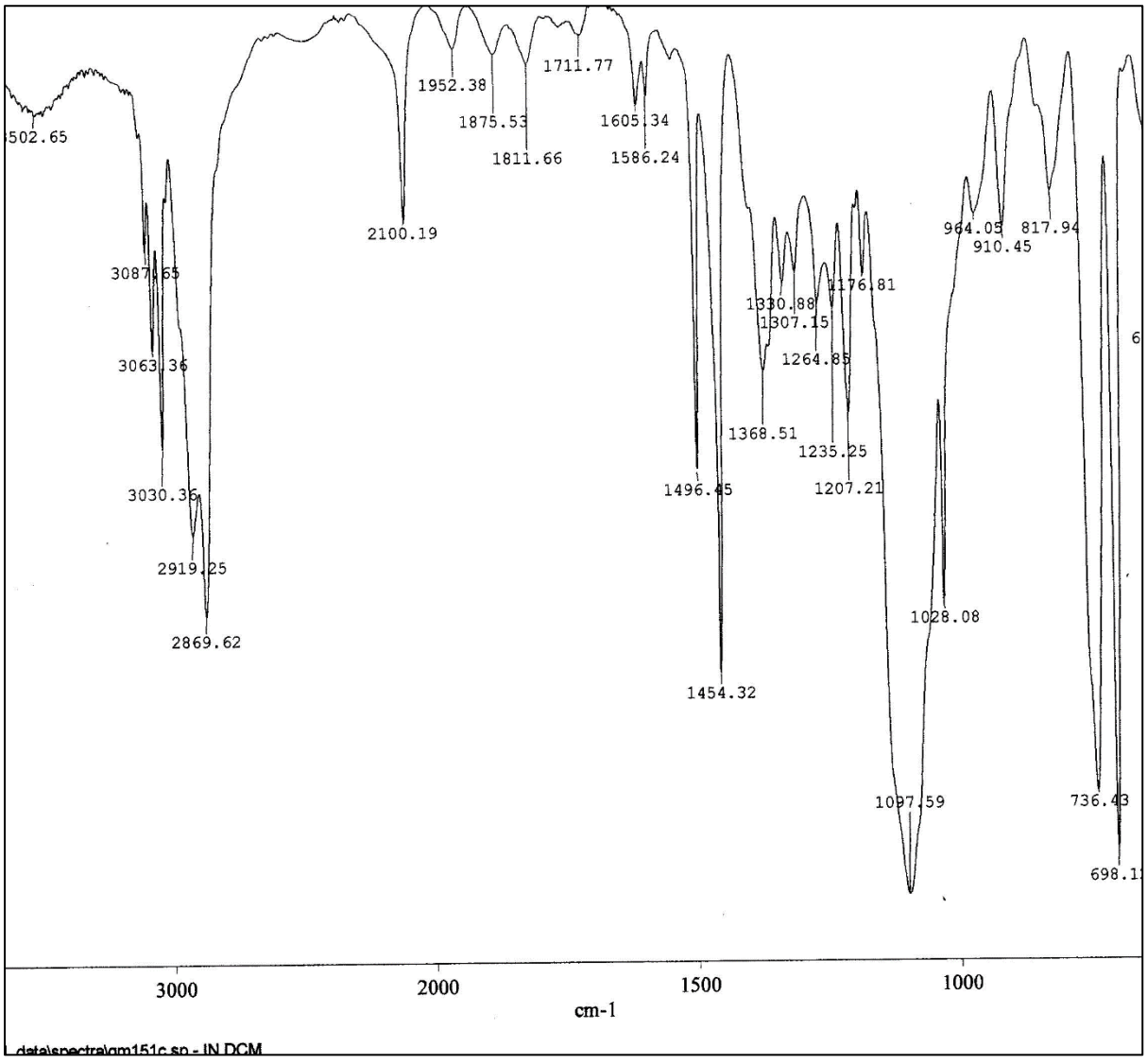
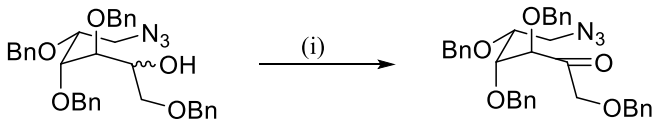


Figure 52: FTIR spectrum of azido alcohol **150**



150 **151**

Reagents and conditions: (i) Dess-Martin periodinane, DCM, 0^0C to RT, 0.5h.

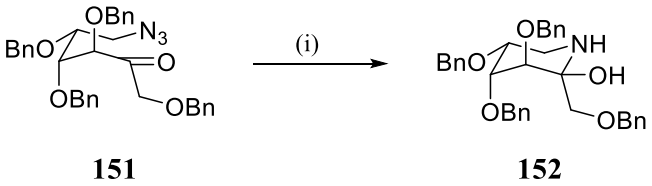
Scheme 42: Oxidation reaction of azido alcohol **150**

Oxidation of azido alcohol **150** to the azido ketone **151** was readily achieved using Dess-Martin periodinane in dichloromethane. Dess-Martin reagent was selected as an oxidant as there was literature precedent for its successful use in oxidising primary alcohols.[76] The oxidation reaction with Dess-Martin periodinane proved successful. Reaction completion was incredibly swift with full consumption of the starting material observed after 30 minutes at room temperature. Only one product was visible by TLC as was expected as any stereochemistry at the C2 position would be lost post oxidation of the hydroxyl group. The single compound was readily purified by column chromatography. The compound was positively identified using a range of methods in addition to ^{1}H NMR. LCMS contained a single peak in the chromatogram that gave the correct mass in the corresponding mass spectrum. Analysis of the product by ^{13}C NMR clearly indicated the presence of a carbon atom constituent of a carbonyl group at 208ppm. The FTIR spectrum of azido ketone **151**, contained a distinctive carbonyl bond stretching absorption at a frequency of 1730cm^{-1} along with the retention of the peak at 2010cm^{-1} that confirmed retention of the azido group. Azido ketone **151** was obtained in a 65% yield.

As the oxidation reaction with Dess-Martin periodinane had had such successful outcomes, it was deemed unnecessary to further develop this reaction using alternative oxidants at this stage.

As an interesting aside, a small scale oxidation reaction of by-product **167** from the nucleophilic displacement reaction with sodium azide was trialled. The same reaction conditions were applied to those described above. The compound did not undergo any reaction at all which reinforced the view that there was no hydroxyl group present in the compound further supporting that the compound was a cyclic species.

3.3.2. Key cyclisation step



151 **152**

Reagents and conditions: (i) PPh_3, THF, H_2O.

Scheme 43: Reduction of the azide group

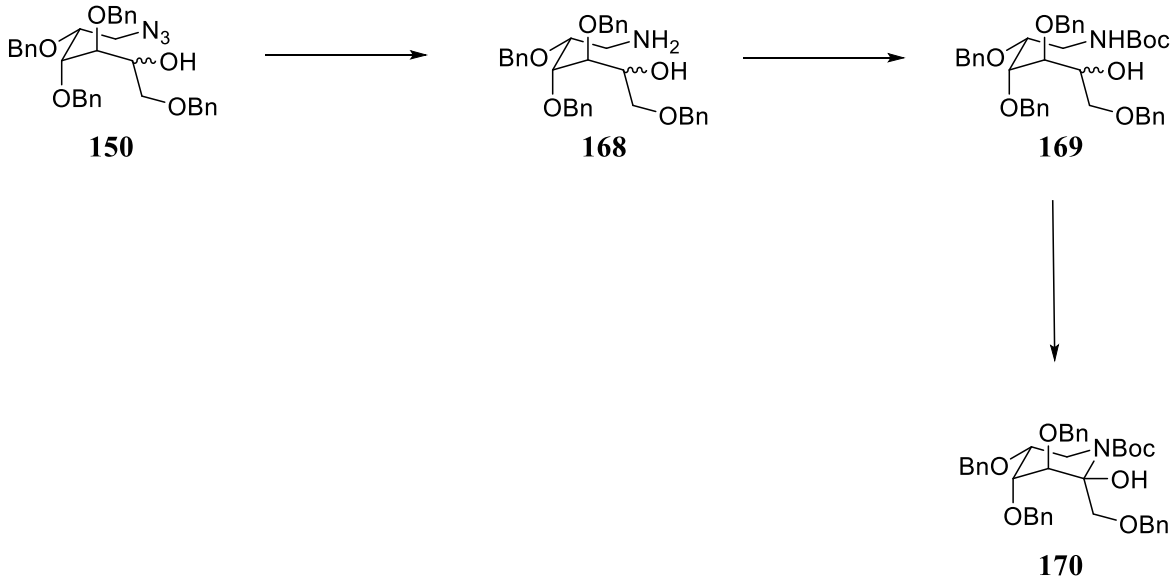
The reduction of the azide group to the corresponding primary amine group is a key step in the synthesis of iminosugars. There is much literature precedent to suggest that the reduction of the azide group, when in the presence of an electrophilic carbonyl group in a suitable position, will result in concomitant reductive amino cyclisation.[102] [103] A variety of methods of reducing the azide group have been reported including hydrogenation and the Staudinger reaction.[104, 105] A Staudinger reaction with triphenylphosphine was attempted to see if the efficient cyclisation of the azido ketone **151** could be induced, leading to the desired cyclic iminosugar **152**. Despite adjusting reaction conditions and attempting the reaction using polystyrene-supported triphenylphosphine, the outcome was either that reaction progression was minimal, in which case unreacted starting material was recovered, or where the reaction did proceed, degradation appeared to occur and no discrete product could be isolated from the reaction. It was assumed that although the azide was being successfully reduced, the resulting primary amine and subsequent intramolecular reaction with the ketone led to a product that was unstable and which undergoes decomposition readily.

The results of this initial Staudinger reaction suggested that the key cyclisation step to give iminosugar **152** may not be as facile as had been hoped. Several different approaches to attempt to successfully induce cyclisation formed the next critical path in the chemistry development work.

- **Stabilising the amine**

The results of the previous Staudinger reaction suggested that improving the stability of both the primary amine and the desired cyclic product required consideration. A reasonable option was thought to be the immediate protection of the primary amine with a BOC group. It was hoped that the BOC group might increase amine stability, facilitate a more controlled intramolecular cyclisation, reduce product degradation and allow for the improved purification of the product. Literature examples demonstrated that the presence of a BOC group does not prevent the reductive amino cyclisation [105,70] and there were literature procedures for one-pot Staudinger reduction and subsequent BOC protection reactions. [106, 107] One-pot reactions were attempted with azido ketone **151** in which the azide was reduced using Staudinger conditions in the presence of *di-tert*-butyl dicarbonate. It was hoped that as the resulting amine formed, it would be "captured" as the BOC-amine conferring additional stability. Disappointingly, despite trialing a series of alternative reaction conditions and introducing BOC-ON ([2-(tert-butoxycarbonyloxymino)-2-phenylacetonitrile]), a successful outcome with isolation of the desired product was not achieved.

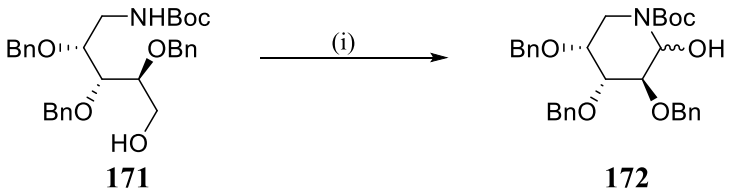
The lack of success in completing the final stage of the planned synthetic route shown in Scheme 34 necessitated the development of an alternative synthetic strategy. Scheme 44 was proposed. In this case, azide reduction and the subsequent BOC protection of the resulting amine occur prior to the oxidation step to generate the corresponding electrophilic site in the molecule at the C2 position.



Reagents and conditions: (i) $LiAlH_4$, THF, 0^0C to RT, 1.5h; (ii) Boc_2O, Et_3N, THF, 0^0-RT, 0.5h; (iii) Dess-Martin periodinane, DCM, 45^0C, o/n.

Scheme 44: Alternative latter stages of synthetic route to *O*-benzyl protected iminosugar

A similar sequence of reactions to that shown in Scheme 44 has been reported although it was employed on a *D*-arabinose derived BOC protected amino alcohol **171** (Scheme 45).[108] Promisingly, the final oxidation step was reported as having resulted in spontaneous oxidative cyclisation to the desired iminosugar **172**.



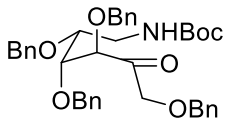
171 172

Reagents and conditions: (i) Oxalyl chloride, DMSO, Et_3N, DCM.

Scheme 45: Final oxidation step in the preparation of a *D*-arabinose iminosugar precursor

The reduction of the azide group using lithium aluminium hydride was successful in reducing the azide group of **150** to furnish the primary amine giving amino alcohol **168**. A 70% yield was achieved and satisfactory analysis of the product was obtained to confirm the isolation of the correct product. The subsequent BOC protection of the primary amine was also a facile and swift reaction. ^{1}H NMR, ^{13}C NMR and LCMS were used to positively identify the BOC-

protected amino alcohol **169**. The subsequent oxidation of the alcohol group with Dess-Martin periodinane required an elevated reaction temperature and longer reaction duration than used in the analogous reaction shown in Scheme 42. The reaction was carried out at 45° C rather than at room temperature and needed approximately 16 hours to achieve completion. This may be as a consequence of the BOC group introducing additional steric hindrance into the molecule making the secondary alcohol group less accessible to the oxidant. It was hoped that the oxidation reaction would result in the formation of the cyclic iminosugar **170** as indicated for the analogue in the literature. [108] Initial analysis of the product of the oxidation reaction by LCMS confirmed the correct mass for the desired product. However, ^{1}H NMR data proved more ambiguous. It was unclear from the data collected whether the desired cyclic compound **170** had been successfully prepared or if the compound had remained in its acyclic form **169**.



173

Figure 53: Possible acyclic product of oxidation reaction of **169**

Additional analysis of the iminosugar suggested that it was the acyclic form **173**. The FTIR spectrum of the product showed two absorption bands within the carbonyl region; the first peak at 1708cm^{-1} corresponded to that present in the FTIR spectrum of BOC protected amino alcohol **169** indicative of the carbonyl bond within the BOC protecting group. The second absorption peak was at a frequency of 1735cm^{-1} and suggested that a second carbonyl bond was present within the compound. This would confirm the ketone functionality in **173**. Also, the FTIR spectrum showed a peak at 3436cm^{-1} which is within the normal frequency range for an N-H bond which would also be present within **173** but not in cyclic **170**. In order to provide further structural evidence, a range of NMR experiments were carried out. ^{1}H NMR spectra were obtained in different deuterated solvents and assignment of protons was aided by 2D COSY experiments. The presence of an "amino" proton represented as a triplet in the spectra was noted. Its presence would concur with the acyclic compound **173**. In addition, three protons attached to the core sugar framework appeared further downfield in the spectrum than the chemical shifts of the corresponding protons in the starting material. This could be explained

by the presence of a ketone at the C2 position in the iminosugar chain. ^{13}C NMR spectra of the material confirmed the correct number of carbon atoms, the assignment of which was aided by a DEPT135 experiment. Peaks at 156ppm and 209ppm suggest the presence of two carbonyl carbon atoms within the molecule. The peak at 156ppm corresponds with the expected resonance of the relevant carbon atom in the BOC group. All of the analytical data collected for the product of the oxidation reaction pointed to the compound being acyclic **173** rather than cyclic **170**.

- **Attempting to induce cyclisation**

As the acyclic product **173** was suspected, efforts were focused on how to induce intramolecular cyclisation of **173** to reform the ring structure and the following investigations were undertaken:

- The oxidation reaction of amino alcohol **169** was repeated at both very high and very low concentrations to establish if reaction volume was critical to cyclisation. There were no observable differences in reaction progression or products at either extreme of concentration.
- Various NMR scale experiments were undertaken in a variety of deuterated solvents to establish if solvent effects altered the cyclisation equilibrium. Each sample was subjected to variable temperatures and the resulting spectra compared to the data initially obtained. No changes were observed in any of the solvents used.
- Each sample had 5% v/v acetic acid added to assess if pH was a factor in inducing cyclisation. ^{1}H NMR data was obtained for each sample immediately following addition of acetic acid and after 12, 24 and 48 hours of standing at room temperature. The samples were then subjected to gentle heating. Again, no changes to NMR spectra were observed.
- The compound was treated with a Lewis acid (zinc chloride/aluminium chloride) in order to attempt to activate the ketone and induce cyclisation. This also proved unsuccessful.

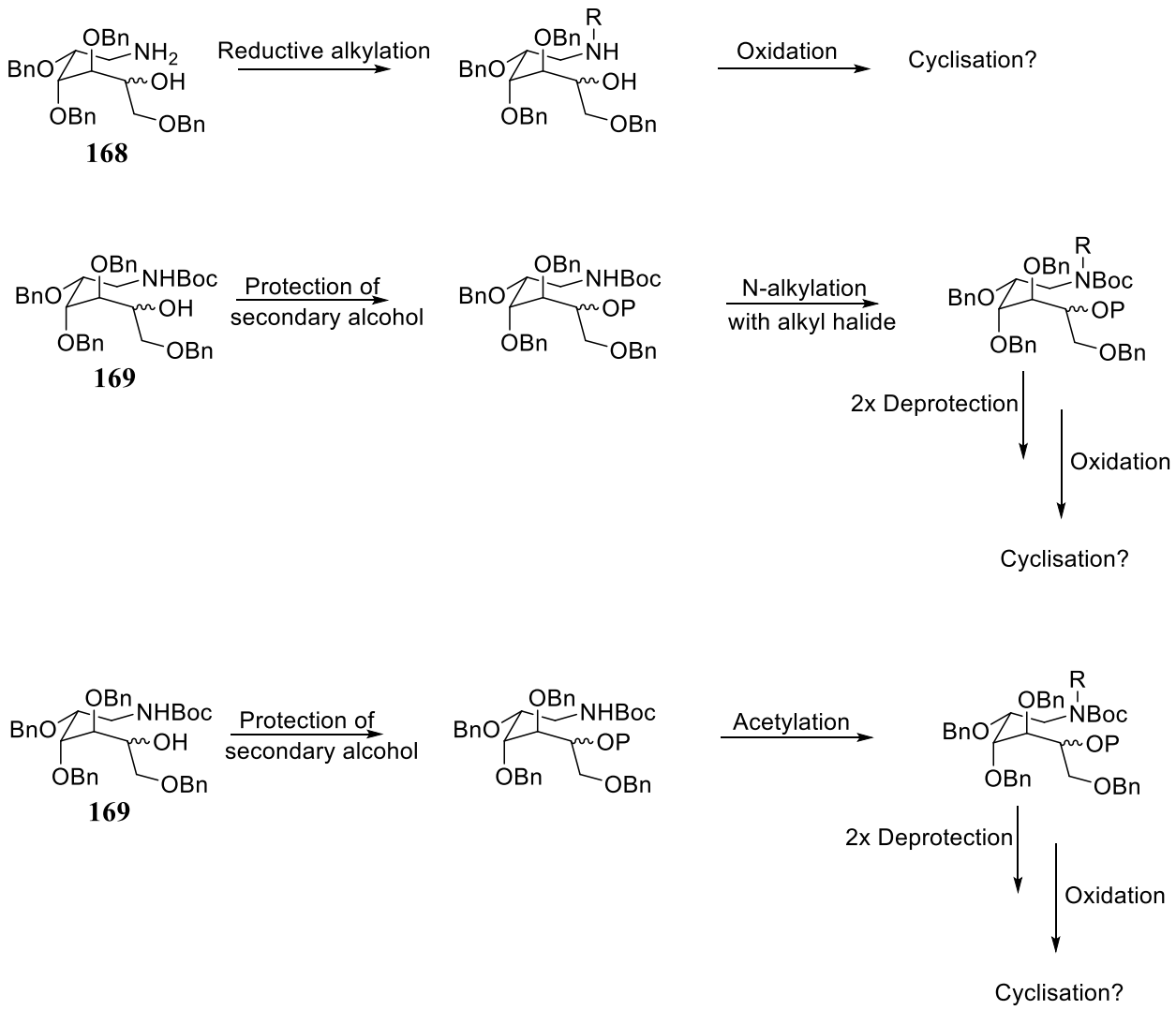
The removal of the BOC protecting group with aqueous hydrochloric acid to afford the primary amine occurred readily in the presence of the ketone. However, isolation of a stable product either as the free amine or as a hydrochloride salt was not achieved. This was not unexpected

as a stable product could not be isolated from the Staudinger reduction of amino ketone **151** which was an attempt to achieve the same outcome but with a different method of activating of the amino group.

Reasons why the iminosugar **173** may prefer an acyclic configuration rather than reforming the six-membered ring and generating a cyclic form were considered in order to establish what modifications could be introduced into the molecule to promote cyclisation. This generated three key ideas, (i) functionalising the amine to alter its nucleophilicity, (ii) removing and/or changing the bulky *O*-benzyl protecting groups, and (iii) modification of the electrophilicity of the ketone that results from the oxidation of the secondary alcohol group by introducing alternative functionality at the C2 position.

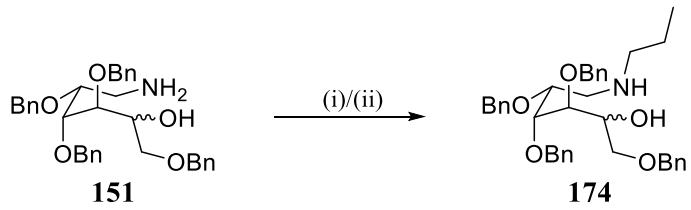
- **Altering the nucleophilicity of the amine**

By modulating the nucleophilicity of the amine, it was hypothesised that the likelihood of forming a cyclic iminosugar could be increased. It has been demonstrated that a primary amine attached to the carbon atom at the C6 position does not lead to a stable product whereas a BOC protected amine appears to reduce the nucleophilicity of the amine sufficiently to prevent cyclisation. The objective was thus to add different electron-withdrawing and electron-donating groups with varying steric demand to the nitrogen atom. The effect of these groups on the outcome of the subsequent oxidation reaction would then be assessed. It was hoped that the results of these model reactions would provide valuable information to support ongoing synthetic chemistry towards the ultimate target compounds **25** - **29**. Starting with amino alcohols **168** and **169**, three main routes to achieving functionalisation of the amine were proposed as shown in Scheme 46.



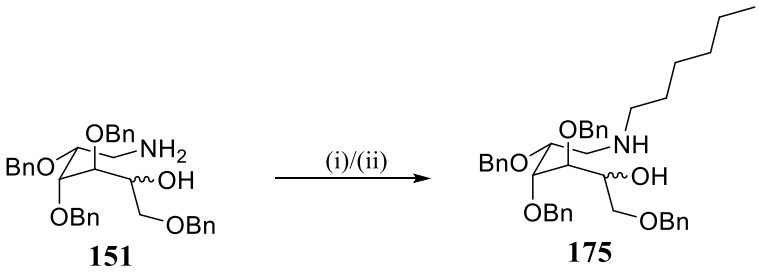
Scheme 46: Proposed generic routes to *N*-functionalisation of amino alcohols **168** and **169**

N-alkylation by reductive alkylation and direct *N*-alkylation with an alkyl halide were considered reasonable methods of introducing an electron-donating group to the amine of **168**. Several small scale reductive alkylation reactions were attempted using a variety of aldehydes, reducing agents (sodium triacetoxyborohydride/sodium cyanoborohydride/sodium borohydride), reaction conditions (pH/temperature/use of dessicants) and addition protocols (all-in reaction/prior imine formation) in order to derive a set of optimised reaction parameters. Supporting ^{1}H NMR experiments were carried out to improve reaction monitoring and to try and understand the kinetics of each reaction in respect of imine and product formation. Small scale reactions showed that compounds **174** and **175**, Schemes 47 and 48, could be successfully synthesised by reductive alkylation with propionaldehyde and hexanal respectively. However, only very small amounts of each compound were isolated.



Reagents and conditions: (i) Propionaldehyde, THF, AcOH, 0.5h; (ii) $NaBH_4$, 40^0C, 2h.

Scheme 47: Preparation of amino alcohol **174**

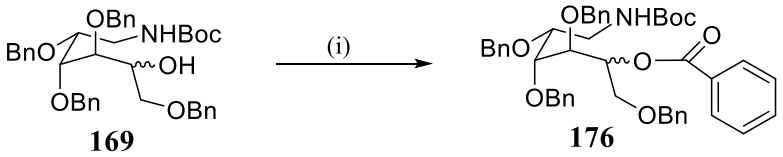


Reagents and conditions: (i) Hexanal, THF, AcOH, 0.5h; (ii) $NaBH_3CN$, 40^0C, 16h.

Scheme 48: Preparation of amino alcohol **175**

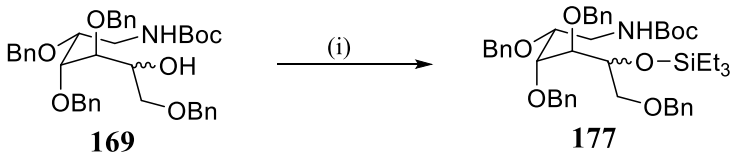
Attempts to scale the reactions up using identical conditions proved frustratingly unsuccessful. Slow reaction progression and complicated reaction profiles were obtained in both cases and the results achieved on the small scale could not be repeated. It was postulated that the secondary alcohol group attached to the carbon at the C2 position interfered with the imine intermediate leading to a capricious reaction. A solution to this would be to orthogonally protect the secondary alcohol group prior to carrying out the reductive alkylation reaction. As protection of the alcohol group was required for both *N*-alklylation and acetylation reactions several protecting groups were considered to ensure flexibility.

It is possible to protect the hydroxyl group in amino alcohol **169** with a variety of protecting groups. An *O*-benzoyl group and a triethyl silyl group were successfully installed in accordance with Schemes 49 and 50 respectively.



Reagents and conditions: (i) BzCl, DCM, Pyridine, RT, 2h.

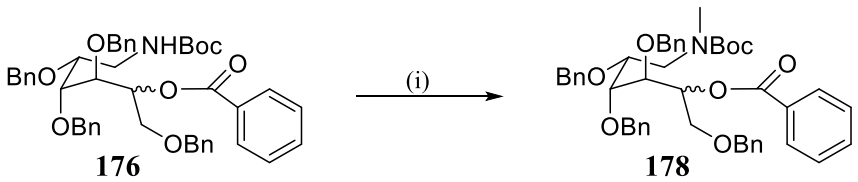
Scheme 49: *O*-benzoyl protection of amino alcohol **169**



Reagents and conditions: (i) TESCl, THF,imidazole, RT, 16h.

Scheme 50: *O*-triethyl silyl protection of amino alcohol **169**

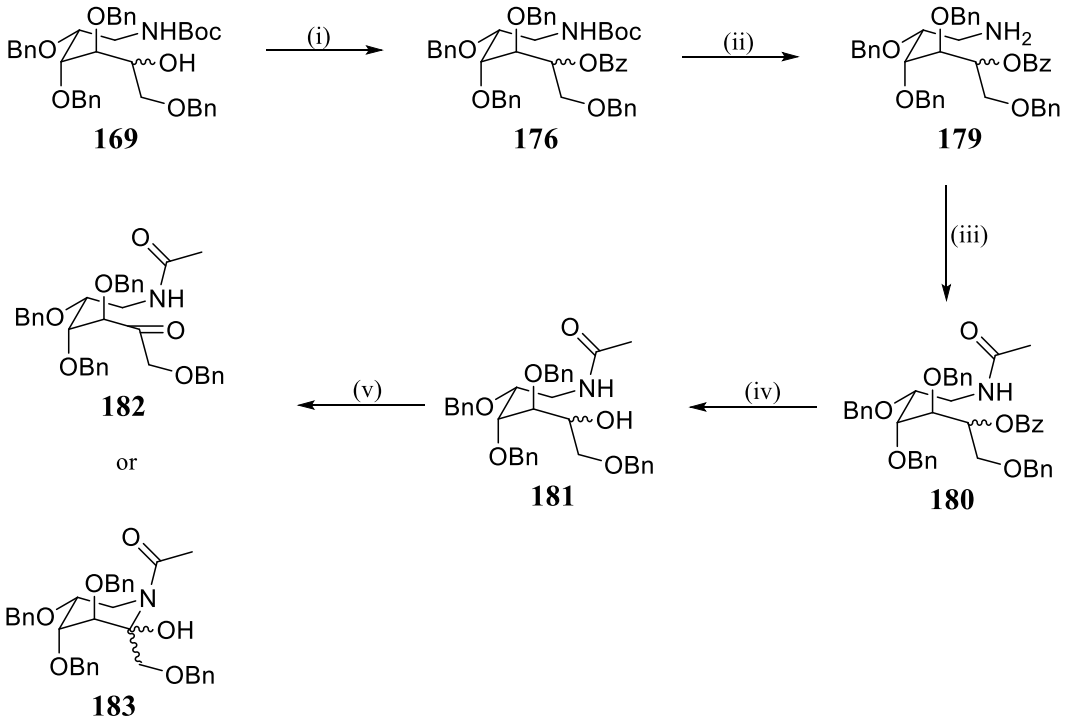
A limited number of small scale direct *N*-alkylation reactions were undertaken on both substrates **176** and **177** using various alkyl halides. Some success with the reaction of **176** with methyl iodide (Scheme 51) was achieved with positive identification of **178** by ^{1}H NMR and LCMS. However, only a very small amount was isolated and the reactions require further optimisation in order to enable the isolation of sufficient material to carry out the sequence of reactions proposed in Scheme 46. Unfortunately the opportunity to undertake further development of these reactions was not available due to time constraints. This would form an interesting area for future development.



Reagents and conditions: (i) MeI, acetonitrile, Et_3N, 50^{0}C, 18h.

Scheme 51: N-alkylation of protected acylic azasugar **176**

The addition of the *O*-benzoyl group to amino alcohol **169** and subsequent deprotection of the *N*-BOC group enabled the acetylation of the nitrogen atom with acetic anhydride. Although an electron withdrawing group, it is significantly less bulky than the *N*-BOC group and it was thought that its reduced size may help to induce intramolecular cyclisation. To test this hypothesis, the reactions detailed in Scheme 52 were carried out to prepare the iminosugar **183**. Similar analysis of the compound that resulted from the final oxidation step to that which was conducted for iminosugar **173** was undertaken to determine whether the cyclic **183** or acyclic **182** iminosugar had been prepared. The ^{1}H NMR, ^{13}C NMR and FTIR spectra of the compound again suggested the acyclic iminosugar **182**.



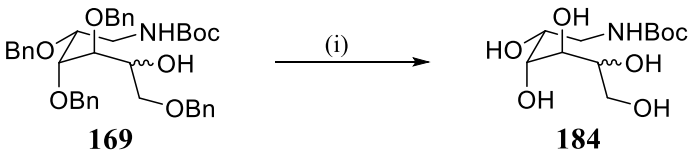
Reagents and conditions: (i) BzCl, DCM, Pyridine, RT, 2h; (ii) DCM, TFA, RT, 2h; (iii) Ac_2O, DCM, Pyridine, RT, 2h; (iv) KOH, MeOH, RT, 16h; (v) Dess-Martin periodinane, DCM, 45^{0}C, 16h.

Scheme 52: Preparation of iminosugar **183**

- **Altering the protecting groups**

Alongside attempts to functionalise the nitrogen atom, two reactions to remove the bulky *O*-benzyl protecting groups from iminosugars **169** and **173** by catalytic hydrogenation were carried out (Schemes 53 and 54). In both cases the reaction was facile with the protecting

groups readily removed. Characterisation of both compounds was achieved by ^{1}H NMR, ^{13}C NMR, FTIR and mass spectroscopy. ^{1}H NMR confirmed that two products were obtained from the hydrogenation of iminosugar **169** as was expected due to the epimer at the C2 position in compound **184**. It was noted that the reaction detailed in Scheme 53 was carried out more as a proof in principle exercise than to progress a line of chemistry. The utility of compound **184** was limited as obtaining a useful product by differentiating and selectively protecting various hydroxyl groups followed by oxidation at the relevant position within the molecule would be very challenging.

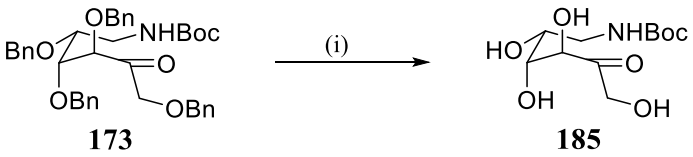


169 **184**

Reagents and conditions: (i) H_2, 10%Pd/C, MeOH, RT, 48h.

Scheme 53: Debenzylation of iminosugar **169**

Iminosugar **185**, as anticipated, was obtained as a single product. The FTIR spectrum of **185** showed two clear peaks indicative of two carbonyl stretches which confirmed that over reduction of the ketone had not occurred during the hydrogenation. Compound **185** was of greater interest than **184** as the retention of the ketone afforded opportunities to undertake further chemistry with the compound to induce the formation of a cyclic iminosugar.

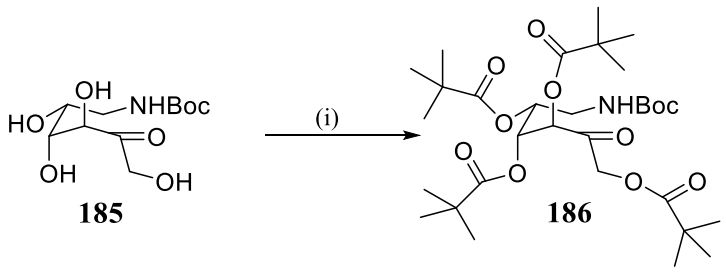


173 **185**

Reagents and conditions: (i) H_2, 10%Pd/C, MeOH, RT, 48h.

Scheme 54: Debenzylation of iminosugar **173**

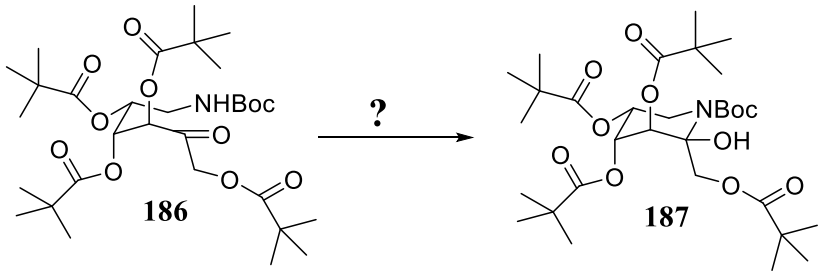
It was successfully demonstrated that the four alcohol groups could be successfully re-protected with pivaloyl groups (Scheme 55). Although the pivaloyl group is sterically large, it is electronically different to an *O*-benzyl group and it was of interest for comparison purposes.



Reagents and conditions: (i) PivCl, pyridine, 0^0C-RT, 8h.

Scheme 55: Protection of **185** with pivaloyl chloride

Had time permitted subsequent reactions would have been conducted with compound **186** to assess whether there was any potential of obtaining a cyclic iminosugar with different functionalisation around the sugar ring. It would be interesting to trial various reaction conditions to see if compound **187** could be obtained. Another potential step would be to ascertain whether the removal of the *N*-BOC group in **186** would result in a stable and isolable product.

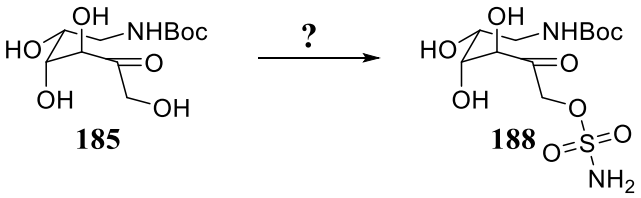


Scheme 56: Potential formation of cyclic iminosugar

Changing the protecting groups does offer scope for further development particularly if combined with the successful functionalisation of the amine. Unfortunately, there was insufficient research time to fully develop these more interesting areas of chemistry that may ultimately lead to a cyclic iminosugar.

- **Modulating the electrophilicity of the ketone**

It was thought that controlled reaction conditions may enable selective functionalisation of the primary alcohol at position C2 in iminosugar **185**. If this could be successfully achieved, it may be possible to modulate the reactivity of the ketone in such a way that stable intramolecular cyclisation is promoted. Reconsidering the ultimate target compounds which are analogues of topiramate, iminosugar **185** may facilitate the installation of the important sulfamate group at the C2 position, **188**, if adequate chemoselectivity could be achieved. This would not only potentially alter the overall reactivity and stability of the iminosugar pre and post cyclisation but also means a key structural feature of the desired compound would be in place.



Scheme 57: Selective functionalisation of deprotected iminosugar **185**

A number of small scale trials of the reaction outlined in Scheme 57 were conducted using conditions that mirrored those successfully employed previously to install a sulfamate group onto a primary alcohol. Positive results were indicated with potential scope for controlling chemoselectivity to the desired primary hydroxyl position. Had further time been available, this was a route that would be interesting to explore and optimise as it could lead to a range of alternative options for onward chemistry development.

3.4. General conclusions

The overall objective of this section of the project was to prepare an iminosugar analogue of topiramate. The *in silico* modelling work had suggested that such a compound would be an active candidate for CA inhibition. As there was no published precedent for an iminosugar that was structurally and stereochemically equivalent to *D*-fructose, the initial aim was to demonstrate the viability of this class of compound. Thus a "model compound", an *O*-benzyl protected variant, was the first synthetic target that would also enable a total synthesis with

appropriate modification. The successful preparation of this model iminosugar via the selected route would enable targeted derivatisation and route development on a sound basis.

As topiramate contains a *D*-fructose core, a synthetic plan was developed that successfully utilised *D*-fructose as a starting material. It has been demonstrated that some of the standard transformations employed in the preparation of iminosugars can be applied to *D*-fructose whose utility in iminosugar synthesis has not been explored fully and as such the exercise was also useful in terms of developing methodology. However, using *D*-fructose as a starting material has not been without challenges, particularly during the isolation and characterisation of its various anomers, a challenge that was necessary during the early stages of the selected synthetic scheme. Literature procedures which had been successfully applied to other monosaccharides required adaptation in order to produce the desired result with *D*-fructose and its derivatives. For example, the standard Fischer glycosidation reaction that has been applied successfully to a range of monosaccharides including glucose and arabinose, proved inefficient with *D*-fructose and an alternative methodology to prepare methyl fructoglycosides was employed. Several of these early stage reactions have been developed and have been shown to be repeatable and, to an extent sufficiently scaleable, to provide multi-gram scale quantities of intermediates. It is recognised that selected reactions within these early stages would benefit from further optimisation and process development but this was not deemed a priority. Each key intermediate was ultimately produced and fully characterised using a variety of techniques applicable to detailed structural elucidation as necessary. Of particular importance during the early stages of chemistry was the differentiation between the furan and pyran forms of derivatives of *D*-fructose. In addition to standard characterisation techniques, several test reactions were conducted on isolated furans so as to provide further positive identification of the undesired compounds for comparison. This was very useful, especially in the absence of significant amounts of literature data. The successful isolation of the desired pyranose isomers in the early stages of the synthetic scheme enabled a fast-track approach to be taken in respect of the downstream chemistry in order to rapidly gain an understanding of the more novel reactions and intermediates. The early stages were successfully scaled up to provide material to enable the more interesting downstream chemistry to be fully explored.

Several adaptations to the synthetic scheme initially proposed were necessary. It was readily established that the presence of the free amine within the compound was unlikely to furnish a stable and isolable iminosugar. Thus, protection of the amine with a BOC group was postulated as a means of introducing additional stability. The synthetic scheme was modified accordingly

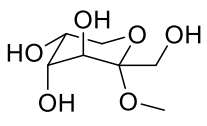
and the new reactions investigated and developed. The successful formation of the BOC protected amino ketone, **171**, provided an important juncture to the overall project. The fact that the compound remained acyclic and that attempts to induce cyclisation were not successful generated several hypotheses that were developed into alternative strategies to attempt to prepare a cyclic iminosugar. The stability and nucleophilicity of the protected amine, the electophilicity of the corresponding ketone and the general stereoelectronics of the molecule were all considered as avenues for consideration and provided scope for further development. This led to the trialling of alternative chemistries that attempted to exploit the different opportunities offered by each potential variation to the compound. Functionalisation of the amino group with both electron donating and electron withdrawing groups generated a supplementary series of experiments that, if pursued, may lead to a cyclic compound. Likewise, the selective addition of the sulfamate group into the molecule in the desired position may influence the reactivity of the ketone sufficiently to facilitate cyclisation. Installing the sulfamate group into the molecule earlier in the synthesis offers opportunities for further investigation, particularly to consider what effect the presence of this key functional group would have on the chemistry attempted here. Alternative hydroxyl protecting strategies may offer a means of altering the overall stereoelectronics of the molecule and although this was only touched upon briefly, there is a wide scope for onward investigation.

Unfortunately, within the confines of this thesis, preparing a cyclic model iminosugar based upon *D*-fructose proved difficult to achieve although this was not immediately surprising given the general lack of precedent contained in the literature. However, the research conducted has provided pertinent information and data about particular reactions of *D*-fructose and several of its derivatives that has led to the suggestion of several potential alternative synthetic pathways towards a cyclic iminosugar.

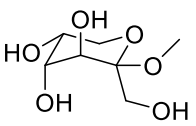
3.5. Experimental details

3.5.1. Synthesis of intermediates of iminosugar analogues of topiramate

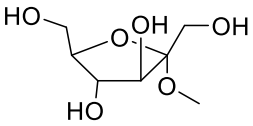
Preparation of methyl-α-D-fructopyranoside **153***/ methyl-β-D-fructopyranoside* **154***/ methyl-α-D-fructofuranoside* **155**/ *methyl-β-D-fructofuranoside* **156**:



153



154



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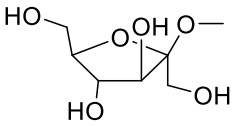
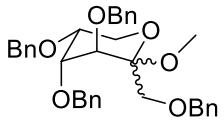


156

D-fructose (750g/4.2mol), ammonium chloride (111g/2.1mol) and methanol (8.25L) were charged to a 10L flange flask fitted with a condenser. The reaction was stirred and heated to reflux (*ca*. 64°C) overnight after which time TLC indicated reaction completion. The reaction was cooled to room temperature and anhydrous sodium hydrogen carbonate (27.5g) was added to raise the pH of the reaction to 7. The reaction was vacuum filtered to remove the solids and concentrated *in vacuo* to give an opaque yellow syrup (861g). This was dissolved in methanol (2.5L), adsorbed onto silica and purified by column chromatography on silica eluted with 7:1.5:1.5 DCM/ ethyl acetate/ methanol. Relevant fractions were combined and the solvent evaporated. A mixture of products was obtained (221g). Mixed fractions were combined, adsorbed onto silica and repurified by column chromatography on silica using the same conditions as employed in the first column. An additional 89.5g of a mixture of products was obtained resulting in a total yield of 39%. In addition, 266g (33%) of an undesired fructofuranose anomer was isolated as a pale yellow syrup. Data for the isolated isomer, assumed to be **155**: ^{1}H NMR (DMSO-*d*6, 270MHz): δ 3.16 (s, 3H, O-CH_3), δ 3.30 – 3.71 (m, 6H, $H_{1,1',3,4,6,6'}$), δ 3.84 (t, 1H, *J*=5.13Hz, H_5), δ 4.29 (t, 1H, *J*=5.67Hz, O*H*), δ 4.73 (t, 1H, *J*=5.13Hz, O*H*), δ 5.06 (d, 1H, *J*=5.9Hz, O*H*), δ 5.14 (d, 1H, *J*=5.7Hz, O*H*). ^{13}C NMR (DMSO-*d*6, 67.5MHz): δ 48.33, 59.77, 61.60, 77.19, 81.07, 82.91, 107.70. MS: m/z of 212 (MH_2O^+) and 217 (MNa^+).

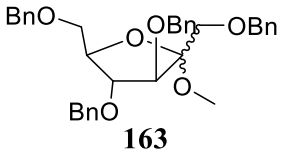
Preparation of 1,3,4,5-tetra-O-benzyl-methyl-α/β-D-fructopyranoside **146**



146

A mixture of **153**, **154** and **156** (310g/ 1.6mol) was dissolved in anhydrous DMF (9L) under N_2 in a 20L flange flask fitted with an overhead stirrer. The solution was cooled to <0°C with an IPA/CO_2 bath. Benzyl bromide (1.14L/9.6mol) was added dropwise maintaining reaction temperature <0°C. Sodium hydride (60% in oil) (393g/9.8mol) was added gradually to prevent exotherms and significant off gassing and to ensure complete dissolution of the sodium hydride. The reaction was stirred at <0°C for a further hour following complete addition of the sodium hydride. The reaction was warmed to room temperature and was stirred under N_2 overnight. A dense precipitate formed. TLC indicated reaction completion. The reaction was cooled to <0°C and methanol (3L) was added to the reaction cautiously to control any exotherm and gas evolution. The reaction was vacuum transferred into a second 20L flask containing water (3L) and was stirred for 30 minutes. Ethyl acetate (5L) was added and the reaction stirred for a further 30 minutes. The organic layer was separated and the aqueous further extracted with ethyl acetate (2 x 1L). The combined organics were washed with water (5 x 1L), brine (1L) and dried over $MgSO_4$. After filtering, the reaction was concentrated *in vacuo* to give a yellow liquid. The crude material was purified by column chromatography on silica eluted with 5-25% ethyl acetate/heptane. Product containing fractions were combined and the solvent evaporated. One product was isolated as a yellow syrup (532g/60%). This was the second undesired fructofuranose, **163**. The other two products were isolated as a mixture as a pale yellow syrup (212.8g/24%). Only one product was visible by ^{1}H NMR. ^{1}H NMR ($CDCl_3$, 270MHz: δ 3.28 (s, 3H, O-CH_3), δ 3.50 (d, 1H, J=12.4Hz, H_6), δ 3.67 (d, 1H, J=9.7Hz, H_1), δ 3.77 – 3.80 (m, 2H (overlapping), $H_{1',3}$), δ 3.88 (dd, 1H, J=1.6, 12.4Hz, $H_{6'}$), δ 3.96 (dd, 1H, J=3.5, 10.2Hz, H_4), δ 4.37 (d, 1H, J= 10.2Hz, H_5), δ 4.41 – 4.94 (m, 8H, O-CH_2-Ph), δ 7.24 – 7.40 (m, 20H, aromatic).

Preparation of 1,3,4,5-tetra-O-benzyl-methyl-α/β-D-fructofuranoside **163**

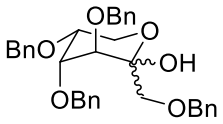


163

Experimental details as for **146**.

^{1}H NMR ($CDCl_3$, 270MHz: δ 3.30 (s, 3H, O-CH_3), δ 3.52 – 3.54 (m, 2H, $H_{1,1'}$), δ 3.64 (s, 2H, $H_{6,6'}$), δ 3.81 (dd, 1H, *J*=2.3, 5.6Hz, H_4), δ 4.04 (d, 1H, *J*=2.3Hz, H_3), δ 4.11 (q, 1H, *J*=5.6, H_5), δ 4.36 – 4.65 (m, 8H, O-CH_2-Ph), δ 4.36 (d, 1H, *J*=10.0Hz, H_1), δ 4.43 (d, 1H, *J*=11.9Hz, O-CH_2-Ph), δ 4.56 - 4.81 (m, 6H, O-CH_2-Ph), δ 7.22 – 7.31 (m, 20H, aromatic).

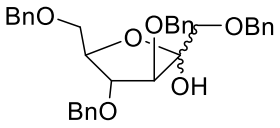
Preparation of 1,3,4,5-tetra-O-benzyl-α/β-D-fructose **147**



147

146 (150g/0.27mol) was dissolved in acetic acid (1.8L) in a 5L flange flask. 1M H_2SO_4 (72ml) was added and the reaction was heated to 50°C and stirred for 4 hours. TLC/LCMS showed consumption of the starting material. The reaction was cooled to room temperature and was vacuum transferred to a 10L flange flask containing water (3L). Ethyl acetate (3L) was added and the reaction stirred for 15 minutes. Solid sodium hydrogen carbonate (136g) was added until rapid effervescence ceased. The organic layer was separated and the aqueous was further extracted with ethyl acetate (3x1.5L). The combined organics were washed with saturated sodium hydrogen carbonate solution (aq) (3x1L), water (3L) brine (3L), dried over $MgSO_4$, vacuum filtered and concentrated *in vacuo* to give a pale yellow syrup (142g). The crude material was purified by column chromatography on silica eluted with 10% - 40% ethyl acetate/ heptane. Relevant fractions were combined and the solvent evaporated to give the products as a yellow syrup (131g/ 90%). LCMS shows two peaks in a ratio of 10:1 both with a m/z of 563 (MNa^+) with a purity of >98%. ^{1}H NMR ($CDCl_3$, 270MHz) (data for major anomer): δ 3.36 (br, 1H, O*H*), δ 3.43 – 3.55 (2xd, 2H, *J*=10.0Hz, $H_{6,6'}$), δ 3.78 – 3.83 (m, 3H, $H_{3,4,5}$), δ 3.95 (s, 2H, $H_{1,1'}$), δ 4.50 – 4.96 (m, 8H, O-CH_2-Ph), δ 7.25 – 7.38 (m, 20H, aromatic). δ 61.02, 71.3, 72.1, 72.2, 73.9, 75.6, 73.3, 75.9, 79.0, 98.2, 128.81 – 130.55(aromatics x20), 137.8, 138.2, 138.5, 138.5.

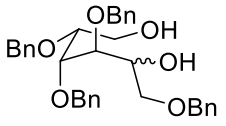
Preparation of 1,3,4,5-tetra-O-benzyl-α/β-D-fructofuranose **164**:



164

163 (0.5g/ 0.9mol) was dissolved in acetic acid (2ml). 1M H_2SO_4 (0.15ml) was added and the reaction was heated to 50°C overnight. TLC/ LCMS showed consumption of the starting material so the reaction was cooled to room temperature and poured over water (10ml) and ethyl acetate (20ml) was added. Anhydrous sodium hydrogen carbonate (5g) was added until rapid effervescence ceased. The organic layer was separated and the aqueous was further extracted with ethyl acetate (3x 5ml). The combined organics were washed with saturated sodium hydrogen carbonate solution (aq) (20ml), water (20ml), brine (20ml) and dried over $MgSO_4$. After vacuum filtering, the reaction was concentrated *in vacuo* to give a colourless syrup (0.421g). The crude material was purified by column chromatography on silica eluted with 10% - 40% ethyl acetate/heptane. Relevant fractions were combined and the solvent evaporated to give the product as a pale yellow syrup (0.28g/57%). LCMS showed a m/z of 563 (MNa^+) with a purity of >98%. ^{1}H NMR ($CDCl_3$, 270MHz): δ 3.32 (br-s, 1H, O*H*), δ 3.53 – 3.58 (m, 2H, $H_{6,6'}$), δ 3.64 (d, 2H, *J*=12.0Hz $H_{1,1'}$), δ 3.81 (dd, 1H, *J*=2.5, 5.4Hz, H_4), δ 4.04 (d, 1H, *J*=2.5Hz, H_3), δ 4.11 (q, 1H, *J*=5.4, H_5), δ 4.36 – 4.65 (m, 8H, O-CH_2-Ph), δ 7.22 – 7.31 (m, 20H, aromatic).

Preparation of 1,3,4,5-tetra-O-benzyl-D-glucitol/1,3,4,5-tetra-O-benzyl-D-mannitol **148**:

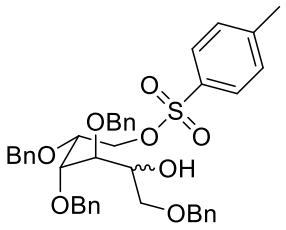


148

147 (122.0g/ 0.23mol) was dissolved in THF (1.95L) and water (0.98L) was added. Solid sodium borohydride (87.0g/ 2.3mol) was added and the reaction was stirred at room temperature for 2 hours. TLC/ LCMS showed no remaining starting material so the reaction was cooled to <10°C with an ice bath and saturated aqueous ammonium chloride solution (aq) (1.5L) was added gradually to control exotherm and gas evolution. The reaction was considered fully quenched once no further gas evolution was observed. Ethyl acetate (3L) was added and the layers separated. The aqueous was further extracted with ethyl acetate (3x1L).

The organics were combined, washed with water (2L), brine (2L), dried over $MgSO_4$, vacuum filtered and concentrated *in vacuo*. A colourless syrup (119.0g/ 95%) was obtained. LCMS showed a single peak at 99% purity with m/z of 543 (MH^+) and 565 (MNa^+). Two epimers visible in the ^1H NMR in a 5:1 ratio. ^1H NMR ($CDCl_3$, 270MHz) (data for major isomer): δ 2.35 (t, 1H, O*H*), δ 2.72 (d, 1H, O*H*), δ 3.38 – 3.51 (m, 2H, $H_{1,1'}$), δ 3.74 – 4.01 (m, 6H, $H_{2,3,4,5,6,6'}$), δ 4.40 – 4.74 (m, 8H, O-CH_2-Ph), δ 7.20 – 7.33 (m, 20H, aromatic). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 62.50, 71.85, 73.35, 74.25, 75.31, 75.69, 79.50, 84.28, 84.56, 127.95 – 128.68 (aromatics x20), 135.01, 136.58, 137.58, 137.95.

Preparation of 1,3,4,5-tetra-O-benzyl-6-toluenesulphonyl-D-glucitol/1,3,4,5-tetra-O-benzyl-6-toluenesulphonyl-D-mannitol **149**:



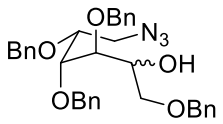
149

148 (118.0g/ 0.217mol) was dissolved in pre-dried DCM (2.36L) under N_2. Triethylamine (60.5ml/0.435mol) and DMAP (2.65g/ 0.022mol) were added. Tosyl chloride (82.0g/ 0.435mol) was added as a solid in portions and the reaction was stirred at room temperature under N_2 for 24 hours. TLC/ LCMS showed reaction completion so the reaction was quenched by the addition of saturated sodium bicarbonate solution (aq) (2L). The organic layer was separated and the aqueous was further extracted with DCM (3x 1L). The organic layers were combined and washed with water (2L), brine (2L) and dried over $MgSO_4$. After vacuum filtering, the solvent was removed *in vacuo* to give a yellow oil (189.4g). The crude material was purified by column chromatography on silica eluted with 7:1.5:1.5 heptane/ DCM/ ethyl acetate. Relevant fractions were combined and the solvent evaporated. The products were obtained as a yellow syrup (128.1g/ 85%). LCMS showed the presence of two products in a ratio of 5:1 with >95% purity. Both peaks showed a m/z of 719 (MNa^+) and 697.5 (MH^+) in the corresponding mass spectrum. ^1H NMR ($CDCl_3$, 270MHz)(for major isomer): δ 2.40 (s,

3H, CH_3), δ 2.50 (d, 1H, *J*=6.2Hz, O*H*), δ 3.34 – 3.48 (m, 2H, $H_{1,1'}$), δ 3.66 (dd, 1H, *J*=5.94, 2.97Hz, H_4), δ 3.85 – 3.97 (m, 3H, $H_{3,5,6}$), δ 4.19 – 4.25 (m, 1H, $H_{6'}$), δ 4.33 – 4.68 (m, 9H, O-CH_2-Ph/ H_2), δ 7.19 – 7.32 (m, 20H, aromatic), δ 7.34 - 7.36 (m, 4H, aromatic (OTs)). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 22.60, 69.3, 71.22, 73.25, 73.35, 74.25, 75.31, 75.69, 78.65, 79.50, 84.28, 127.65 – 129.75 (aromatics x20), 134.50, 135.62, 136.77, 138.44, 140.30, 145.20.

The bis-tosylated product was also isolated in small amounts from the reaction. LCMS confirmed the compound had a m/z of 873.6 (MNa^+). ^{1}H NMR ($CDCl_3$, 270MHz): δ 2.34 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), δ 3.34 (dd, 1H, *J*=5.4, 11.34Hz, H_1), δ 3.58 (dd, 1H, *J*=5.4, 11.34Hz, $H_{1'}$), δ 3.74 – 3.76 (m, 2H, $H_{4,5}$), δ 3.88 – 3.92 (m, 1H, H_3), δ 4.08 – 4.54 (m, 10H, O-CH_2-Ph/ $H_{6,6'}$), δ 4.83 – 4.88 (m, 1H, H_2), δ 7.08 – 7.26 (m, 20H, aromatic (OBn)), δ 7.66 – 7.71 (4xd, 8H, *J*=2.8Hz, aromatic (OTs)).

Preparation of **150**:

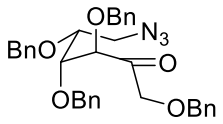


150

149 (128.1g/ 0.18mol) was dissolved in anhydrous DMF (3.2L) under N_2. Sodium azide (21.1g/ 0.324mol) was added and the reaction was heated to 50°C and stirred for 24 hours. TLC/ LCMS indicated full consumption of the starting material so the reaction was cooled to room temperature and water (4L) was added cautiously. Ethyl acetate (3L) was added and the reaction stirred for 30 minutes. The organic layer was separated and the aqueous further extracted with ethyl acetate (3x 1L). The organics were combined and were washed with water (6x 1L), brine (1L) and dried over $MgSO_4$. The reaction was vacuum filtered and concentrated *in vacuo* to give a yellow oil (103.4g). The crude material was purified by column chromatography on silica eluted with 8:1:1 heptane/ ethyl acetate/ DCM. Relevant fractions were collected and the solvent evaporated. The products were obtained as a colourless syrup (48.5g/58%). LCMS showed two peaks in a ratio of 12:1 both with m/z 590.6 (MNa^+) in >97% purity. ^{1}H NMR ($CDCl_3$, 270MHz)(data for major epimer): δ 2.61 (d, 1H, *J*=6.2Hz, O*H*), δ

3.35 – 3.48 (m, 2H, $H_{1,1'}$), δ 3.53 – 3.55 (m, 2H, $H_{3,5}$), δ 3.67 (dd, 2H, *J*=2.7, 5.9Hz, H_4), δ 3.81 – 3.85 (m, 1H, H_6), δ 3.90 – 3.96 (m, 2H, $H_{6',2}$), δ 4.39 – 4.73 (m, 8H, O-CH_2-Ph), δ 7.21 – 7.34 (m, 20H, aromatic). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 50.96, 71.22, 71.25, 73.35, 74.25, 75.65, 75.98, 78.55, 81.52, 82.28, 127.10 – 129.25 (aromatics x20), 135.50, 135.62, 135.99, 138.49. FTIR (CH_2Cl_2): 2102.8 (N_3).

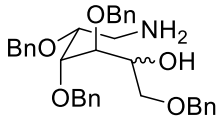
Preparation of **151**:



151

150 (1.39g/ 2.45mmol) was dissolved in pre-dried DCM (10ml) under N_2 and cooled to 0°C. Dess-Martin periodinane (1.25g/ 2.94mmol) as a suspension in DCM (5ml) was added dropwise maintaining the reaction temperature at 0°C. The reaction was stirred for a further 10 minutes at 0°C and then warmed to room temperature. After 30 minutes of stirring, TLC and LCMS indicated consumption of the starting material. Ethyl acetate (40ml) was added to the reaction and it was cooled to <5°C. Saturated sodium thiosulfate solution (aq) (10ml) was added to quench any remaining oxidant followed by saturated sodium bicarbonate solution (aq) (10ml). The organic layer was separated and the aqueous further extracted with DCM (2x 10ml). The organics were combined and washed with saturated sodium bicarbonate solution (aq) (30ml), brine (30ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a clear colourless syrup (1.19g). The syrup was purified by column chromatography on silica eluted with 8:1:1 heptane/ ethyl acetate/ DCM. Relevant fractions were collected and the solvent evaporated. The product was obtained as a colourless syrup (0.91g/ 65%). LCMS showed a peak with m/z 588.6 (MNa^+) in 96% purity. ^{1}H NMR ($CDCl_3$, 270MHz): δ 3.35 (dd, 1H, *J*=4.1, 13.0Hz, H_6), δ 3.69 (d, 1H, *J*=13.0Hz, $H_{6'}$), δ 3.74 – 3.83 (m, 1H, H_4), δ 4.09 – 4.13 (m, 1H, H_5), δ 4.21 – 4.69 (m, 10H, O-CH_2-Ph, $H_{1,1'}$), δ 7.17 – 7.35 (m, 20H, aromatic). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ50.1, 72.1, 73.4, 74.0, 74.3, 74.9, 78.2, 79.2, 84.3, 128.0 – 128.7 (aromatics x20), 136.95, 137.52, 137.98, 138.26, 208.0. FTIR (CH_2Cl_2): 2101.4 (N_3), 1730.5 (C=O).

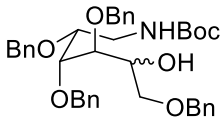
Preparation of **168**:



168

Lithium aluminium hydride (1M solution in THF) (147ml) was charged to a flask under N_2 and was cooled to -5°C. **150** (41.90g/ 0.07mol) was dissolved in THF (1L) and the solution added dropwise to the solution of lithium aluminium hydride. The reaction was stirred at <0°C for 30 minutes and was then allowed to warm gradually to room temperature. After 2 hours, LCMS confirmed reaction completion and the formation of the desired product. The reaction was cooled to 0°C and water (5.6ml) was added dropwise ensuring that the rate of addition controlled any exotherms and off gassing. 15% sodium hydroxide (aq) (5.6ml) was added cautiously followed by water (16.7ml). After warming to room temperature, the reaction was stirred for 30 minutes. A gelatinous solid formed in the flask. The reaction was filtered through a pad of Celite which was rinsed with THF (350ml). The THF was removed *in vacuo* and the clear residue was dissolved in ethyl acetate (250ml). The reaction was washed with water (150ml), brine (150ml) and dried over magnesium sulfate. After filtering, the reaction was concentrated *in vacuo* to give a colourless syrup (28.0g/70%) that solidified to a white solid on standing. LCMS purity of 96% with corresponding mass spectrum showing m/z of 564 (MNa^+) and 542 (MH^+). 96% purity by NMR assay. ^{1}H NMR ($CDCl_3$, 270MHz): δ 2.75 (br-s, 1H, O*H*), δ 3.04 – 3.06 (m, 2H, $H_{6,6'}$), δ 3.39 – 3.53 (m, 2H, $H_{1,1'}$), δ 3.70 (dd, 1H, *J*=5.4Hz, H_4), δ 3.81 – 3.85 (m, 1H, H_5), δ 3.93 – 4.00 (m, 2H, $H_{2,3}$), δ 4.38 – 4.72 (m, 8H, O-CH_2-Ph), δ 7.19 -7.24 (m, 3H, aromatic), δ 7.26 -7.34 (m, 17H, aromatic), δ 7.57 (br-s, 2H, NH_2). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 69.32, 72.02, 73.36, 74.30, 74.62, 74.72, 75.50, 75.91 78.35, 79.50, 127.81 – 128.55(aromatics x20), 136.50, 138.62, 139.45, 139.69.

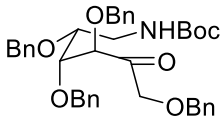
Preparation of **169**:



169

169 (31.39g/ 0.06mol) was dissolved in THF (775ml) and charged to a 1L flask under N_2 and cooled to <5°C with an ice bath. Triethylamine (16.73ml/0.12mol) was added portionwise over 30 minutes and the reaction stirred for an additional 30 minutes. Boc anhydride (16.5ml/0.072mol) as a solution in THF (20ml) was added to the reaction dropwise maintaining the reaction temperature at <5°C. The reaction was stirred for 30 minutes and then allowed to warm to room temperature gradually. TLC/LCMS confirmed reaction completion with complete consumption of starting material. The reaction was concentrated *in vacuo* to remove THF and the resulting residue was dissolved in ethyl acetate (300ml) and the solution washed with water (300ml), brine (300ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a yellow oil (41.41g). The oil was purified by column chromatography on silica eluted with 10-20% ethyl acetate/heptane. Product fractions were combined and the solvent evaporated to afford the product as a yellow syrup (25.41g/63%). LCMS purity of 97% was recorded with corresponding mass spectrum showing m/z of 665 (MNa^+) and 543 ($M\text{-}Boc^+$). ^1H NMR shows two isomeric products in a roughly 5:1 ratio as identified by two OH signals. 99% purity by NMR assay. ^1H NMR ($CDCl_3$, 270MHz)(data for major product): δ 1.42 (s, 9H, Boc), δ 2.78 (d, 1H, *J*=6.2Hz, O*H*), δ 3.38 – 3.53 (m, 4H, $H_{6,6',1,1'}$), δ 3.71 (dd, 1H, *J*=5.4H, H_4), δ 3.77 – 3.80 (m, 1H, H_2), δ 3.92 – 4.01 (m, 2H, $H_{5,3}$), δ 4.36 – 4.74 (m, 8H, O-CH_2-Ph), δ 4.92 (t, 1H, *J*=5.0Hz, N*H*), δ 7.19 -7.23 (m, 2H, aromatic), δ 7.26 -7.36 (m, 18H, aromatic). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 28.42, 42.20, 69.32, 71.20, 72.98, 74.50, 74.82, 74.98, 75.54, 75.98, 78.65, 79.65, 127.41 – 129.52 (aromatics x20), 136.50, 138.62, 139.45, 139.69, 155.62.

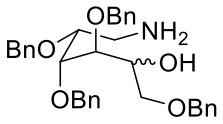
Preparation of **171**:



171

169 (1.28g/ 1.99mmol) was dissolved in pre-dried DCM (25ml) under N_2. Dess-Martin periodinane (1.00g/2.39mmol) was added. The reaction was heated to 40°C in an oil bath and stirred for 12 hours. TLC and LCMS indicated consumption of the starting material so the reaction was cooled to room temperature. DCM (25ml) was added to the reaction and it was cooled to <5°C. Saturated sodium thiosulfate solution (aq) (10ml) was added to quench any remaining oxidant followed by saturated sodium bicarbonate solution (aq) (10ml). The organic layer was separated and the aqueous further extracted with DCM (2x10ml). The combined organics were washed with saturated sodium bicarbonate solution (aq) (30ml), brine (30ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a yellow syrup (1.31g). The syrup was purified by column chromatography on silica eluted with 8:1:1 heptane/ ethyl acetate/ DCM. Relevant fractions were collected and the solvent evaporated. The product was obtained as an opaque colourless syrup (0.97g/76%). LCMS showed a peak with m/z 662.7 (MNa^+) and 540.5 ($M\text{-}Boc^+$) in 97% purity. ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.44 (s, 9H, Boc), δ 3.44 – 3.56 (m, 2H, $H_{1,1'}$), δ 3.67 – 3.72 (m, 2H, $H_{5,3}$), δ 3.99 (dd, 1H, J=3.78, 7.83Hz, H_4), δ 4.18 – 4.60 (m, 11H, O-CH_2-Ph, $H_{6,6',2}$), δ 4.70 (br, 1H, NH), δ 7.18 – 7.36 (m, 20H, aromatic). ^{13}C NMR ($CDCl_3$, 67.5MHz: δ28.36, 42.60, 71.19, 73.27, 74.14, 74.32, 74.71, 78.56, 79.28, 79.44, 84.10, 127.71 – 128.53 (aromatics x22), 136.78, 137.19, 155.85, 208.48.

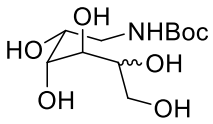
Preparation of **168**:



168

169 (1.0g/ 1.56 mmol) was dissolved in DCM (20ml) and TFA (2ml) was added. The reaction was stirred at room temperature. After two hours, TLC and LCMS indicated complete consumption of starting material. The reaction was poured over saturated sodium bicarbonate solution (aq) (40ml). The organic layer was separated. The aqueous was further extracted with DCM (3x10ml). The organic layers were combined, washed with saturated sodium bicarbonate solution (aq) (40ml), water (40ml), brine (40ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a pale yellow syrup (0.82mg/97%) that solidified on standing. LCMS purity of 93% with corresponding mass spectrum showing m/z of 564 (MNa^+) and 542 (MH^+). 97% purity by NMR assay. ^{1}H NMR ($CDCl_3$, 270MHz): δ 2.75 (br-s, 1H, O*H*), δ 3.04 – 3.06 (m, 2H, $H_{1,1'}$), δ 3.39 – 3.53 (m, 2H, $H_{6,6'}$), δ 3.70 (dd, 1H, *J*=5.4Hz, H_4), δ 3.81 – 3.85 (m, 1H, H_5), δ 3.93 – 4.00 (m, 2H, $H_{2,3}$), δ 4.38 – 4.72 (m, 8H, O-CH_2-Ph), δ 7.19 -7.24 (m, 3H, aromatic), δ 7.26 -7.34 (m, 17H, aromatic), δ 7.57 (br-s, 2H, NH_2). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 69.32, 72.02, 73.36, 74.30, 74.62, 74.72, 75.50, 75.91 78.35, 79.50, 127.81 – 128.55(aromatics x20), 136.50, 138.62, 139.45, 139.69.

Preparation of **184**:

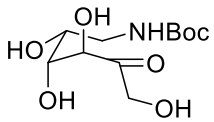


184

169 (1.10g/1.72mmol) was dissolved in methanol (20ml). The solution was charged to a suspension of palladium on carbon (10%) (0.366g/3.44mmol) in methanol (20ml) under N_2. Hydrogen was sparged into the reaction at atmospheric pressure. The reaction was heated to 40˚C in an oil bath. After 7 hours the reaction was sampled. Analysis by LCMS and ^{1}H NMR indicated that although there had been reaction progression, the reaction was incomplete. The

reaction was stirred under a hydrogen atmosphere at room temperature overnight. The hydrogen sparge was reinstated and the reaction reheated to 40˚C. After 7 hours the reaction was sampled. Analysis by LCMS and ^{1}H NMR indicated that there was no remaining starting material within the crude reaction mixture but the reaction remained incomplete. The reaction was stirred under a hydrogen atmosphere at room temperature overnight. The hydrogen sparge was reinstated and the reaction reheated to 40˚C. After 4 hours the reaction was sampled. ^{1}H NMR indicated reaction completion with no remaining *O*-benzyl groups signals visible. The reaction was cooled to room temperature and placed under a nitrogen atmosphere. The reaction was filtered through a pad of Celite which was rinsed with methanol (150ml). The solvent was removed *in vacuo* to give a colourless syrup (599mg). The crude material was purified by column chromatography on silica eluted with 10% methanol/DCM. Product fractions were combined and the solvent removed *in vacuo* to give a pale yellow syrup (0.441g/91%). Alcohol groups were identified by a D_2O shake. MS recorded a m/z of 304 (MNa^+) and 182 ($M\text{-}Boc^+$) which would correspond with the desired product. ^{1}H NMR (DMSO-*d*6, 270MHz) (major product): δ 1.38 (s, 9H, Boc), δ 2.83 – 2.91 (m, 1H, H_1), δ 3.26 – 3.33 (m, 2H, $H_{1',6}$), δ 3.41 – 3.55 (m, 4H, $H_{3,4,5,6'}$), δ 3.62 – 3.67 (m, 1H, H_2), δ 4.10 (d, 1H, *J*=7.0Hz, O*H*), δ 4.46 – 4.51 (m, 2H, 2xO*H*), δ 4.57 (d, 1H, *J*=4.6Hz, O*H*), δ 4.64 (d, 1H, *J*=5.6Hz, O*H*), δ 6.43 (t, 1H, *J*=5.40Hz, N*H*). ^{13}C NMR (DMSO-*d*6, 67.5MHz): δ 28.82, 44.54, 63.04, 69.26, 70.36, 73.70, 74.25, 78.19, 156.51. ^{1}H NMR (DMSO-*d*6, 270MHz): (identifiable signals from minor product; integrals as a fraction of 1H equivalent of major product. All are exchangeables by D_2O shake.) δ 4.29 – 4.35 (m, 0.2H, O*H*), δ 4.42 (t, 0.2H, *J*=6.21Hz, O*H*), δ 5.10 (d, 0.2H, *J*=5.94Hz, O*H*), δ 6.69 (t, 0.2H, *J*=5.67Hz, N*H*).

Preparation of **185**:

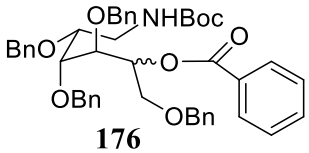


185

173 (5.0g/7.8mmol) was dissolved in methanol (25ml). The solution was charged to a suspension of palladium on carbon (10%) (1.66g/15.6mmol) in methanol (75ml) under nitrogen. Hydrogen was sparged into the reaction at atmospheric pressure. The reaction was heated to 40˚C. After 7 hours the reaction was sampled. Analysis by LCMS and ^{1}H NMR

indicated that although there had been reaction progression, the reaction was incomplete. The reaction was stirred under a hydrogen atmosphere at room temperature overnight. The hydrogen sparge was reinstated and the reaction reheated to 40˚C. After four hours the reaction was sampled. ^{1}H NMR indicated reaction completion with no remaining *O*-benzyl groups visible. The reaction was cooled to room temperature and placed under a nitrogen atmosphere. The reaction was filtered through a pad of Celite which was rinsed with methanol (500ml). The solvent was removed *in vacuo* to give a colourless syrup (4.70g). The crude material was purified by column chromatography on silica eluted with 5-10% methanol in DCM. Product fractions were combined and the solvent removed *in vacuo* to give a colourless syrup (1.818g/83%). The syrup solidified to a white solid on standing overnight. ^{1}H NMR in DMSO-*d*6 showed the correct number of alcohol groups were present in the product as identified by D_2O shake. MS showed a m/z of 302 (MNa^+) which corresponded with the desired product. ^{1}H NMR (DMSO-*d*6, 270MHz): δ 1.37 (s, 9H, Boc), δ 3.09 – 3.12 (m, 2H, $H_{1,1'}$), δ 3.23 (m, 2H, $H_{4,5}$), δ 3.52 – 3.56 (m, 1H, H_3), δ 3.73 – 3.85 (m, 2H, $H_{6,6'}$), δ 4.71 (t, 1H, *J*=6.21Hz, O*H*), δ 4.79 (d, 1H, *J*=6.48Hz, O*H*), δ 5.14 – 5.17 (m, 1H, O*H*), δ 5.60 (d, 1H, *J*=21.06Hz, O*H*), δ 6.43 (t, 1H, *J*=5.40Hz, N*H*). ^{13}C NMR (DMSO-*d*6, 67.5MHz): δ 28.26, 48.62, 62.86, 75.46, 76.38, 77.69, 79.02, 102.07, 155.58.

Preparation of **176**:

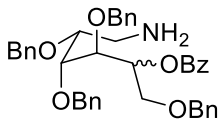


176

169 (1.0g/ 1.56mmol) was dissolved in pre-dried DCM (12.5ml) under nitrogen. Pyridine (2.5ml) was added and the reaction was cooled to *ca* 0°C with an ice bath and stirred for 30 minutes. Benzoyl chloride (0.26ml) was added dropwise and the reaction was stirred at 0°C for 30 minutes then was gradually warmed to room temperature and stirred under nitrogen overnight. HPLC showed that approximately 9% of starting material was remaining. An additional charge of benzoyl chloride (0.13ml/ 0.6mmol) was made. After one hour of stirring, TLC and HPLC indicated consumption of the starting material. DCM (5ml) was added and the reaction was washed with water (10ml), 1M sodium hydroxide (10ml), 1M hydrochloric acid (10ml), brine (10ml) and dried over $MgSO_4$. After vacuum filtering, the solvent was removed *in vacuo* to give a yellow oil (1.42g). The crude material was purified by column

chromatography on silica (20g) eluted with 10% ethyl acetate/ heptane (500ml). Relevant fractions were collected and the solvent evaporated. The product was obtained as a clear colourless syrup (0.89g/ 76%). LCMS showed two peaks with m/z 768 (MNa^+) and 646 (M-Boc^+) in 10:1 ratio. ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.40 (s, 9H, Boc), δ 3.42 – 3.54 (m, 2H, $H_{1,1'}$), δ 3.62 – 3.71 (m, 3H, $H_{6,6',3}$), δ 3.72 – 3.84 (m, 1H, H_5), δ 3.99 (dd, 1H, J = 7.2, 2.7Hz, H_4), δ 4.33 – 4.75 (m, 8H, O-CH_2-Ph), δ 4.89 (br, 1H, N*H*), δ 5.55 (dd, 1H, J = 3.7, 7.8Hz, H_2), δ 7.23 – 7.27 (m, 20H, aromatic), δ 7.43 (t, 2H, J = 7.8Hz aromatic), δ 7.56 (t, 1H, *J*=7.8Hz aromatic), δ 8.02 (d, 2H, *J*=7.8Hz aromatic).

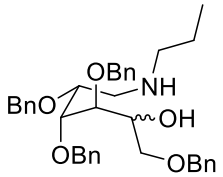
Preparation of **179**:



179

176 (0.89g/ 1.19 mmol) was dissolved in DCM (20ml) and TFA (0.2ml) was added. The reaction was stirred at room temperature and after two hours, TLC and HPLC indicated complete consumption of starting material. The reaction was poured over saturated sodium bicarbonate solution (aq) (40ml) and the organic layer was separated. The aqueous was further extracted with DCM (3 x 10ml). The organic layers were combined, washed with saturated sodium bicarbonate solution (aq) (40ml), water (40ml), brine (40ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a pale yellow syrup (0.61mg/ 79%). LCMS purity of 95% with corresponding mass spectrum showing m/z of 668 (MNa^+) and 647 (MH^+). ^{1}H NMR ($CDCl_3$, 270MHz): δ 2.95 – 3.02 (m, 2H, $H_{1,1}$), δ 3.60 – 3.79 (m, 3H, $H_{3,6,6}$), δ 3.92 (t, 1H, *J*=4.1Hz, H_5), δ 4.05 (d, 1H, *J*=4.1Hz , H_4), δ 4.40 – 4.53 (m, 4H, O-CH_2-Ph), δ 4.64 – 4.53 (m, 4H, O-CH_2-Ph), δ 5.57 (dd, 1H, *J*=5.1, 4.56Hz , H_2), δ 7.20 -7.30 (m, 20H, aromatic), δ 7.42 – 7.48 (m, 2H, aromatic), δ 7.56 – 7.61 (m, 1H, aromatic), δ 8.06 – 8.09 (m, 2H, aromatic).

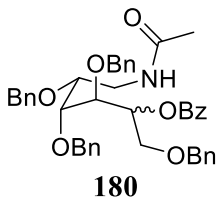
Preparation of **174**:



174

151 (50mg/0.09mmol) was dissolved in pre-dried THF (1ml) under nitrogen. Propionaldehyde (64µl of a 10% v/v solution in THF/0.11mmol) was added followed by acetic acid (55µl of a 10% v/v solution in THF/ 0.09mmol). The reaction was stirred under nitrogen at room temperature for 30 minutes. Sodium borohydride (8.1mg/0.13mmol) was added and after two hours TLC indicated minimal reaction progression. The reaction was warmed to 40°C for two hours and TLC indicated no remaining starting material. The reaction was poured over saturated sodium bicarbonate solution (aq) (10ml). The aqueous was extracted with DCM (3 x 5ml) and the organic layers combined, washed with water (10ml), brine (10ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a pale yellow oil (55mg). The crude material was purified by column chromatography on silica eluted with 5% methanol/ DCM. Relevant fractions were collected and the solvent evaporated. The product was obtained as a clear colourless syrup (11mg/20%). LCMS showed a peak with m/z 585 (MH^+). ^{1}H NMR ($CDCl_3$, 270MHz): δ 0.82 (t, 3H, *J*=7.6Hz, C*H*$_3$), δ 1.40 – 1.51 (m, 2H, C*H*$_2$), δ 2.54 (t, 2H, *J*=7.6Hz, C*H*$_2$), δ 3.00 (2xd overlapped, 2H, *H*$_{1,1'}$), δ 3.41 – 3.52 (m, 3H, *H*$_{6,3,5}$), δ 3.70 (dd, 3H, *J*=2.2, 6.5Hz, *H*$_4$), δ 3.98 – 4.07 (m, 3H, *H*$_{6',2,OH}$), δ 4.39 – 4.74 (m, 8H, O-C*H*$_2$-Ph), δ 7.22 – 7.30 (m, 20H, aromatic).

Preparation of **180**:

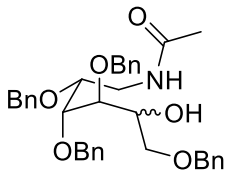


180

179 (100mg/0.16mmol) was dissolved in pre-dried DCM (1.5ml) and pyridine (1ml) under nitrogen. The reaction was cooled to *ca* 0°C with an ice bath. Acetic anhydride (15.8µl as a solution in 0.2ml DCM/0.16mmol) was added dropwise and the reaction stirred at 0°C for one

hour before the reaction was gradually warmed to room temperature. After two hours, TLC/ LCMS confirmed reaction completion. DCM (10ml) and water (10ml) were added and the organic layer separated. The aqueous was further extracted with DCM (3x5ml). The organics were combined, washed with saturated ammonium chloride solution (aq) (20ml), brine (20ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a yellow syrup (104mg). The crude material was purified by column chromatography on silica eluted with 5%-25% ethyl acetate/heptane. Relevant fractions were combined and the solvent evaporated. The product was obtained as a yellow syrup (59mg/54%). LCMS showed two adjacent peaks each with m/z 688 (MH^+) and 710 (MNa^+) in 92% purity. ^{1}H NMR ($CDCl_3$, 270MHz)(data for major epimer): δ 1.72 (s, 3H, CH_3), δ 3.35 – 3.41 (m, 1H, H_1), δ 3.61 – 3.73 (m, 4H, $H_{1',6,6',5}$), δ 3.89 (dd, 1H, J=2.7, 6.21Hz, H_4), δ 3.96 – 3.98 (m, 1H, H_3), δ 4.39 – 4.77 (m, 8H, O-CH_2-Ph), δ 5.55 (dd, 1H, J=2.7, 6.21Hz, H_2), δ 5.81 – 5.85 (m, 1H, NH), δ 7.19 – 7.29 (m, 20H, aromatic), δ 7.41 – 7.47 (m, 2H, aromatic), δ 7.55 – 7.58 (m, 1H, aromatic), δ 8.02 – 8.06 (m, 2H, aromatic).

Preparation of **181**:

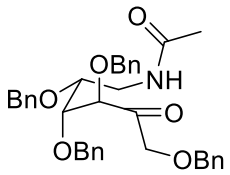


181

180 (150mg/0.5mmol) was dissolved in methanol (3ml). Potassium hydroxide (37mg/0.66mmol) was added and the reaction stirred at room temperature overnight. The reaction was sampled and no starting material was present by TLC or LCMS. 6N HCl in ethanol (0.24ml) was added dropwise to raise the pH of the reaction to *ca* 7. DCM (10ml) was added and the reaction was washed with water (10ml), brine (10ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a pale yellow syrup (142mg). The crude material was purified by column chromatography on silica eluted with 4-10% ethyl acetate in heptane (100ml). Relevant fractions were combined and the solvent removed *in vacuo.* The product was obtained as a pale yellow syrup (109mg/85%). LCMS showed one peak only with m/z 584 (MH^+) and 606 (MNa^+) in 92% purity. ^{1}H NMR ($CDCl_3$, 270MHz)(data for major isomer): δ 1.75 (s, 1H, CH_3), δ 2.77 (d, 1H, J=6.2Hz, OH), δ 3.31 – 3.52 (m, 3H, $H_{1,1',6}$), δ 3.67 – 3.76 (m, 3H, $H_{6',4,5}$), δ 3.95 – 3.98 (m, 2H, $H_{2,3}$), δ 4.45 – 4.74

(m, 8H, O-CH_2-Ph), δ 5.85 – 5.87 (m, 1H, N*H*), δ 7.20 – 7.35 (m, 20H, aromatic). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ23.45, 38.59, 72.65, 74.30, 74.52, 74.69, 74.95, 78.52 79.80, 83.78, 127.95 – 128.68 (aromatics x20), 135.01, 136.58, 137.58, 137.95, 170.07.

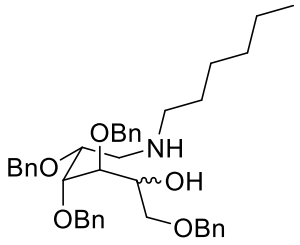
Preparation of **182**:



182

181 (100mg/0.17mmol) was dissolved in DCM (2ml) and Dess-Martin periodinane (89mg/0.21mmol) was added. The reaction was stirred under nitrogen at 45°C for 16 hours. TLC/ LCMS indicated no remaining starting material. The reaction was diluted with DCM (5ml) and cooled to room temperature. Saturated sodium thiosulfate solution (aq) (2ml) was added to quench any remaining oxidant followed by saturated sodium bicarbonate solution (aq) (5ml). The organic layer was separated and the aqueous further extracted with DCM (2x 5ml). The combined organics were washed with saturated sodium bicarbonate solution (aq) (10ml), brine (10ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a colourless syrup (108mg). The crude material was purified by column chromatography on silica eluted with 1:1 heptane/ethyl acetate. Relevant fractions were collected and the solvent evaporated. The product was obtained as a colourless syrup (83mg/84%). LCMS showed a peak with m/z 582.5 (MH^+) and 604.1 (MNa^+). ^{1}H NMR ($CDCl_3$, 270MHz): δ 1.78 (s, 3H, CH_3), δ 3.48 – 3.63 (m, 2H, $H_{1,1'}$), δ 3.64 – 3.67 (m, 1H, H_5), δ 3.95 (dd, 1H, J=3.5, 6.8Hz, H_4), δ 4.18 – 4.56 (m, 11H, O-CH_2-Ph, $H_{6,6',3}$), δ 5.50 (t, 1H, J=10.5Hz, N*H*), δ 7.18 – 7.35 (m, 20H, aromatic). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ23.21, 37.92, 71.35, 73.30, 74.19, 74.31, 74.58, 79.80, 83.78, 127.95 – 128.68 (aromatics x20), 135.01, 136.58, 137.58, 137.95, 170.07, 208.26.

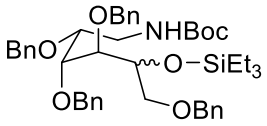
Preparation of **175**:



175

151 (100mg/0.19mmol) was dissolved in pre-dried THF (2ml) under nitrogen. Hexanal (18.5mg/019mmol) was added and the reaction was stirred under nitrogen at room temperature for two hours. Sodium cyanoborohydride (14mg/0.19mmol) was added and the reaction was stirred at room temperature for 16 hours and TLC indicated no remaining starting material. The reaction was poured over saturated sodium bicarbonate solution (aq) (10ml). The aqueous was extracted with DCM (3x5ml) and the organic layers combined, washed with water (10ml), brine (10ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a yellow syrup (97mg). The crude material was purified by column chromatography on silica eluted with 1-5% methanol/DCM. Relevant fractions were collected and the solvent evaporated. The product was obtained as a clear colourless syrup (39mg/34%). LCMS showed a peak with m/z 626 (MH^+). ^{1}H NMR ($CDCl_3$, 270MHz): δ 0.85(s, 3H, Me), δ 1.21 – 1.42 (m, 8H, 4xCH_2), δ 2.55 (t, 2H, *J*=7.8Hz, N-CH_2), δ 2.97 (2xd overlapping, 2H, $H_{1,1'}$), δ 3.40 – 3.47 (m, 2H, $H_{6,6'}$), δ 3.71 (dd, 1H, J=1.9, 6.2Hz, H_4), δ 3.98 – 4.02 (m, 3H, $H_{2,3,5}$), δ 4.40 – 4.72 (m, 18H, O-CH_2-Ph), δ 7.21 – 7.30 (m, 20H, aromatic).

Preparation of **177**:

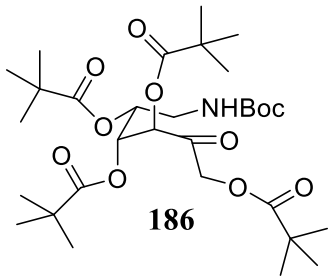


177

169 (500mg/0.78mmol) was dissolved in THF (10ml) under nitrogen and cooled to 0° C. Imidazole (106mg/1.56mmol) was added and the reaction stirred for 30 minutes to ensure full dissolution. TESCl (1.17ml/0.78mmol) was added. The reaction was stirred at 0° C for one

hour before allowing it to warm to room temperature gradually. The reaction was stirred under nitrogen at room temperature for 16 hours. TLC/LCMS indicated no remaining starting material. Water (10ml) was added to the reaction followed by DCM (10ml). The organic layer was separated and the aqueous further extracted with DCM (3x5ml). The combined organics were washed with water (25ml), brine (25ml) and dried over $MgSO_4$. After filtering, the solvent was removed *in vacuo* to give a clear oil (570mg). The crude material was purified by column chromatography on silica eluted with 1-5% ethyl acetate/heptane. Relevant fractions were collected and the solvent evaporated. Two distinct products were obtained as a clear colourless syrups (428mg/73%). LCMS showed two peaks with m/z 757 (MH^+) in a 12:1 ratio. ^{1}H NMR ($CDCl_3$, 270MHz)(data for major isomer): δ 0.46 – 0.59 (m, 6H, 3xSiH_2), δ 0.87 – 0.99 (m, 9H, 3xCH_3), δ 1.41 (s, 9H, Boc), δ 3.35 – 3.52 (m, 4H, $H_{6,6',1,1'}$), δ 3.70 (dd, 1H, J=2.2, 6.8Hz, H_4), δ 3.76 (br, 1H, H_2), δ 3.91 – 4.00 (m, 2H, $H_{3,5}$), δ 4.40 – 4.73 (m, 8H, O-CH_2-Ph), δ 4.92 (br, 1H, NH), δ 7.18 – 7.29 (m, 20H, aromatic).

Preparation of **186**:



186

185 (100mg/0.36mmol) was dissolved in pyridine (1ml) under nitrogen and cooled to <5° C. Pivaloyl chloride (173mg/1.44mmol) was added and the reaction was stirred at 0° C for one hour before allowing it to warm to room temperature gradually. The reaction was stirred under nitrogen at room temperature for 16 hours after which time TLC indicated no remaining starting material. The reaction was filtered and DCM (10ml) was added. The reaction was washed with 1M HCl (10ml), saturated sodium bicarbonate (aq.) (10ml) and brine (10ml). The reaction was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow syrup (84mg). The crude material was purified by column chromatography on silica eluted with 10-50% ethyl acetate/heptane. Relevant fractions were collected and the solvent evaporated. MS

reported a m/z 616 (MH^+) and 638 (MNa^+). ^1H NMR ($CDCl_3$, 270MHz)(data for major isomer): δ 1.15 – 1.31 (m, 36H, 4xO-Piv), δ 1.43 (s, 9H, Boc), δ 3.37 – 3.59 (m, 2H, $H_{1,1'}$), δ 3.79 (d, 1H, H_4), δ 3.96 – 4.37 (m, 4H, $H_{5,3,6,6'}$), δ 4.78 (br, 1H, N*H*).

REFERENCES

1. Winchester, B.G., *Tetrahdron Asymmetry*, **2009**, 20, 645 - 651.
2. Nortey, S. O., Wu, W-N., Maryanoff, B.E., *Carbohydrate Research*, **1997**, 304, 29 - 39.
3. Maryanoff, B.E., Nortey, S. O., Gardocki, J. F., Shank, R. P., Dodgson, S. P., *Journal of Medicinal Chemistry*, **1987**, 30, 880 - 887.
4. Sills, P. E., Leach, J. P., Kilpatrick, W. S., Fraser, C. M., Thompson, G. G., Brodie, M. J. , *Epilepsia*, **2000**, 41 (Suppl. 1) S30 - S34.
5. M.H.R.A., **2011**, PL 35507/ 0072-5.
6. Britzi, M., Perucca, E., Soback, S., Levy, R. H., Fattore, C., Crema, F., Gatti, G., Doose, D. R., Maryanoff, B. E., Bialer, M., *Epilepsia*, **2005**, 46, 378 - 384.
7. F.D.A., Department of Health and Human Services, **2004**.
8. F.D.A., Department of Health and Human Services http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm312468.htm, (accessed 17/07/2014).
9. Gadde, K. M., Allison, D. B., Ryan, D. H., Peterson, C. A., Troupin, B., Schwiers, M. L., Day, W. W., *Lancet*, **2011**, 377, 1341 - 1352.
10. Abou-Khalil, B., Topiramate YOL Study Group., *Epilepsia*, **2000**, 41 (Suppl. 1), S72 - S76.
11. Perucca, E., *Pharmacological Research*, **1997**, 35, 241 - 256.
12. Sachedo, R.C., Sachedo, S. K., Levy, R. H., Streeter, A. J., Bishop, F. E., Kunze, K. L., Mather, G. G., Roskos, L. K., Shen, D. D., Thummel, K. E., Trager, W. F., Curtin, C. R., Doose, D. R., Gisclon, L. G., and Bialer, M. , *Epilepsia*, **2002**, 43, 691 - 696.
13. Garnett, W.R., *Epilepsia*, **2000**, 41 (Suppl. 1), S61 - S65.
14. Shank, R.P., Gardocki, J.F., Streeter, A. J., Maryanoff, B. E., *Epilepsia*, **2000**, 41 (Suppl. 1), S3 - S9.
15. Zona, C., Ciotti, M. T., Avoli, M., *Neuroscience Letters*, **1997**, 231, 123 - 126.
16. Zhang X.-l.,Velumian, A. A., Jones, O. T., Carlen, P. L., *Epilepsia*, **2000**, 41 (Suppl.1), S52 - S60.
17. White, H.S.,Brown, S. D., Woodhead, J. H., Skeen, G, A., Wolf, H. H., *Epilepsia*, **2000**, 41 (Suppl.1), S17 - S20.
18. McLean, M.J., Bukhari, A. A., Wamil, A. W., *Epilepsia*, **2000**, 41 (Suppl. 1), S21 - S24.
19. Bromfield, E.B., Cavazos, J. E., Sirven, J. I., ed., *An Introduction to Epilepsy*, American Epilepsy Society, **2006**.
20. Gibbs III, J.W., Sombati, S., DeLorenzo, R., Coulter, D. A., *Epilepsia*, **2000**, 41 (Suppl. 1), S10 - S16.
21. White, H.S., Brown, S. D., Skeen, G, A., Twyman, R.E., *Epilepsia*, **1995**, 36 (S4), 34.
22. Gordey, M., DeLorey, T. M., Olsen, R., *Epilepsia*, **2000**, 41 (Suppl. 1), S25 - S29.
23. Aggarwal, M., Kondeti, B., McKenna, R., *Expert Opin. Ther. Patents*, **2013**, 23, 717 - 724.
24. Dodgson, S.J., Shank, R. P., Maryanoff, B. E., *Epilepsia*, **2000**, 41 (Suppl. 1) S35 - S39.
25. Nishimori, I., Vullo, D., Innocenti, A., Scozzafava, A., Mastrolorenzo, A., Supuran, C. T., *Journal of Medicinal Chemistry*, **2005**, 48, 7860 - 7866.
26. Supuran, C.T., *Nature Reviews: Drug Discovery*, **2008**, 7, 168 - 181.
27. Supuran, C.T., *Bioorganic and Medicianal Chemistry Letters*, **2010**, 20, 3467.
28. Alterio, V., Di Fiore, A., D'Ambrosia, K., Supuran, C. T., De Simone, G., *Chemical Reviews*, **2012**, 112, 4421 - 4468.

29. McKenna, R., Supuran, C. T., in *Carbonic Anhydrase: Mechanism, Regulation, Links to Disease and Industrial Application*, ed. Frost, S.C., McKenna, R., Springer, **2014**, vol. 75.
30. Christianson, J.D., *Ann. Rev. Biochem.*, **1999**, 68, 33 - 57.
31. McKenna, R., Frost, S. C., in *Carbonic Anhydrase: Mechanism, regulation, links to disease, industrial applications*, ed. Harris, J.R., Springer, **2014**, vol. 75.
32. Boone, C.D., Pinard, M., McKenna, R., Silverman, D., in *Carbonic Anhydrase: mechanism, regulation, links to disease and industrial application*, ed. Frost, S.C., McKenna, R., Springer, **2014**, vol. 75.
33. Voet, D., Voet, J., *Biochemistry*, John Wiley and Sons, 4th edn., **2011**.
34. Temperini, C., Scozzafava, A., Supuran, C.T., in *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural and Disease Applications*, ed. Supuran, C.T., Winum, J.-Y., Wiley, Hoboken, NJ, **2009**.
35. Sly, W.S. and Hu, P. Y., *Annu. Rev. Biochem.*, **1995**, 64, 375 - 401.
36. Winum C.J., Supuran, C. T., *Bioorganic and Medicinal Chemistry*, **2013**, 21, 1419 - 1426.
37. Carta, F., Temperini, C., Innocenti, A., Scozzafava, A., Kaila, K., Supuran, C. T., *Journal of Medicinal Chemistry*, **2010**, 53, 5511 - 5522.
38. Recacha, R., Costanzo, M. J., Maryanoff, B. E., Chattopadhyay, D., *Biochemistry Journal*, **2002**, 361, 437 - 441.
39. Poulsen, S.A., Wilkinson, B. L., Innocenti, A., Vullo, D., Supuran, C. T., *Bioorganic and Medicianal Chemistry Letters*, **2008**, 18, 4624 - 4627.
40. Picard, F., Deshaies, Y., Lalonde, J., Samson, P., Richard, D., *Obesity Research*, **2000**, 8, 656 - 663.
41. Vitale, R.M., Pedone, C., Amodeo, P., Antel, J., Wurl, M., Scozzafava, A., Supuran, C. T., De Simone, G., *Bioorganic and Medicianal Chemistry*, **2007**, 15, 4152 - 4158.
42. Leppik, I.E., *Seizure*, **2004**, 13S, S5-9.
43. Armitage, I., Berne, A. M., Elliott, E. L., Fu, M., Hicks, F., McCubbin, Q., Zhu, L., *Organic Letters*, **2012**, 10, 2626-2629.
44. Woo, L.W.L., Lightowler, M., Purohit, A., Reed, M.J., Potter, B. V. L., *Journal of Steroid Biochemical and Molecular Biology*, **1996**, 57, 79 - 88.
45. Okada, M., Iwashita S., Koizumi, N., *Tetrahedron Letters*, **2000**, 41, 7047 - 7051.
46. Howarth, N.M., Purohit, A., Reed, M. J., Potter, B. V. L., *Journal of Medicinal Chemistry*, **1994**, 37, 219 - 221.
47. Appel, R., Berger G., *Chem. Ber.*, **1958**, 91, 1339 - 1341.
48. Armitage, I., Elliott, E. L., Hicks, F., Langston, M., McCarron, A., McCubbin, Q. J., O'Brien, E., Stirling, M., Zhu, L., *Organic Process Research and Development*, **2015**, 19, 1299 - 1307.
49. Miller, D.C., Carbain, B., Beale, G. S., Alhasan, S. F., Reeves, H. L., Baisch, U., Newell, D. R., Golding, B. T., Griffin, R. J., *Organic and Biomolecular Chemistry*, **2015**, 13, (18) 5279 - 5284.
50. Maryanoff, B.E., Costanzo, M. J., Shank, R. P., Schupsky, J. J., Ortegon, M. E., Vaught, J. L., *Bioorganic and Medicinal Chemistry Letters*, **1993**, 3, 2653 - 2656.
51. Alho, M.A.M., D'Accorso, N., *ARKIVOC*, **2011**, 7, 136 - 148.
52. Alho, M.A.M., D'Accorso, N. B., *Journal of Hetereocyclic Chemistry*, **1999**, 36, 177-182.
53. Izquierdo, I., Plaza, M.T., *Carbohydrate Research*, **1990**, 205, 293-296.
54. Lowe, R.W., Szarek, W. A., Jones, J. K. N., *Carbohydrate Research*, **1973**, 28, 281 - 293.

55. Koos, M., Steiner, B., *Chem. Pap.*, **1996**, 50, 15 - 20.
56. Harfinest, M., Heuser, D.J., Joyner, C. T., Batchelor, J. F., White, H. L., *Jour. Med.* ,39, 1857 - 1862.
57. Ilies, M.A., Vullo, D., Pastorek, J., Scozzafava, A., Ilies, M., Caproiu, M.T., Pastorekova, S., Supuran, C.T., *J. Med. Chem.*, **2003**, 46.
58. Vullo, D., Franchi, M., Gallori, E., Antel, J., Scozzafava, A., Supuran, C.T., *J. Med. Chem.*, **2004**, 47.
59. Maryanoff, B.E., Costanzo, M. J., Nortey, S. O., Greco, M. N., Shank, R. P., Schupsky, J. J., Ortegon, M. E., Vaught, J. L., *Jour. Med. Chem.*, **1998**, 41, 1315 - 1343.
60. Klier, A.H., Alvea, R. J., Prado, M. A. F., Filho, J. D., D'accorso, N. B. , *Synthetic Communications*, **2000**, 30, 4361 - 4374.
61. Gutmann, B.R., J-P., Roberge, D. Kappe, C. O., *Angew. Chem.*, **2010**, 49, 7101.
62. Khalifah, R.G., *J. Biol. Chem.*, **1971**, 246.
63. Winchester, B.G., Fleet, G.W., *Glycobiology*, **1992**, 2, 119 - 210.
64. Horne, G., *Topics in Medicinal Chemistry*, **2014**, 12, 23 - 52.
65. Asano, N., Nash, R. J., Molyneux, R. J., Fleet, G. W. J., *Tetrahdron Asymmetry*, **2000**, 11, 1645 - 1680.
66. Inouye, S., Tsuruoka, T., Niida, T., *Journal of Antibiotics*, **1966**, 19.
67. Compain, P., Martin, O.R, ed., *Iminosugars: from synthesis to therapeutic applications*, John Wiley and Sons Ltd, Chichester,UK., **2007**.
68. Nash, R.J., Bell, E. A., Williams, J. M. , *Phytochemistry*, **1985**, 24, 1620 - 1630.
69. Stocker, B.L., Dangerfield, E. M., Win-Mason, A. L., Haslett, G. W., Timmer, M. S. M., *European Journal of Organic Chemistry*, **2012**, 1615 - 1637.
70. Pearson, M.S.M., Mathe-Allainmat, M., Fargeas, V., Lebreton, J., *European Journal of Organic Chemistry*, **2005**, 2159 - 2191.
71. Compain, P., Chagnault, V., Martin, O.R, *Tetrahedron: Asymmetry*, **2009**, 20, 672 - 711.
72. Butters, T.D., F. M. Platt, *Glycobiology*, **2005**, 15, 43R - 52R.
73. Moriyama, H., Tsukida, T., Inoue, Y., Yokota, K., Yoshino, K., Kondo, H., Miura, N., Nishimura, S-I., *Jour. Med. Chem.*, **2004**, 47, 1930 - 1938.
74. Lenagh-Snow, G.M.J., Jenkinson, S. F., Newberry, S. J., Kato, A., Nakagawa, S., Adachi, I., Wormald, M. R., Yoshihars, A., Morimoto, K., Akimitsu, K., Izumori, K., Fleet, G. W. J., *Organic Letters*, **2012**, 14, 2050 - 2053.
75. Inouye, S., Tsuruoka, T., Ito, T., Niida, T., *Tetrahedron*, **1968**, 23, 2125 - 2144.
76. Best, D., Doctor of Philosopy (D.Phil), University of Oxford, **2010**.
77. Cipolla, L., Lay, L., Nicotra, F., Pangrazio, C., Panza, L., *Tetrahedron*, **1995**, 52, 4679 - 4690.
78. Fowler, P.A., Haines, A.H., Taylor, R.J.K., Chrystal, E.J.T., Gravestock, M.B., *Carbohydrate Research*, **1993**, 246, 377 - 381.
79. Maughan, M.A.T., Davies, I. G., Claridge, T. D. W., Courtney, S., Hay, P., Davis, B. G., *Angew. Chem. Int. Ed.*, **2003**, 42, 3788 - 3792.
80. Godin, G., Compain, P., Masson, G., Martin, O. R., *Journal of Organic Chemistry*, **2002**, 67, 6960 - 6970.
81. Spreitz, J., Stutz, A. E., Wrodnigg, T. M., *Carbohydrate Research*, **2002**, 337, 183 - 186.
82. Martin, O., Saavdra, O. M., Xie, K., Liu, L., Picasso, S., Vogel, P., Kizu, H., Asano, N., *Bioorganic and Medicianal Chemistry*, **2001**, 9, 1269 - 1278.
83. Martin, O., Liu, L., Yang, F., *Tetrahedron Letters*, **1996**, 37, 1991 - 1994.

84. Goujon, J.Y., Gueyard, D., Compain, P., Martin, O., Ikeda, K., Kato, A., Asano, N., *Bioorganic and Medicianal Chemistry*, **2005**, 13, 2313 - 2324.
85. Banba, Y., Abe, C., Nemoto, H., Kato, A., Adachi, I., Takahata, H., *Tetrahdron Asymmetry*, **2001**, 12, 817 - 819.
86. Felpin, F.X., Lebreton, J. , *European Journal of Organic Chemistry*, **2003**, 3693 - 3712.
87. Kummeter, M., Kazmaier, U., *European Journal of Organic Chemistry*, **2003**, 3330 - 3334.
88. Haukaas, M.H., O'Doherty, G.A., *Organic Letters*, **2001**, 3, 401 - 401.
89. Banwell, M.G., Ma, X., Asano, N., Ikeda, K., Lambert, J. N., *Organic and Biomolecular Chemistry*, **2003**, 3, 2035 - 2037.
90. Schuster, M., *Bioorganic and Medicianal Chemistry Letters*, **1999**, 9, 615 - 618.
91. Schurer, S.C., Blechert, S., *Tetrahedron Letters*, **1999**, 40, 1877 - 1880.
92. Dhavale, D.D., Matin, M. M., *ARKIVOC*, **2005**, iii, 110 - 132.
93. Reuillon, A.B.T., R. J. Griffin, D.C. Miller, B. T. Golding, *Organic and Biomolecular Chemistry*, **2012**, 10.
94. Joseph, C.C., Regeling, H., Zwanenburg, B., Chittenden, G. J. F., *Carbohydrate Research*, **2002**, 337, 1083 - 1087.
95. Furneaux, R.H., Tyler, P. C., Whitehouse, L. A., *Tetrahdron Letters*, **1993**, 34, 3613 - 3616.
96. Furneaux, R.H., P. C., Whitehouse, L. A., *Tetrahedron Letters*, **1993**, 34, 3613 - 3616.
97. Hollingsworth, R.I., Wang, G., *Chemical Reviews*, **2000**, 100, 4267 - 4282.
98. Helleur, R., Rao, V. S., Perlin, A. S., *Carbohydrate Research*, **1981**, 89, 83 -90.
99. Ramstadius, C., Boklund, M., Cumpstey, I., *Tetrahedron: Asymmetry*, **2011**, 22, 399 - 405.
100. Jitian Liu, Y.T., Kaigui Wu, Caifeng Bi, Qui Cui, *Carbohydrate Research*, **2012**, 350, 20-24.
101. Nurminen, E.,Poijarvi, P., Koskua, K., Hovinen, J., *Journal of Chemical Education*, **2007**, 84, 1480-1482.
102. La Ferla, B., Cipolla, L., Nicotra, F., in *Iminosugars: from synthesis to therapeutic applications*, ed. P. Compain, Martin, O.R, John Wiley and Sons Ltd., Chichester, **2007**, Chapter 3.
103. Pawar, N.J., Parihar, V.J., Chavan, S.T., Joshi, R., Joshi, P. V., Sabarwal, S.G., Puranik, V.G., Dhavale, D.D., *Journal of Organic Chemistry*, **2012**, 77, 7873 - 7882.
104. Jenkinson, S.F., Best, D., Saville, A. W., Mui, J., Martinez, R. F., Nakagawa, S., Kunimatsu, T., Alonzi, D. S., Butters, T. D., Norez, C., Becq, F., Bleriot, Y., Wilson, F. X, Weymouth-Wilson, A. C., Kato, A., Fleet, G. W. J, *Journal of Organic Chemistry*, **2013**, 78, 7380 - 7397.
105. Sadhu, P.S., Santhoshi, A., Rao, V. J., *Bull. Korean Chem. Soc.*, **2012**, 33.
106. Afonso, C.A.M., *Tetrahedron Letters*, **1995**, 36, 8857 - 8858.
107. Ariza, X., Urpi, F., Viladomat, C., Vilarrasa, J., *Tetrahedron Letters*, **1998**, 39, 9101 - 9102.
108. Suzuki, K., Hashimoto, H., *Tetrahedron Letters*, **1994**, 35, 4119 - 4122.