The Synthesis of Azadisaccharides and Aminopyrrolidines as Potential Glycosidase Inhibitors

Thesis submitted for the degree of

Doctor of Philosophy at the University of Leicester

by

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Statement

The accompanying thesis submitted for the degree of Ph.D. entitled "The Synthesis of Azadisaccharides and Aminopyrrolidines as Potential Glycosidase Inhibitors" is based on work conducted by the author in the Department of Chemistry at the University of Leicester between the period October 2001 to September 2004.

All the work recorded in this thesis is original unless otherwise acknowledged in the text or by references. None of the work has been submitted for another degree in this or any other university.

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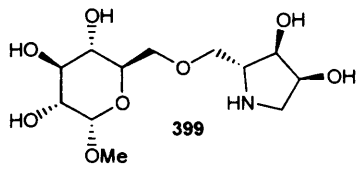
Abstract

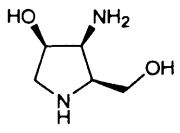
Azadisaccharides *e.g.* **399** (Figure 1) are known to be potent selective inhibitors of glycosidases and thus have potential as anti-viral, anti-cancer and anti-diabetic agents. We have developed new, efficient, versatile and stereoselective routes to azadisaccharides using non-carbohydrate starting materials. Pinacol methodology has been successfully used to synthesise two (2→6) linked homoaza-*O*-disaccharides mimics and due to the versatility of the synthetic route, modification has enabled the synthesis of a (2→6) linked homoaza-*O*-trisaccharide. Complementary to this, RCM (Grubbs catalyst) and stereoselective dihydroxylation (OsO_4) has been utilised in the synthesis of (1→6) linked 5-deoxy-pyrrolidine and piperidine homoaza-*O*- and *N*- disaccharides as single diastereoisomers. Preliminary biological screening of these compounds has identified **399** as a weak selective inhibitor of naringinase.



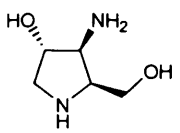
399

Figure 1

Aminopyrrolidines are compounds of great chemical and biochemical interest and are versatile chiral intermediates in the synthesis of compounds of considerable therapeutic value. Three novel diastereomeric 2-hydroxymethyl-3-amino-4-hydroxylpyrrolidines have been synthesised from one key homoallylic carbamate. Using a tethered aminohydroxylation and a regio- and stereoselective intramolecular epoxide opening the novel (2*S*, 3*S*, 4*R*) isomer **488** and (2*S*, 3*S*, 4*S*) isomer **489** (Figure 2) have been synthesised respectively. Access to a third (2*S*, 3*R*, 4*R*) isomer was also achieved using an unprecedented regioselective intermolecular epoxide opening. Preliminary biological screening of **488** and **489** has identified both as weak inhibitors of β-galactosidase.



488
(2*S*, 3*S*, 4*R*)



489
(2*S*, 3*S*, 4*S*)

Figure 2

Contents

Acknowledgements

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Finally, I would like to say a big thank you to both my parents and David for their continued support and encouragement throughout my PhD.

Abbreviations

Ac	acetyl
acac	acetylacetone
app	apparent
Ar	aryl
aq.	aqueous
Bn	benzyl
$(Boc)_2O$	di-*tert* butyl dicarbonate
^{t}Bu	*tert*-butyl
Bz	benzoyl
Cbz	benzyloxycarbonyl
^{13}C	carbon
mCPBA	*meta*-chloroperoxybenzoic acid
D-AB1	1,4-dideoxy-1,4-imino-D-arabinitol
DHQD	dihydroquinidine
DHQ	dihydroquinine
DMF	*N,N*-dimethylformamide
DMP	Dess-Martin periodinane
DMAP	dimethylaminopyridine
DNJ	deoxynojirimycin
dppf	[1,1'-Bis(diphenylphophino)ferrocene]
e.g.	*exempli gratia* (latin "for example")
Equiv	equivalents
EI	electron impact
ES	electrospray
Et	ethyl
FAB	fast atom bombardment
^1H	proton
h	hour
HCl	hydrochloric acid
HOBt	hydroxybenzotriazole
liq.	liquid
M	molar
Me	methyl
MHz	megaHertz
min	minute(s)

Mp	melting point
NBA	*N*-bromoacetamide
NBS	*N*-bromosuccinimide
NMO	*N*-methyl-morpholine *N*-oxide
NMR	nuclear magnetic resonance
NJ	nojirimycin
nOe	nuclear Overhauser effect
Nuc	nucleophile
P	protecting Group
Ph	phenyl
Pd/C	palladium on carbon
^{i}Pr	*iso*-propyl
^{n}Pr	*n*-propyl
PMP	*para*-methoxyphenyl
Py	pyridine
RCM	ring closing metathesis
R_f	retention factor
r.t.	room temperature
TBAF	tetrabutylammonium fluoride
TBDPSCl	tert-butyldiphenylsilyl chloride
tert	tertiary
Tf	trifluoromethanesulfonic
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Z	benzyloxycarbonyl

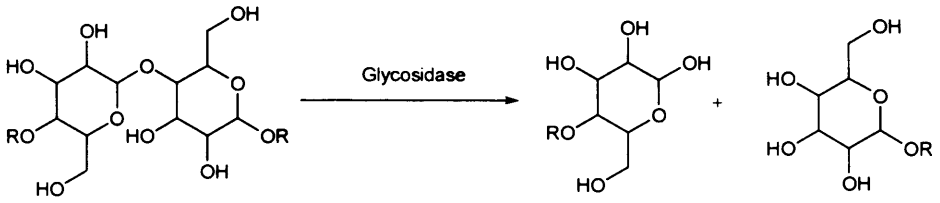
Chapter 1
Introduction

1.0 Introduction

1.1 Glycosidase enzymes and azasugars

1.1.1 The glycosidic bond

The glycosidic bond that links two glucose residues is the most stable linkage within naturally occurring biopolymers; for example the half-life for the spontaneous hydrolysis of starch is in the range of 5 million years.[1] The enzymes responsible for cleaving the glycosidic bond are known as glycosidases (Scheme 1) and these catalysts are very proficient, accomplishing hydrolysis with rate constants up to $1000s^{-1}$.[1] Glycosidases have considerable biological importance as they are involved in a wide range of vital natural processes, such as intestinal digestion, post-translational processing of glycoproteins and the lysosomal catabolism of glycoconjugates.[2]



Scheme 1

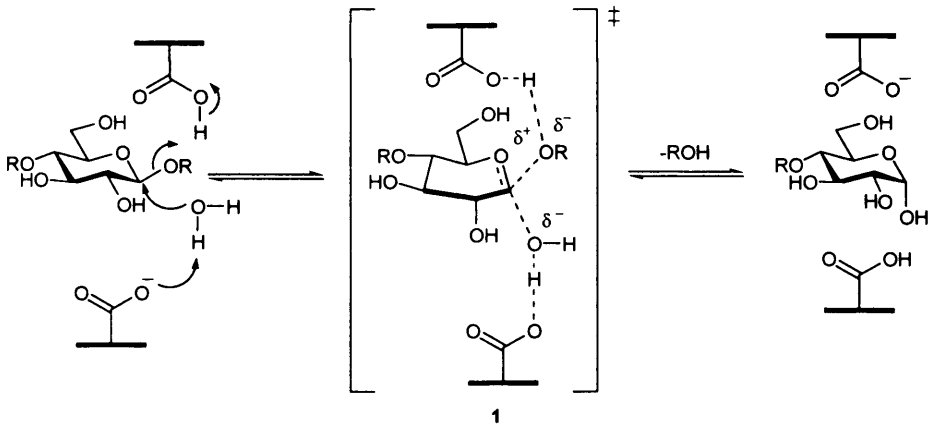
1.1.2 Inhibition of glycosidases

There has been substantial interest in recent years in designing, synthesising and testing glycosidase inhibitors, both to enable a greater insight into the active site structures and mechanisms of these interesting enzymes, and to also generate new therapeutics.[3] Strong selective inhibitors of glycosidic cleaving enzymes have many potential applications and certain sugar mimics have aroused increasing interest as potential anti-viral, anti-cancer, anti-diabetic agents and agrochemicals.[4] Much of the effort toward the synthesis of glycosidase inhibitors has gone into the formation of nitrogen-containing sugar analogues *i.e.* azasugars, of which there are several classes. Designs for these have been inspired both by mechanistic information and the structures of naturally occurring azasugar inhibitors.[3]

1.1.3 Mechanism of glycosidases[1]

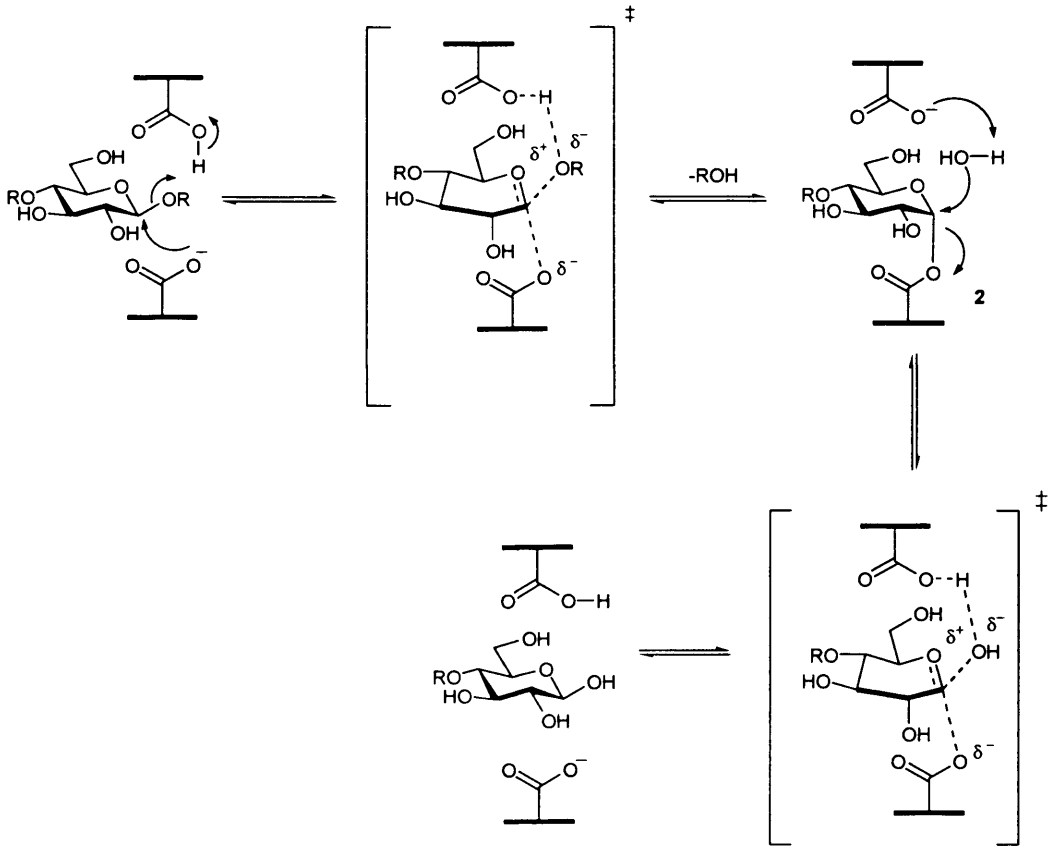
Hydrolysis of the glycosidic bond can occur with one of two possible stereochemical outcomes: inversion or retention at the anomeric configuration. This observation can be explained by the existence of two different enzyme mechanisms. Inverting enzymes catalyse glycosidic cleavage *via* a single displacement mechanism involving an oxocarbenium ion-like transition state **1** (Scheme 2). The two carboxyl groups in the active site of inverting

glycosidases act as general acid and general base catalysts and are suitably positioned (~10 Å apart) to allow the substrate and a water molecule to bind between them.



Scheme 2

In contrast, the carboxyl groups in the active site of retaining glycosidases are only 5.5 Å apart, consistent with a double displacement mechanism. This is a two-step mechanism involving a covalent glycosyl intermediate **2** (Scheme 3) and both steps occur *via* transition states with substantial oxocarbenium ion character.



Scheme 3

Both mechanisms of glycosyl hydrolysis occur *via* a related transition state **1** (Figure 1). An attractive approach to designing and synthesising potent azasugar inhibitors has been to mimic this transition state, the rationale being that the transition state has the highest degree of enzymatic stabilization. Direct information about the transition state has been obtained from kinetic isotope studies.[4] These indicate various degrees of sp^2 character at the anomeric carbon, supporting the formation of the oxocarbenium ion-like structure. It is also proposed the transition state adopts a half-chair conformation to accommodate this cationic character (Figure 1).[4]

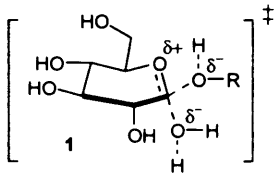


Figure 1

1.1.4 Naturally occurring azasugars[2]

Strong inhibition of glycosidases by naturally occurring azasugars has been known for 30 years and studied intensively for the last 15 years.[5] Naturally occurring azasugars are believed to be widespread in plants and microorganisms and are classified into five structural classes: polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines and nortropanes. This area has been extensively reviewed by Asano and co-workers[2] and a few examples will now be discussed.

In 1966, the piperidine, nojirimycin (NJ) **3** (Figure 2) was discovered as the first natural glucose mimic, being produced by *Streptomyces roseochromogens* R-468 and was shown to be a potent inhibitor of α- and β-glucosidases from various sources.

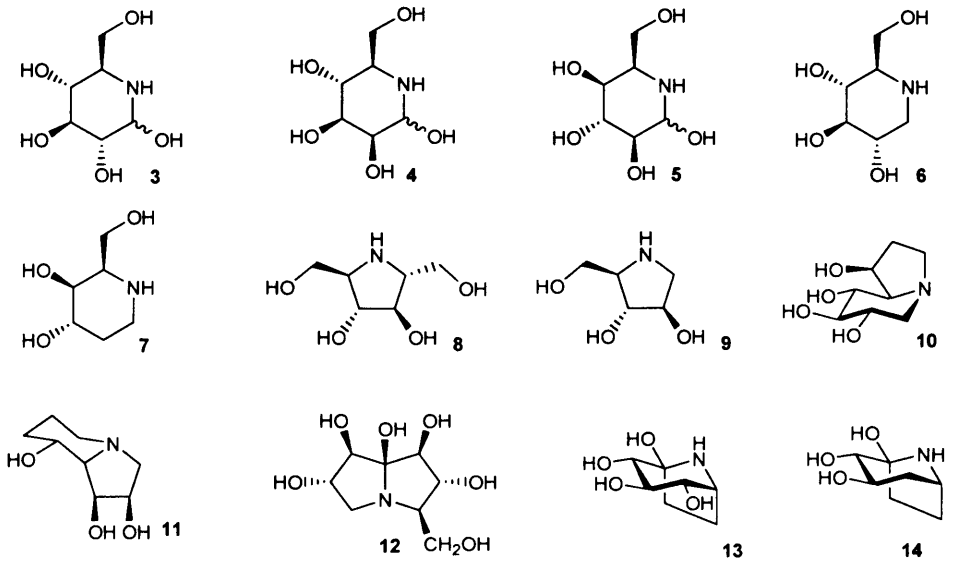


Figure 2

Soon after, the related compounds nojirimycin B (*manno*-NJ) **4** and galactostatin (*galacto*-NJ) **5** were also isolated from the fermentation broths of species of *Streptomyces*. Since then many other naturally occurring azasugars from each structural class have been isolated.

1-Deoxynojirimycin (DNJ) **6** was firstly isolated from Mulberry trees but is also produced by many strains of *Bacillus* and *Streptomyces* and 1,2-dideoxy-nojirimycin (fagomine) **7**, was isolated from the seeds of Japanese buckwheat (*Fagopyrum esculentum*) and the Moreton Bay chestnut (black bean). In 1976 the pyrrolidine 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) **8** was found in leaves of the legume *Dertris elliptica*[2] and the pyrrolidine 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1) **9** was first isolated from the fruits of the legume *Anglyocalyx boutiquenus*.[6] The toxicity to livestock of the legumes *Swainsona canescens* and *Castanospermum australe* in Australia led to the isolation of the toxic indolizidines swainsonine **10** and castanospermine **11** (Figure 2). The pyrrolizidine, casuarine **12** was isolated from the bark of *Casuarina equisetifolia* and the nortropane containing calystegines *e.g.* **13** and **14**, were found to be abundant in the underground organs and root exudates of *Calystegia sepium*, *Convolvulus arvensis* and *Atropa belladonna*.

Naturally occurring azasugars have a high affinity for glycosidases, binding 10^3-10^4 times more strongly than glucose, and thus are strong inhibitors.[7] As a result these sugar mimics have been found to exhibit varying therapeutic properties. For example, the origin and isolation of deoxynojirimycin (DNJ) **6** was prompted by the knowledge that extracts of Mulberry were able to suppress the rise in blood glucose that follows eating, and thus **6** has a potential application in the treatment of diabetes.[2] In enzyme assays D-AB1 **9** has been found to be a potent inhibitor of hepatic glycogen phosphorylase *in vitro* and *in vivo* and therefore has a potential use in the treatment of type II diabetes, where hepatic glucose production is elevated.[6] Casuarine **12** has been prescribed in Western Samoa for the treatment of breast cancer and the calystegine B_2 **14** and swainsonine **10** have been implicated in lysosomal storage diseases.[2] Castanospermine **11** and derivatives have been shown to inhibit replication of the HIV virus and swainsonine **10** has attracted attention in the area of tumour metastasis as it inhibits tumour growth and stimulates the immune response.[2]

1.2 Design of glycosidase inhibitors

1.2.1 Design of azasugars as glycosidase inhibitors

In view of transition-state information and the existence of naturally occurring azasugars, many studies have been undertaken in an attempt to understand the factors that contribute to a good and potent glycosidase inhibitor. This area has been extensively reviewed

by Lundt and Madsen[8] where it has been proposed the following features of azasugar analogues are of importance for their inhibitory potency:

i. Position of the basic (cationic) centre. Basic sites can form salt bridges/hydrogen bonds with acid catalytic groups.

ii. Geometry at the anomeric position - to resemble the oxocarbenium-ion like transition state

iii. Hydroxylation pattern and ring size.

iv. Multiple interactions - greater selectivity of inhibition can be observed if the azasugar is attached to another sugar moiety forming an azadisaccharide. This will be discussed in more detail in section 1.2.3.

1.2.2 The design and synthesis of glycosidase inhibitors of non-natural product origin[4]

There have been many reports in the literature regarding the synthesis of non-natural sugar mimics that have been designed to encompass the factors above and this area has been recently reviewed by Bols and co-workers.[4] To illustrate the area, a few examples will be summarised.

1.2.2.1 Position of the cationic centre

In an attempt to investigate how the position of the cationic centre influences inhibition, a variety of derivatives have been synthesised, some of which are illustrated in Figure 3.

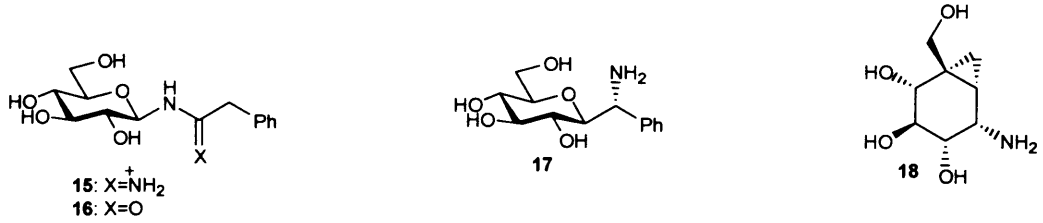


Figure 3

It is believed, in the early stages of glycoside cleavage of an ordinary *O*-glycoside, protonation of the exocyclic oxygen of the glycosidic bond occurs. Thus, it is proposed there may be considerable charge on this exocyclic atom during the early stages of hydrolysis[4] and a variety of different analogues have hence been designed and synthesised to try and mimic this.

For example Hiratake and co-workers[9] reported the synthesis of the glycosylamidine **15**, which was found to be both an active and selective glycosidase inhibitor, displaying activity against glucosidases but not galactosidases. The importance of the positive charge was shown by synthesising and testing the neutral glucosylamide equivalent **16**, which was consequently found to be a very weak inhibitor of all glycosidases. In comparison, Schmidt and co-workers[10] synthesised the substituted phenylglucoside **17** in an attempt to mimic exocyclic

positive charge, and interestingly, this compound was determined to be a potent inhibitor against a β-glucosidase (K_i = 70μM). As an alternative approach to imitate exocyclic charge development, Tanaka and co-workers synthesised the bicyclo-[4.1.0] heptane derivative **18**. This compound was also found to be remarkably potent, displaying inhibition against α-glucosidase (K_i = 0.1μM).[4]

1.2.2.2 Geometry at the anomeric centre

In glycoside hydrolysis the actual transition state is thought to closely resemble the intermediate **1** (Figure 1) where there is substantial positive charge on the endocyclic oxygen atom. It is believed the activity of nojirimycin **3** as a very potent glycosidase inhibitor is because of its ability to undergo a reversible dehydration to form the iminium ion **19**, which closely resembles both the charge and shape of this transition state (Figure 4).[8] The inhibitory properties of related natural azasugars *e.g.* DNJ **6** is thought to stem from their ability to become protonated in the active site forming a cation, which can interact strongly with an anionic group (carboxylate) present at the enzyme active site. Yet, there is some dispute as to whether these are true transition state mimics, as DNJ **6** and other azasugars adopt a chair conformation instead of the expected half-chair conformation of the transition state **1** or the iminium ion **19**. [11] As a result these compounds cannot be expected to be perfect transition state analogues and consequently, substantial effort has gone into synthesising improved transition state analogues that mimic both the charge and shape of the oxocarbenium-ion intermediate (Figure 4).

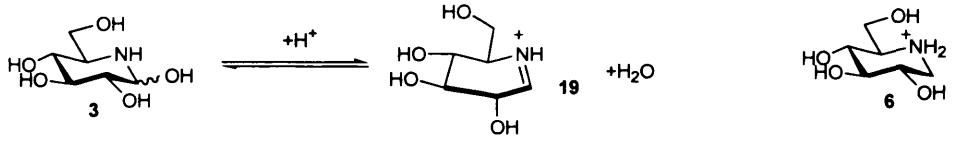


Figure 4

For example, Tatsuta and co-workers have synthesised a variety of compounds incorporating an sp^2 hybridised *C*-1 centre, *i.e.* the imidazole derivative **20** (Figure 5).[12] The authors proposed this compound would be an improved transition state analogue since it would more closely reflect the partially planar nature of the sugar ring and the build up of positive charge on *C*-1. Furthermore, it was also thought the presence of an exocyclic heteroatom on *C*-1 would additionally mimic the departing aglycon. This theory was somewhat supported as **20** was found to be a potent inhibitor of β-glucosidase from almonds (K_i = 0.1μM). Vasella and co-workers[11] have also synthesised related compounds, *e.g.* the

tetrazole derivative **21** and inhibition studies have determined this derivative to be an effective inhibitor of bovine kidney β-glucosaminidase (K_i = 0.2μM).

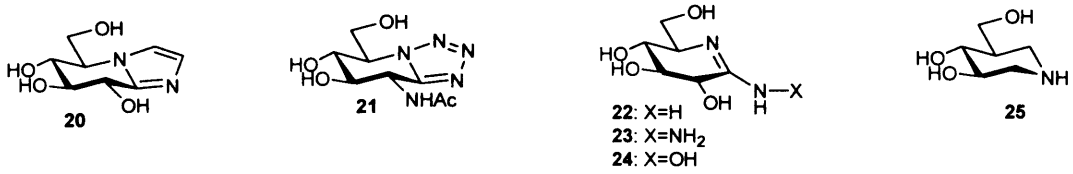


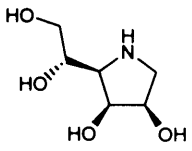
Figure 5

An alternative approach to mimic transition state charge and shape was reported by Ganem and co-workers[13] where the synthesis of the amidine, hydrazine and oxime structures **22-24** is described. Biological screening of these compounds revealed them to be potent, broad spectrum competitive inhibitors of glucosidases, mannosidases and galactosidases.

In 1994 Bols and co-workers[14] reported the first synthesis of the azasugar derivative **25**, where a nitrogen atom had been incorporated in place of C-1 *i.e.* in the pseudo-anomeric position (Figure 5). For stability reasons the 2-hydroxy group was omitted and because of the close resemblance to fagomine **7**, the compound was named isofagomine. The authors proposed that by having the endocyclic nitrogen located at the position corresponding to the anomeric centre this would possibly mimic the charge generated at this centre in the transition state. Subsequent testing of **25** revealed it to exhibit considerable activity, inhibiting β-glycosidase from almonds 500-fold more effectively than DNJ **6**.

1.2.2.3 Hydroxylation pattern and ring size

In addition to piperidines, polyhydroxylated pyrrolidines and azepanes have also been shown to exhibit considerable inhibitory activity (Figure 6).[8] At first sight, though the pyrrolidine type compounds *e.g.* **26** and **27** bear less structural resemblance to the oxocarbenium-like transition state, they have been shown to inhibit many glycosidases with 100-fold greater activity than DNJ **6**.[8] The proposed reason for this is thought to be due to the pyrrolidine–type inhibitors adopting a planar, half-chair conformation with greater similarity to the transition state and additionally because the hydroxyl groups are better orientated to interact with the active site. In contrast azepanes *e.g.* **28** are more flexible than the corresponding pyrrolidines and piperidines and can adopt many puckered low-energy conformations. As a result they are more flexible and can adapt to the space filling and polar requirements of glycosidase active sites. Consequently **28** is a moderate but non-specific inhibitor of a range of glycosidases.[8]



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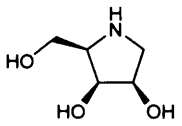

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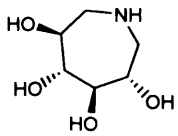


28

Figure 6

To summarise, there are many factors that contribute to a potent glycosidase inhibitor and some of these have been discussed. However, it is proposed[15] that not only potency, but greater selectivity of inhibition can be observed if the number of enzyme-inhibitor interactions is increased. This can be achieved if the azasugar is attached to a natural sugar *i.e.* forming an azadisaccharide and consequently, the design and synthesis of these relatively new sugar mimics will be the main focus of the remainder of this introduction.

1.2.3 Azadisaccharides as glycosidase inhibitors

As discussed above, most of the glycosidase inhibitors that have been synthesised to date are analogs of the glycon part of the substrate or the transition state. Whilst some very potent inhibitors have been discovered, these can inhibit a broad spectrum of glycosidase enzymes and therefore detrimental effects could potentially result if they are used as therapeutics. The aglycon 'R' (Figure 1) is also part of the transition state and its interactions with the corresponding element of the active site are very important in recognition and catalysis.[8] Consequently, it has been proposed[15] that di- or oligosaccharides incorporating an azasugar could be more accurate transition state analogues and consequently more selective inhibitors. This theory has been reinforced by the discovery of naturally biologically active azadisaccharides and by the synthesis of disaccharide derivatives that also display considerable activity.

1.2.4 Naturally occurring azadisaccharides

In 1968 validamycin A **29** was discovered by the researchers of Takeda Chemical Industries as the major and most active component of the validamycin family, isolated from the cultured broth of *Streptomyces hygroscopics var. limoneus*.[6] Takeda commercialised validamycin A for the control of the pathogen responsible for sheath blight disease in rice plants as well as for treating diseases in vegetable seedlings. Validamycin A **29** contains the pseudodisaccharide validoxylamine A **30** and an additional glucose unit and both validamycin **29** and validoxylamine A **30** are powerful inhibitors of the trehalase enzymes found in *R. solani* and other organisms. Since the discovery of these two compounds many other trehalase inhibitors have been discovered and these have all been found to be pseudodisaccharide in

structure *e.g.*, the naturally occurring trehazolin **31**, salbostatin **32** and casuarine-6-*O*-α-glucopyranoside **33**, and the synthetic azadisaccharide derivative MDL 25637 **34** (Figure 7) (the synthesis and more detailed analysis of **34** will be discussed in section 1.3.2). These compounds are powerful competitive inhibitors of porcine kidney trehalases with K_i values in the nanomolar range. There are two sub-sites in the enzyme active site, one for catalysis and one for recognition. It is proposed the extremely high affinity of pseudodisaccharide inhibitors derives from the synergistic interactions of an alkaloid unit and a sugar unit in these two sub-sites.[6]

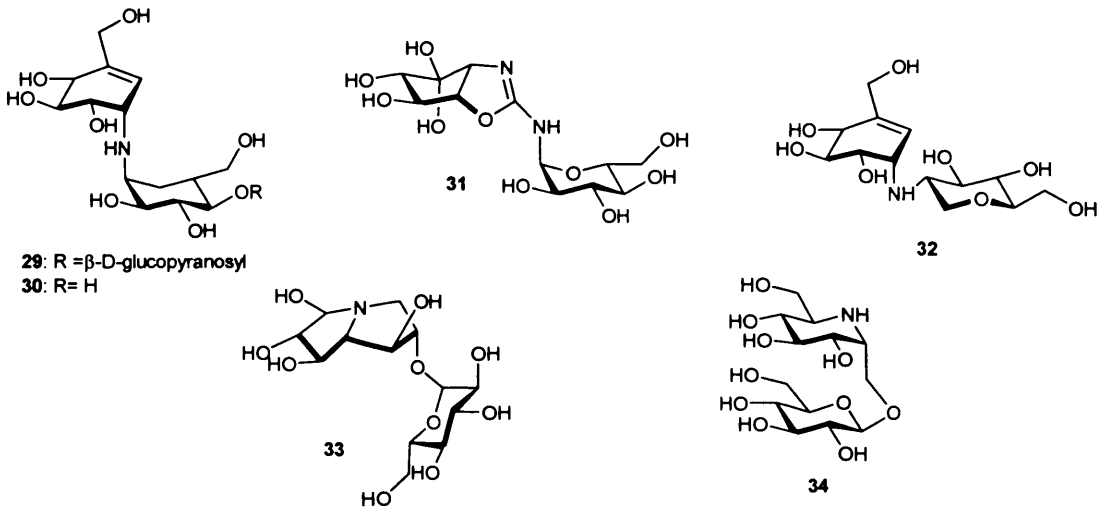


Figure 7

As part of an attempt to isolate new natural glycosidase inhibitors Asano and co-workers isolated four piperidine azadisaccharides **35-38** from the leaves and roots of *Morus alba* together with the pyrrolidine azadisaccharide **39** and a number of monosaccharide inhibitors (Figure 8).[16] Compounds **35-38** were found to selectively inhibit α-glucosidase (Rice) with IC_{50} values in μM range (*e.g.* **35**: IC_{50} = 0.95μM) where as the pyrrolidine **39** was determined to only be a weak inhibitor of this enzyme (IC_{50} = 0.46mM).

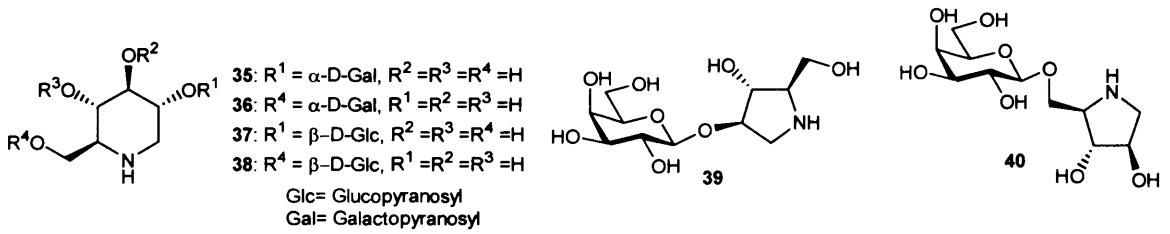
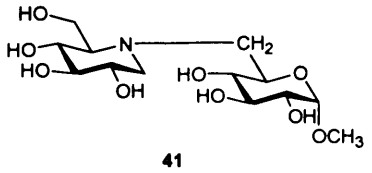


Figure 8

In a later study the same authors also isolated the same pyrrolidine **39** together with **40** and a range of other monosaccharide inhibitors from the bark and pods of *A. pynaetii*, a plant growing in tropical African forests.[17] Compounds **39** and **40** were tested against a range of glycosidase enzymes, although unfortunately no significant biological activity was detected.

As discussed previously (section 1.1.4), DNJ **6** had been found to suppress the rise in blood glucose that follows eating and hence was found to be a potent inhibitor of mammalian α-glucosidases *in vitro*. This opened the possibility of a therapeutic application for DNJ, however the activity of **6** *in vivo* was only moderate. Therefore a large number of DNJ derivatives were prepared with the aim of increasing *in vivo* activity.[18] One of these compounds was MDL 25637 **34**[19] (mentioned above) and the other the *N*-linked azadisaccharide **41** (Figure 9).[20]



41

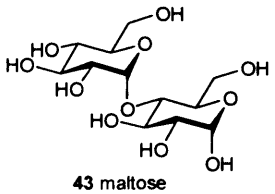
Figure 9

These, together with other selected compounds, were found to effectively reduce postprandial elevations of blood glucose and plasma insulin in animal loading tests with starch and sucrose. In particular **41** was shown to have a long lasting inhibitory effect, reported to be caused by quasi-irreversible binding to α-glucosidases.[20]

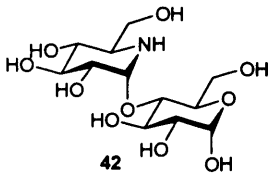
1.2.5 Approaches to azadisaccharide design and synthesis

The area of azadisaccharide design, synthesis and biological testing is relatively new (in comparison to monosaccharide azasugars). Yet, due to the recent emergence of these compounds as potential therapeutics there has been a surge of reports regarding the synthesis of azadisaccharide analogues and this area has recently been reviewed by Martin.[21]

When designing and synthesising an azadisaccharide in which the azasugar is intended to mimic the glycon part of the substrate, there are limitations in how the azasugar can be linked to the natural aglycon.



43 maltose



42

Figure 10

If an exact aza-analog of a disaccharide is made *e.g.* **42** for maltose **43** (Figure 10), this would be expected to undergo rapid cleavage within the active site of the enzyme (if not before) due to the lability of the *O,N*-acetal function. Consequently, these compounds would not be very good inhibitors and therefore would be of little use as biological probes.[21] In order to generate

stable structures simulating aza-analogs of glycoconjugates, chemists have used several inventive solutions.

The majority of the literature reports concerning azadisaccharides involve the synthesis of aza-*C*-disaccharides, where the oxygen atom of the *O,N*-acetal is replaced by a methylene group *e.g.* **44**[22] (Figure 11). However another class of azadisaccharides, which are less documented, are the homoaza-*O*-disaccharides where a methylene group is inserted into the C-O bond of the *O,N*-acetal *e.g.* **34**[19,23] (Figure 11). Analogs of azadisaccharides have also been prepared by replacing the interglycosidic oxygen atom by sulphur **46** or nitrogen **47**[24] by linking the aglycon directly to the nitrogen atom *e.g.* **48**[25] and by linking the aglycon *via* an amidine *e.g.* **49**[26] (Figure 11).

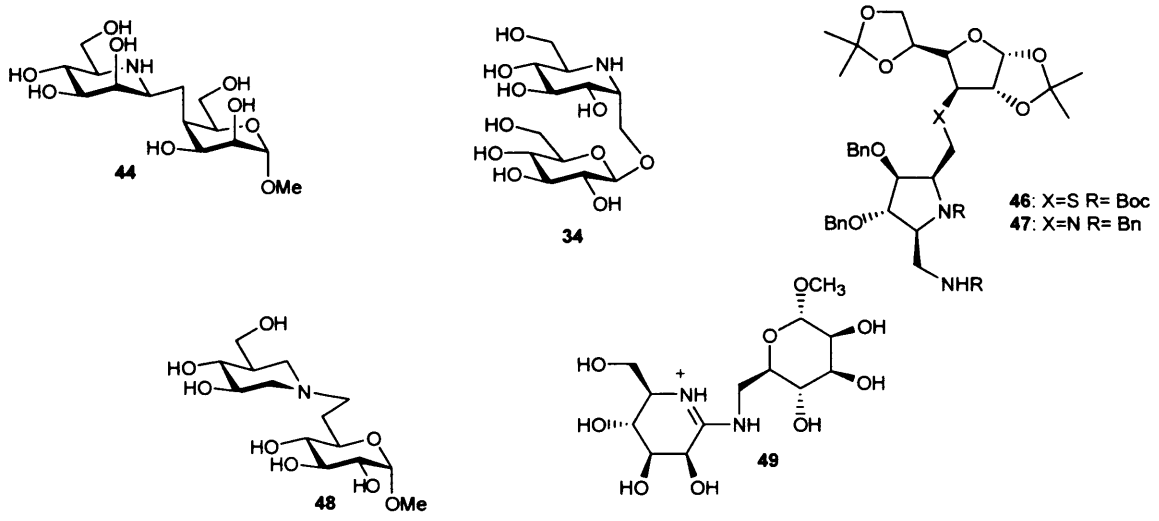


Figure 11

1.3 Current routes to azadisaccharides

As discussed above, the term azadisaccharide covers a wide range of different compounds. To try and summarise the literature within this area, four main classes of azadisaccharide will be discussed:

i. Aza-*C*-disaccharides

ii. Homoaza-*O*-disaccharides

iii. Aza-*N*- and *S*-disaccharides

iv. Miscellaneous (*e.g.* amidine linked)

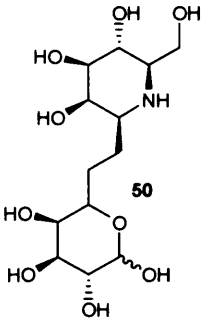
To summarise the literature, each heading will be divided into sections depending on how the azasugar is linked to the aglycon. For example **44** (Figure 11) is a (1→4) linked aza-*C*-disaccharide.

1.3.1 Aza-*C*-disaccharides

Aza-*C*-disaccharides are an emerging class of carbohydrate mimics and are the most common form of azadisaccharide reported within the literature. The first example of an aza-*C*-disaccharide was D-azaMan-β-(1→6)-D-Gal **50** (Figure 12) synthesised by a Johnson and co-workers.[27] Shortly after Martin and co-workers[28] synthesised a hydroxymethylene (1→6) linked aza-*C*-disaccharide and Van Boom[29], Dondoni[30], Wightman[31] and Argyropoulos[32] have all synthesised (1→6) linked aza-*C*-disaccharides (also termed linear azadisaccharides). Vogel and co-workers[33] reported the first synthesis of a branched system, and have also synthesised (1→3),[34,35] (1→4)[36] and (1→2)[37] linked aza-*C*-disaccharides using varying methodologies. Johnson and co-workers in addition to their first report have also achieved the synthesis of (1→1) and (1→4) linked compounds[22], and Brandi and co-workers have synthesised both (1→3) and (1→2) linked aza-*C*-disaccharides.[38] As it is not possible to cover exhaustively all of this literature, a brief review of this work will be considered here.

1.3.1.1 Linear aza-C- disaccharides; (1→6) linked

Johnson and co-workers[27] synthesised the first aza-*C*-disaccharide **50**. This compound fully resembles the parent disaccharide 6-*O*-β-D-mannopyranosyl-D-galactose **51** (Figure 12).



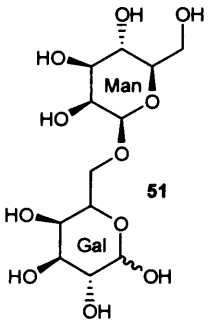
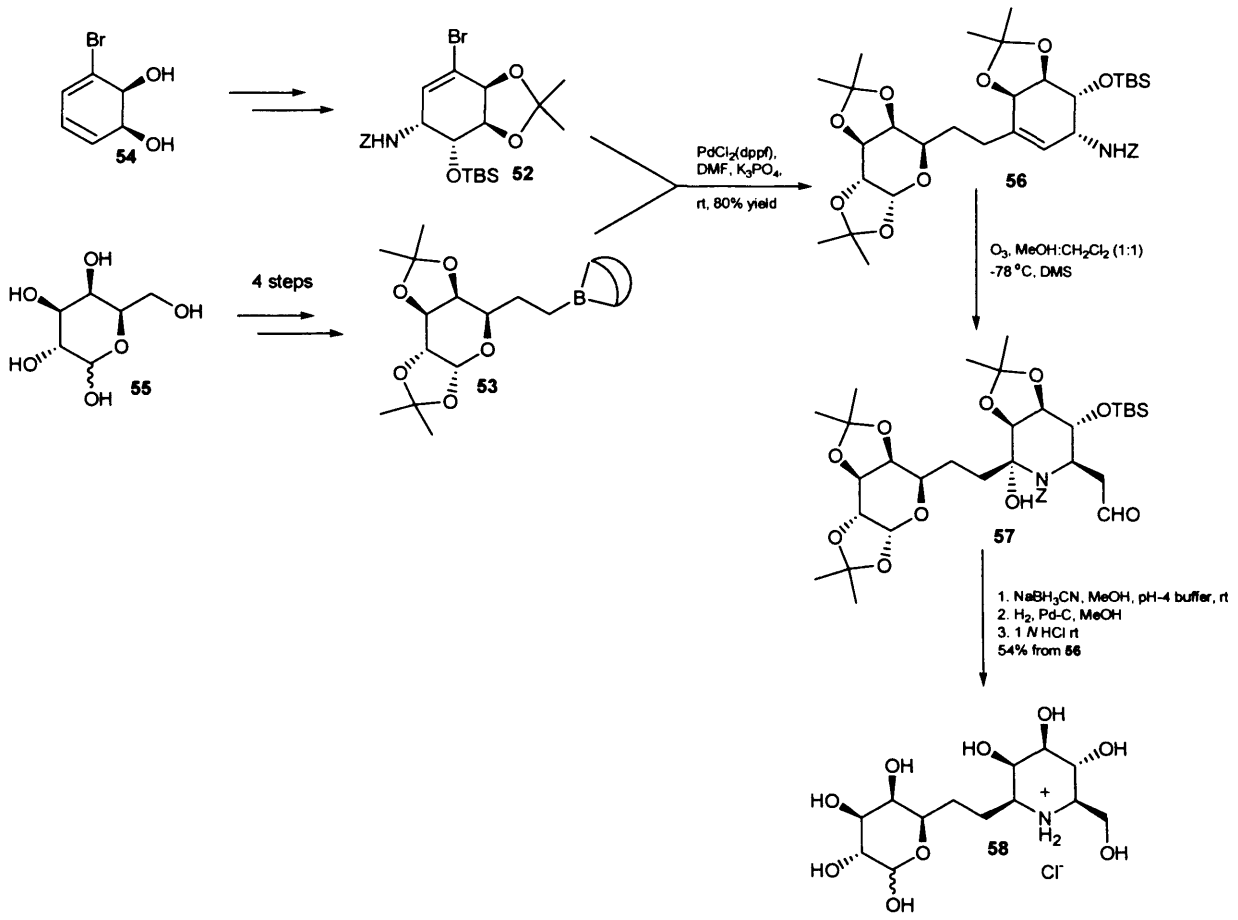


Figure 12

A palladium-catalysed Suzuki coupling of a vinyl bromide **52**, with an alkylboron transmetallation partner **53** was used as the key synthetic step to synthesise **50** (Scheme 4). The vinyl bromide **52** was prepared from the enantiopure bromodiol **54**, a product from microbial oxidation of bromobenzene. The alkyl boron reagent **53** was generated by hydroboration of the corresponding olefin, which was synthesised from D-galactose **55** in four steps. Suzuki coupling of **52** and **53** was achieved in 80% yield and was followed by ozonolysis of the cyclohexene **56** with a reductive work-up to give the keto-aldehyde, which existed predominantly in the cyclic form **57**. Chemoselective reduction of the resulting aminal gave the β-pseudo anomer as a single diastereoisomer which subsequently underwent acidic deprotection to afford the hydrochloric salt of **50** *i.e.* **58** as an anomeric mixture (α:β; 1:2) (Scheme 4).



Scheme 4

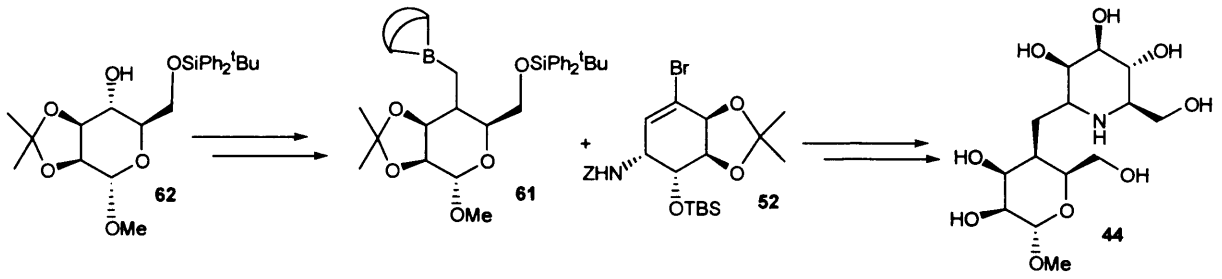
By manipulating D-mannose or D-glucose in place of **55**, this methodology has also been used to synthesise D-azaMan-β-(1→6)-D-Man **59** and D-azaMan-β-(1→6)-D-Glc **60** respectively (Figure 13).[22]

Figure 13

Compounds **59** and **60** were shown to inhibit amyloglucosidase however no activity was found with many commercially available glycosidase inhibitors (*e.g.* α- and β-galactosidases, β-glucosidase and α and β-mannosidase). The biological activity of **50** was not reported.

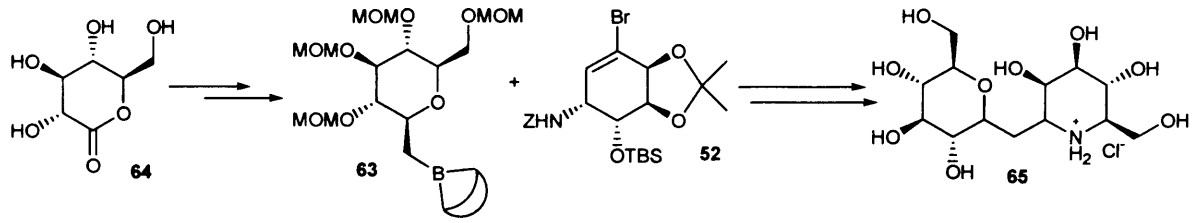
In addition to synthesising (1→6) linked aza-*C*-disaccharides, (1→4) and (1→1) linked compounds have been also been produced by Johnson using the same Suzuki coupling methodology.[22] The D-azaMan-β-(1→4)-D-Talo derivative **44** was synthesised by coupling the alkyl boron reagent **61** (derived from **62** by PCC oxidation followed by Tebbe methylation

and subsequent hydroboration) to the same vinyl bromide **52**. Again ozonolysis of the product followed by the previously described sequence led to the azadisaccharide derivative **44** (Scheme 5).



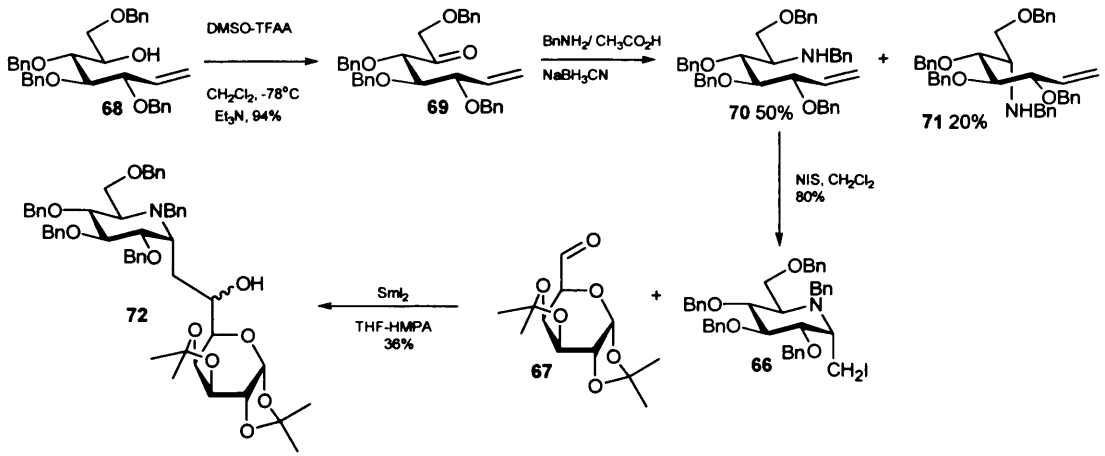
Scheme 5

Similarly by replacing the alkylboron **61** with the analogous reactant **63** (derived from γ-D-gluconolactone **64**) the authors used the same methodology to synthesise D-azaMan-β-(1→1)-D-β-Glc **65** (Scheme 6). As with the linear azadisaccharides **59** and **60**, compounds **44** and **65** showed inhibition only against amyloglucosidase. Interestingly, none of these derivatives inhibited the mannosidase enzymes, even though the piperidine ring stereochemistry corresponds to the parent mannojirimycin, which is a potent mannosidase inhibitor.



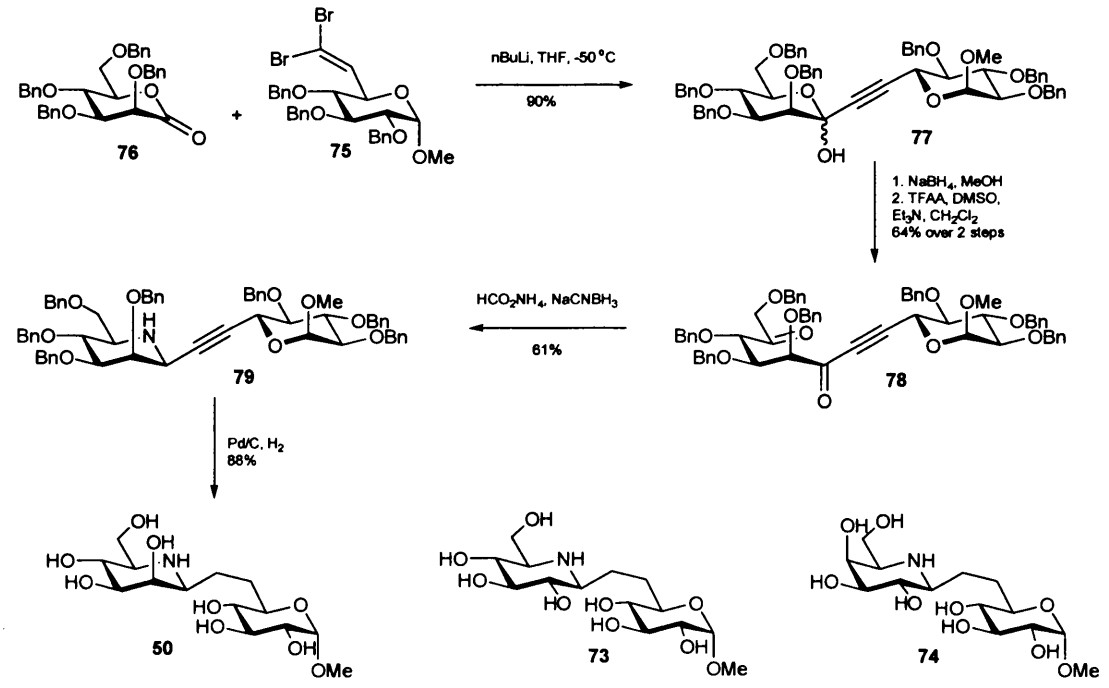
Scheme 6

In an attempt to access novel linear (1→6) linked aza-*C*-disaccharides Martin and co-workers[28] employed a samarium mediated Barbier coupling to connect an azasugar derivative **66** to an aldehydo-sugar **67** (Scheme 7). Thus, oxidation of heptenitol **68** (readily available from tetra-*O*-benzyl-D-glucohexopyranose) afforded the unsaturated hexulose derivative **69** in 94% yield, which underwent reductive amination with benzylamine (in the presence of $NaBH_3CN$) to give D-*gluco* and L-*ido* aminoheptenitols **70** and **71** respectively. An NIS-mediated cyclisation of **70** subsequently afforded the iodide **66** and coupling of **66** together with the aldehyde-sugar **67** using SmI_2 yielded the product **72** in 36% yield as a mixture of easily separable stereoisomers (2:1 ratio). The authors describe this product as an intermediate precursor of the aza-*C*-analogue of D-Glc-α-(1→6)-D-Gal, although further elaboration of **72** into the free aza-*C*-disaccharide was not reported.



Scheme 7

Van Boom and co-workers[29] have also synthesised novel (1→6) linked aza-*C*-disaccharides **73** and **74** together with the aza-*C*-mannoside **50** already reported by Johnson and co-workers (Scheme 8).[27] Their synthetic approach involved the use of a double reductive amination of a suitable diketone to generate the azasugar component of the disaccharide. Thus, treating **75** with an excess of *n*-butyllithium, followed by the addition of 2,3,4,6-tetra-*O*-benzyl-D-mannopyranolactone **76** afforded the ketose **77** in 90% yield. Reduction using sodium borohydride followed by a DMSO-mediated oxidation gave the suitably derivatised diketone **78**. Compound **78** then underwent a double reductive amination using ammonium formate and sodium cyanoborohydride to yield the protected aza-*C*-disaccharide **79**. Hydrogenolysis of the benzyl groups and concomitant reduction of the triple bond yielded the known azaMan-β-C-(1→6)-D-Glc **50**.

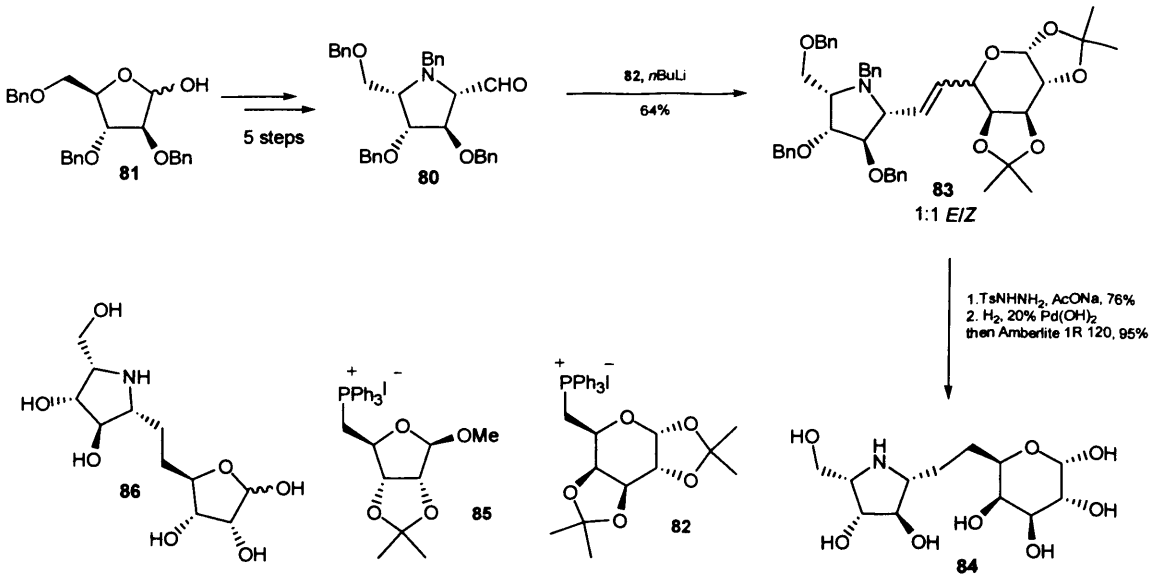


Scheme 8

The synthesis of the novel disaccharides **73** and **74** were accomplished using the same synthetic route, utilising the gluco- and galactopyranolactone respectively in place of **76**.

Compounds **59**, **73** and **74** were screened against a range of glycosidases. The results observed with **50** were comparable to those reported by Johnson and co-workers[27] in that no activity was observed against most glycosidases. However, **73** exhibited activity against rice-α-glucosidase and **74** showed strong inhibition of α-galactosidases from coffee beans and *E. coli.*

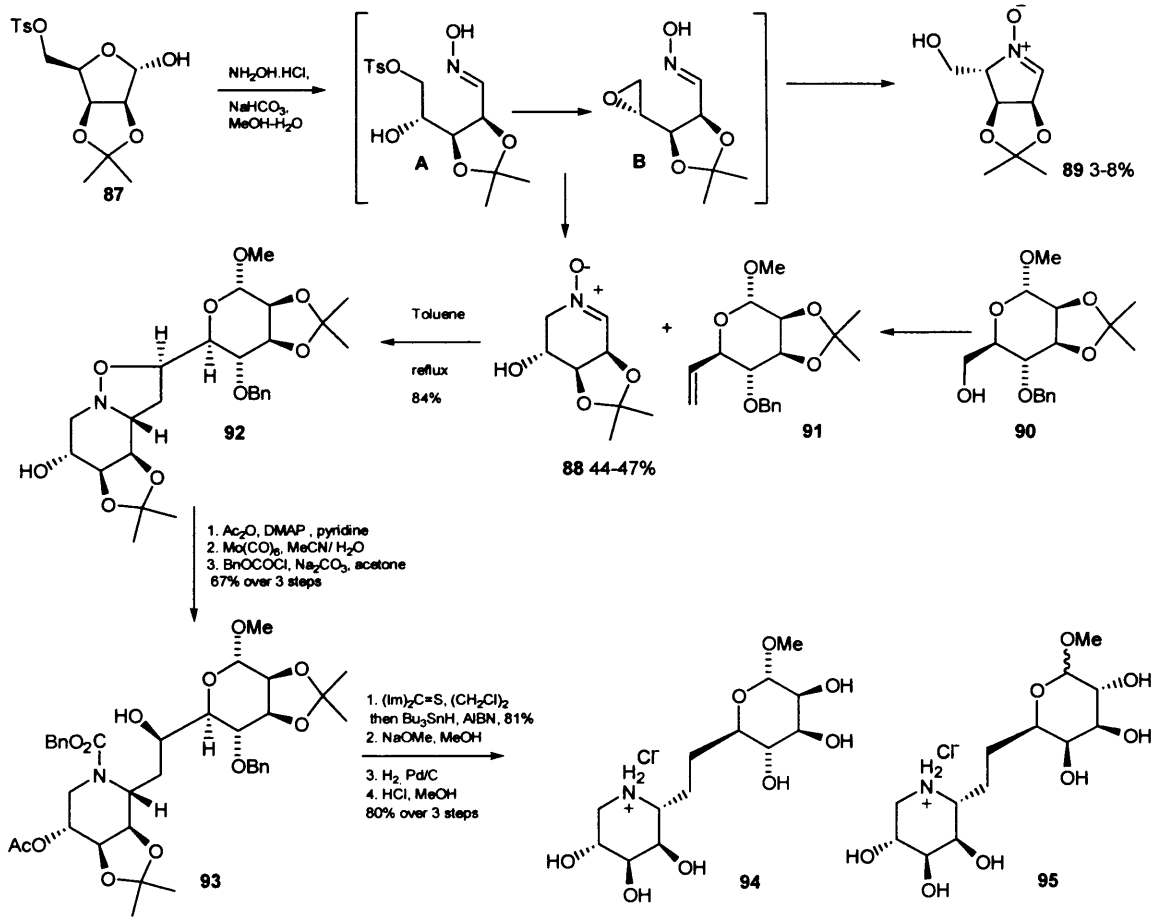
Using a Wittig coupling Dondoni and co-workers[30,39] have also synthesised (1→6) linked aza-*C*-disaccharides. The pyrrolidine aldehyde **80** (prepared in five steps from the readily available 2,3,5,-tri-*O*-benzyl-D-arabino-furanose **81**) underwent reaction with the ylid generated *in situ* from D-galactopyranose phosphonium iodide **82** yielding the olefin **83** in 64% yield as a mixture of *E* and *Z* isomers (*ca.* 1:1 ratio) (Scheme 9). The double bond was then reduced using diimide generated *in situ* from *p*-toluenesulfonylhydrazine, and the oxygen and nitrogen protecting groups were removed using standard conditions to yield the free (1→6) linked azadisaccharide **84**.



Scheme 9

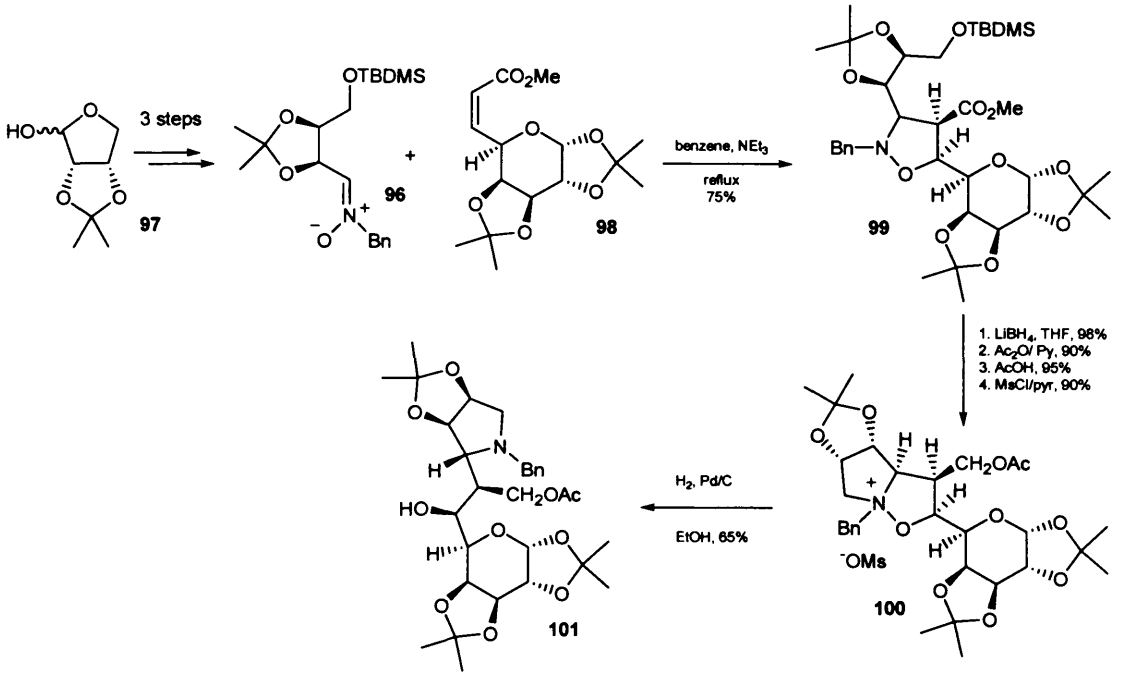
Using the same reaction sequence and incorporating the ribofuranosyl phosphonium iodide **85** in the key Wittig step the (1→5) linked system **86** was also synthesised. The flexibility of this synthetic route was additionally demonstrated by the use of alternative pyrrolidine aldehydes in the Wittig reaction (with **82** and **85**) generating many different azadisaccharides; however unfortunately, no biological testing of these compounds was reported.

Wightman and co-workers[31] utilised cycloaddition reactions of functionalised cyclic nitrones to access two (1→6) linked azadisaccharides (Scheme 10). Treatment of 2,3-*O*-isopropylidene-D-lyxose with tosyl chloride led to the relatively unstable tosylate **87** which was directly treated with excess hydroxylamine to give predominantly the nitrone **88** together with smaller amounts of **89**, thought to have been formed *via* the intermediates **A** and **B**. The methyl-α-D-mannopyranoside derivative **90** was then routinely oxidised and converted to the alkene **91**. Cycloaddition of **91** and nitrone **88** led to the crystalline *anti-, exo*-cycloadduct **92**, which was subsequently acetylated. The N-O bond was then reduced using $Mo(CO)_6$ to produce, after amine protection **93**. Radical deoxygenation using thiocarbonyldiimidazole followed by routine deprotection then gave the azadisaccharide **94** (Scheme 10). Reaction of the nitrone **88** with the D-galactopyranosyl derived alkene analogue of **91** led, after a similar series of transformations, to the related azadisaccharide **95**.



Scheme 10

Most recently, Argyropoulos and co-workers[32] utilised a similar nitrone cycloaddition to access a novel azadisaccharide (Scheme 11).

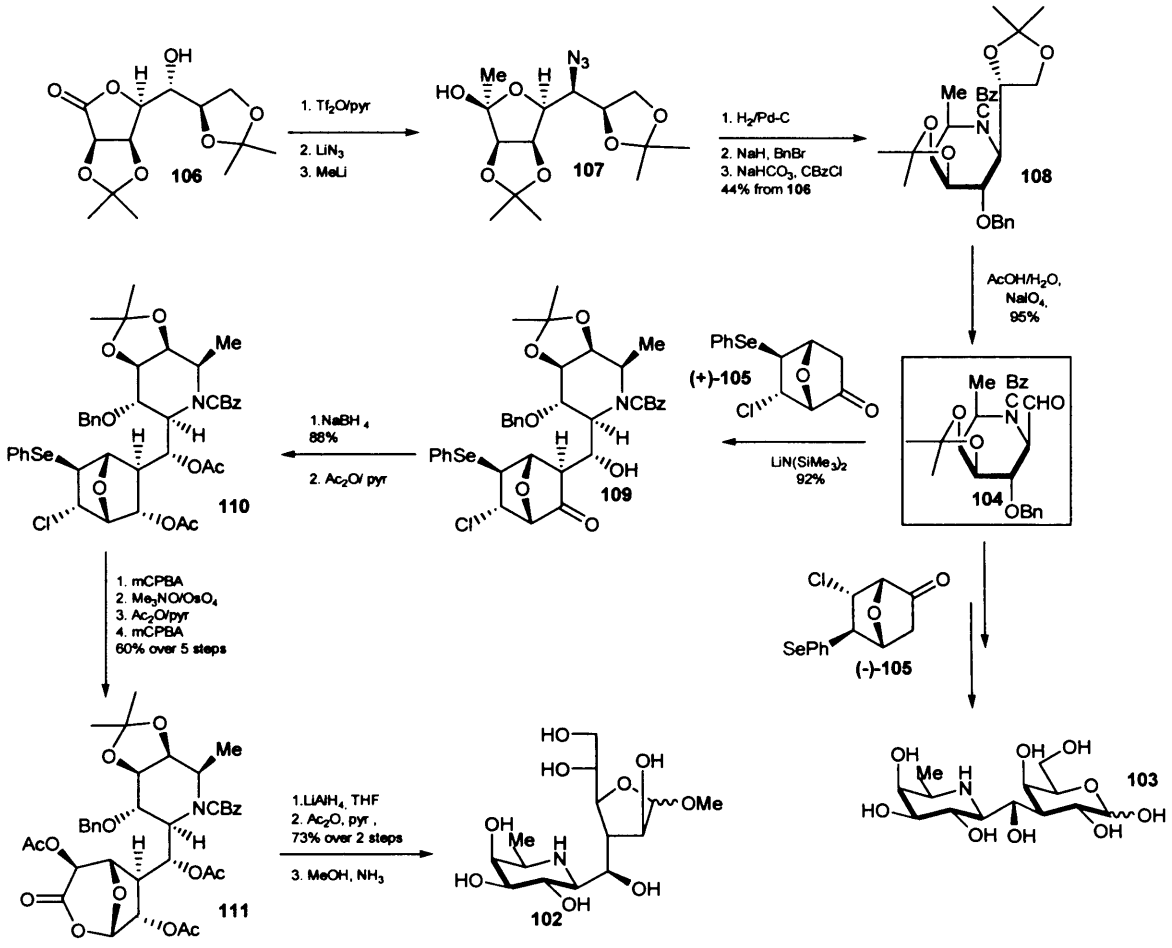


Scheme 11

The key nitrone **96** was synthesised in three steps from L-erythrose monoacetonide **97** and this was subsequently reacted with the known alkene **98**. After heating the reaction mixture in refluxing benzene for two days, only one stereoisomer **99** was isolated in 75% yield. Extensive NMR studies confirmed the stereochemistry shown where the cycloaddition step follows *exo*-attack of the alkene **98** to the *Re* face of sugar Z-nitrone **96**. Reduction of the ester was then carried out using $LiBH_4$ and the resulting alcohol was acetylated. The TBDMS group was then deprotected using acetic acid and the mesylate salt **100** was subsequently produced. This underwent catalytic hydrogenolysis (H_2, Pd/C) to yield the desired final compound **101**. Unfortunately, manipulation of this material to the fully deprotected disaccharide and biological testing was not reported.

1.3.1.2 Branched aza-C-disaccharides; (1→3) linked

In 1996 Vogel and co-workers reported the first synthesis of a branched (1→3) linked aza-*C*-disaccharide.[33,40] Their first publication details the synthesis of two disaccharides **102** and **103**. Both compounds have the 1,5,6-trideoxy-1,5-imino-β-D-galactose unit in common, one has a (1→3) link to methyl D-altrofuranoside (**102**), and the other a (1→3) link to D-galactose (**103**). An approach involving cross aldolisation of aldehyde **104** with both enantiomers of the ketone **105** was used to synthesise both compounds (Scheme 12).

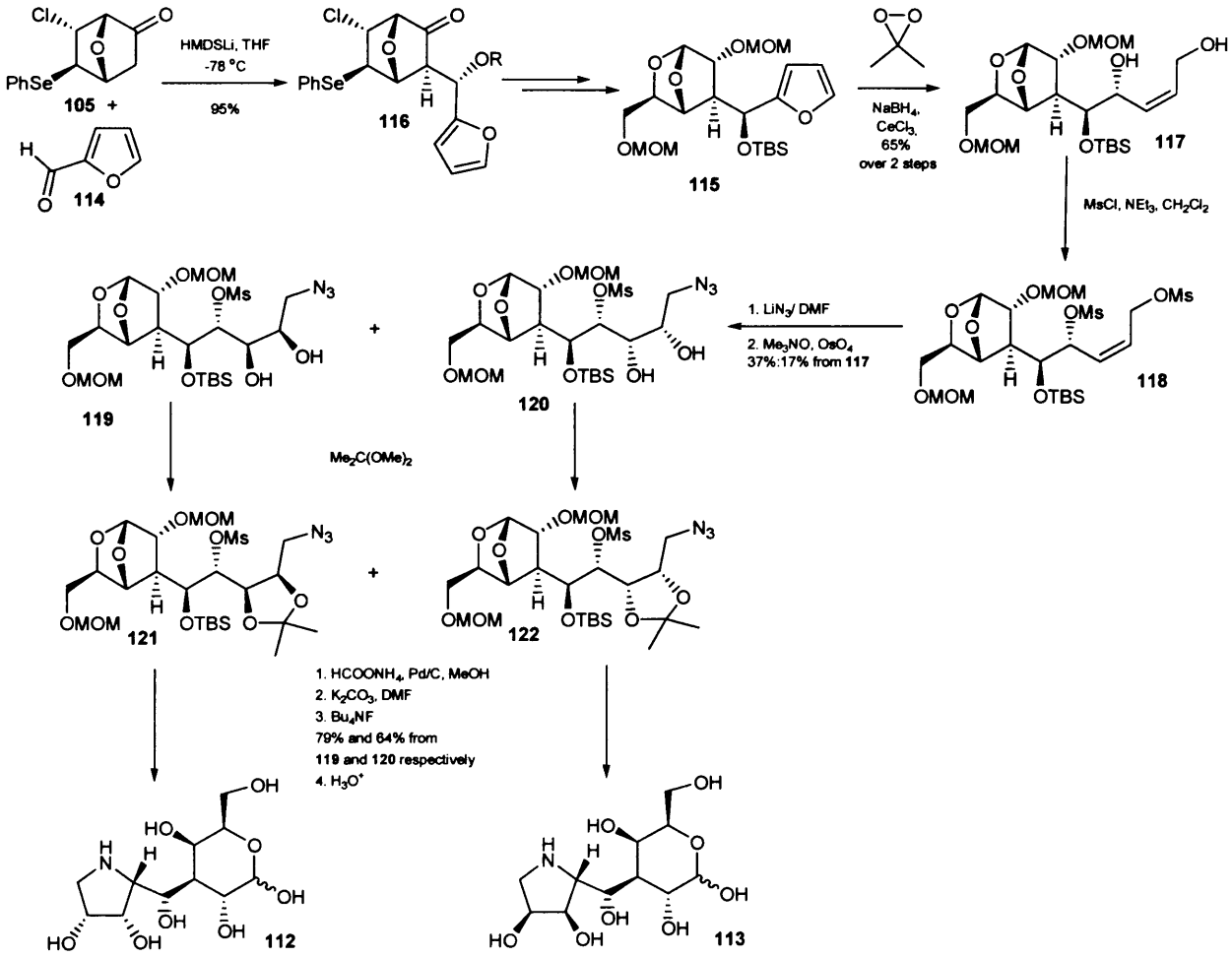


Scheme 12

For the synthesis of **102**, the commercially available D-glycero-D-gulo-heptono-1,4-lactone was firstly converted to the *bis*-acetonide **106** (Scheme 12). This compound was then transformed to an azide through an S_N2 displacement of an intermediate triflate. Addition of MeLi then afforded the hemiacetal **107**. Hydrogenation to the primary amine led to equilibration with the corresponding imine, resulting from intramolecular addition onto the ulose moiety. The latter was hydrogenated to give a semi-protected imino octitol, which after benzyl and CBz protection gave **108**. Treatment of **108** with 8:1 $AcOH/H_2O$ and $NaIO_4$ led to selective hydrolysis of the least substituted isopropylidene group and oxidative cleavage of the resulting 1,2-diol gave the key aldehyde **104**. Condensation of **104** with the lithium enolate of (+)-**105** yielded **109** as the major aldol product. Reduction of **109** with $NaBH_4$ followed by acetylation then gave **110**. Oxidation (mCPBA) of the selenide in **110** (with *in situ* selenoxide elimination) followed by hydroxylation of the resulting olefin, acetylation and Baeyer-Villiger oxidation then provided uronolactone **111**, which was converted into a 5:1 α: β mixture of the azadisaccharide **102** (Scheme 12). Using the other enantiomer of ketone **105**, this cross aldol methodology was also used to synthesise **103** (Scheme 12). Both **102** and **103** were screened against many commercially available glycosidases, however neither exhibited any significant biological activity.

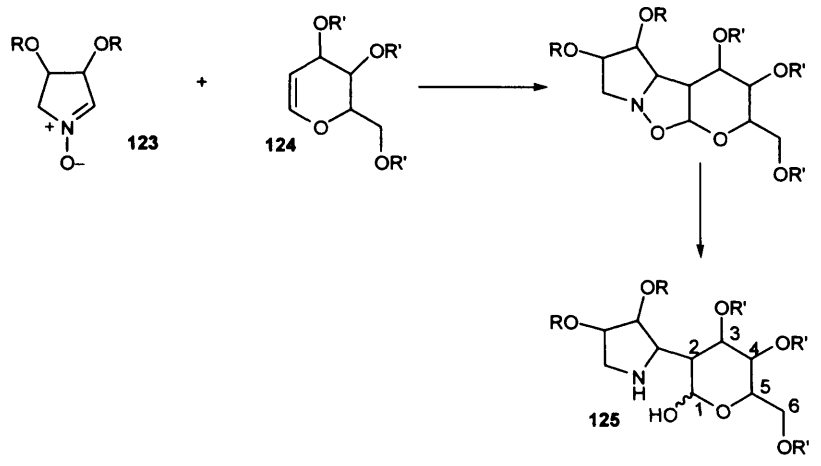
Vogel also employed a similar approach to access disaccharides **112** and **113**, connecting position C-1 of 4-amino-4-deoxyerythrofuranose to position C-3 of galactose (Scheme 13).[41] This time an aldol reaction between the lithium enolate of **105** and a furfural **114** was used to access the branched chain galactose derivative **115** (*via* **116**) as an intermediate. Treatment of **115** with dimethyldioxirane led to a γ-oxo-(Z)-enal which was directly reduced to the corresponding enediol **117** under Luche conditions ($NaBH_4$, $CeCl_3$, MeOH, 0 °C). Di-mesylation of the enediol **117** gave **118**, which was subsequently treated with lithium azide. Without purification, the alkene was dihydroxylated affording a mixture of two diols **119** and **120** (37% and 11% yield respectively from **117**), which were separated and purified by flash column chromatography.

For each of **119** and **120**, the diols were firstly protected ($Me_2C(OMe)_2$) to yield the acetonides **121** and **122** (73% and 79% respectively). Reduction of the azide to the amine ($HCOONH_4$, Pd/C) was then carried out and cyclisation followed by deprotection (K_2CO_3, DMF, Bu_4NF and H_3O^+) yielded the novel azadisaccharides **112** and **113**. These were both tested as potential inhibitors of 25 available glycosidases and interestingly **113** was found to be a moderate selective inhibitor of α-mannosidase



Scheme 13

In addition to the syntheses by Vogel, there have been further reports of branched aza-*C*-disaccharides. For example, in 1997 Brandi and co-workers[38,42] reported the first synthesis of novel (1→2) linked azadisaccharides using 1,3-dipolar cycloadditions of enantiopure polyhydroxylated pyrroline *N*-oxides **123** to 1,2-glycals **124** (Scheme 14). Variation of the 1,2-glycal moiety enabled the synthesis of many cycloaddition adducts using this methodology and subsequent deprotection and N-O bond reduction duly gave access to a range of (1→2) linked aza-*C*-disaccharides **125**.



Scheme 14

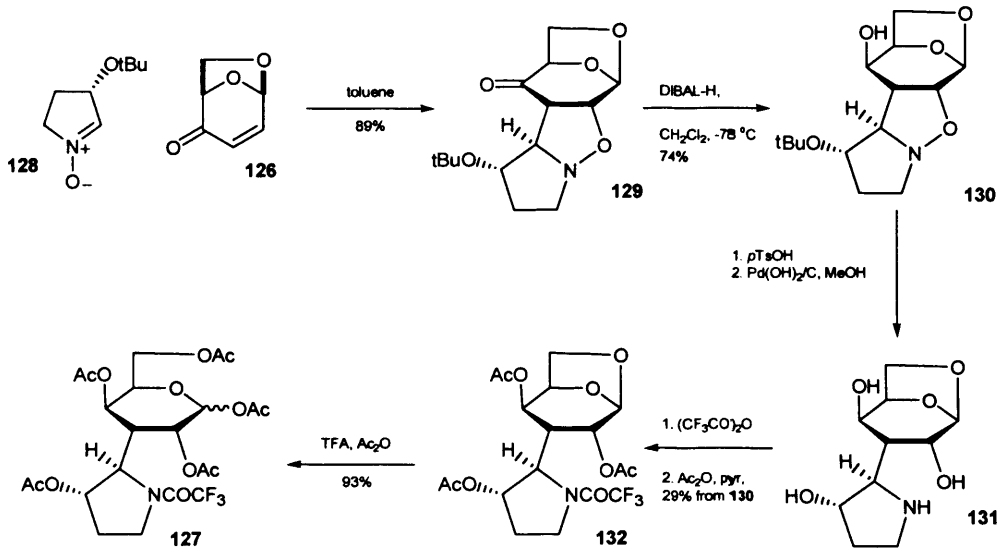
The authors found a major limitation of this approach was the low reactivity of the nitrones towards glycals in the key cycloaddition step. This meant three equivalents of glycal, high temperatures and long reaction times were required to obtain reasonable yields. This problem was partially resolved by performing the cycloaddition reactions at high pressure.[43]

However, utilising isolevoglucosenone **126** as the dipolarophile, this methodology has been successfully used to synthesise a novel (1→3) aza-*C*-disaccharide **127** (Scheme 15).[42] The authors envisaged that isolevoglucosenone **126** would be an excellent dipolarophile in 1,3-dipolar cycloadditions to nitrones and hence the harsh reaction conditions used in previous examples, would not be required.

Isolevoglucosenone **126** was thus reacted with the L-malic acid derived nitrone **128** in toluene at room temperature and after 1.5 hours only a single adduct **129** was isolated in 89% yield. Through extensive NMR studies, evidence was gathered confirming the reaction had been completely regio- and stereoselective yielding the stereoisomer shown in Scheme 15. It was proposed this product was formed as a result of the nitrone adding to the lower face of isolevoglucosenone **126** in an *exo* fashion.

The lactone moiety of **129** was reduced using DIBAL yielding the sole *endo* alcohol **130** (reaction with $LiAlH_4$ and $NaBH_4$ yielded mixtures). The tertiary butyl ether was then deprotected and N-O bond cleavage was achieved by hydrogenation to give **131**. Protection of

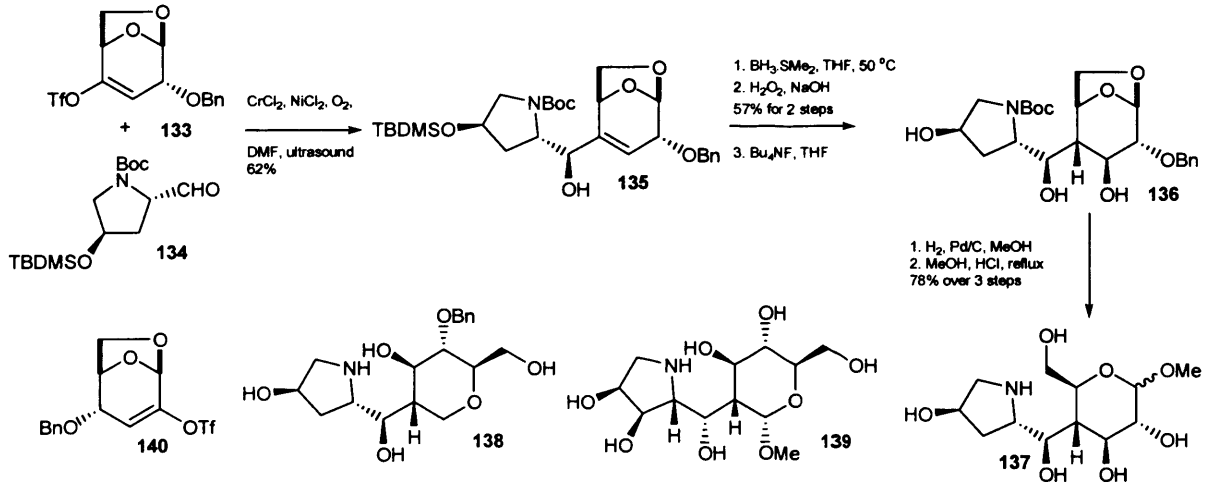
the amine in **131** as a trifluoroacetamide, followed by peracetylation subsequently gave the triacetate **132**. Finally, acetolysis of **132** with acetic anhydride and trifluoroacetic acid afforded **127** as a 1.4:1 mixture of α- and β-anomers. Unfortunately, although the authors had demonstrated this methodology could be used to access these sugar mimics; further deprotection to yield the 'naked' disaccharide was not described in the report.



Scheme 15

1.3.1.3 Branched aza-C-disaccharides; (1→2) and (1→4) linked

Vogel and co-workers, in addition to synthesising (1→3) linked aza-*C*-disaccharides, have also reported the synthesis of (1→2) and (1→4) sugar mimics.[36,37] Utilising isolevoglucosenone **126** as a key starting material, novel sugar mimics were accessed using a Nozaki-Kishi coupling (Scheme 16).



Scheme 16

Coupling of the triflate **133** (derived from isolevoglucosenone **126**) and the hydroxyproline-derived carbaldehyde **134** (Scheme 16) yielded the allylic alcohol **135** as the major isomer in 62% yield. Subsequent hydroboration, oxidation and desilylation then

furnished the tetrol **136**. Compound **136** then underwent debenzylation followed by Boc deprotection under acidic conditions to afford the (1→4) linked aza-*C*-disaccharide **137** (2:1 α:β mixture). Using similar transformations, the (1→2) linked aza-*C*-disaccharides **138** and **139** have also been synthesised utilising the levoglucosenone derived triflate **140** in the Nozaki-Kishi coupling step.[37] These compounds have been screened against a range of glycosidases and **139** was found to be a weak inhibitor of α-mannosidases from jack bean and almonds.

1.3.1.4 C-linked azadisaccharides that are joined through the ring nitrogen

As discussed previously (section 1.2.2.2), the non-natural sugar mimic, isofagomine **25** was identified as a potent inhibitor of glycosidase enzymes. In an effort to try and increase the selectivity and potency of isofagomine, Bols and co-workers[25] synthesised the azadisaccharide **48** (Figure 14). Subsequent screening of compound **48** against a range of glycosidases revealed a 60-fold increase in the inhibition of glucoamylase compared to isofagomine, which clearly suggested that substitution at *N*-1 with the extra glucose residue increased affinity by mimicking the sugar moiety of the leaving group.

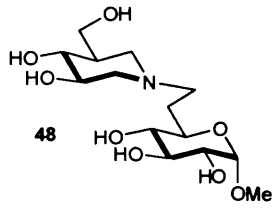
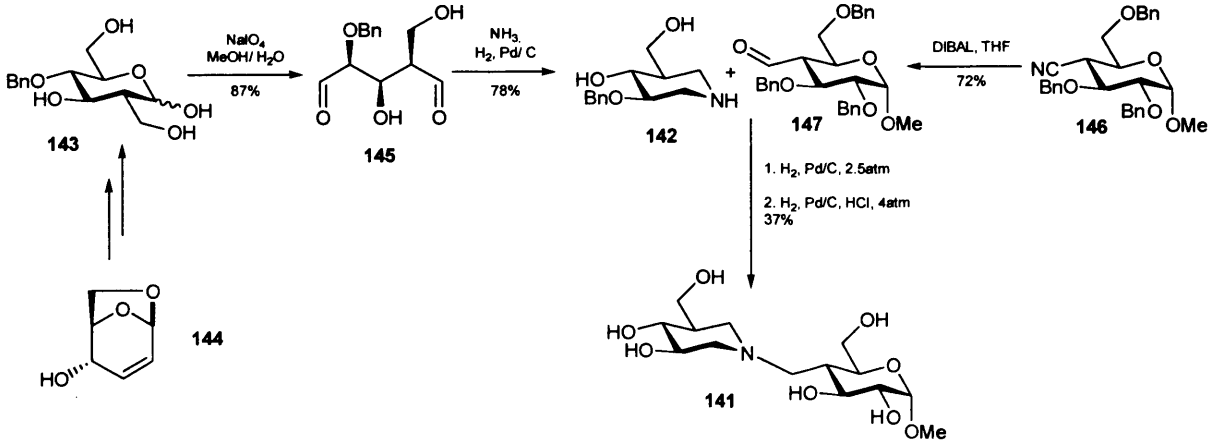


Figure 14

However, the authors recognised that the natural substrate of glucoamylase is the α-1,4-linked glucose units of starch, and therefore proposed the disaccharide **48** (which mimics a (1→6) linked disaccharide) was not an optimal transition state mimic. Therefore, the synthesis of the suitably (1→4) linked system **141** was undertaken (Scheme 17).[15]

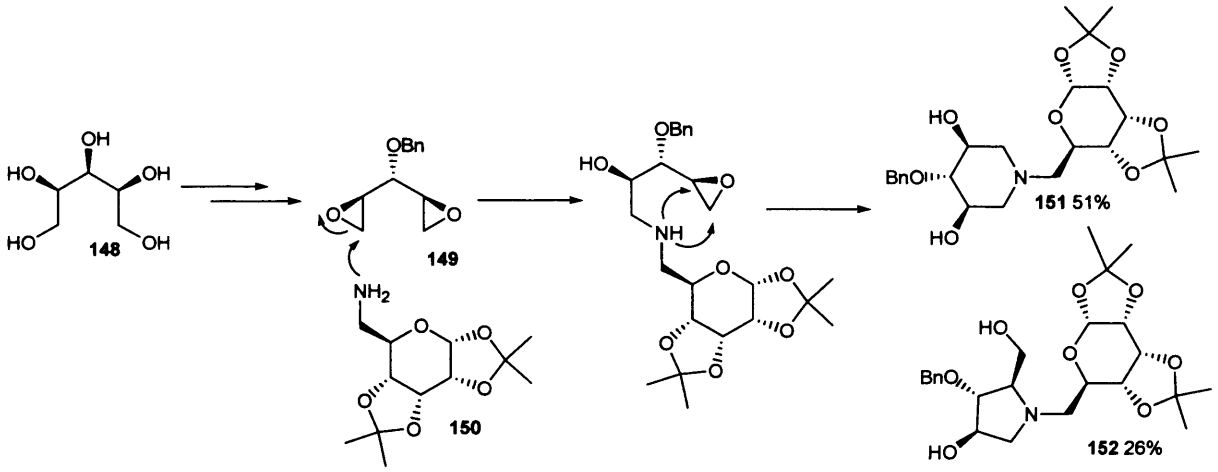


Scheme 17

The route to the azasugar component **142** was adapted from a previous synthesis in which the derivative **143** was synthesised in 7 steps from levoglucosan **144** (Scheme 17).[44] Conversion of **143** into the dialdehyde **145** by periodate cleavage was followed by a double reductive amination with ammonia to give 3-*O*-benzylisofagomine **142.**

Meanwhile nitrile **146** was synthesised from D-galactose in 6 steps and this was converted to the aldehyde **147.** Reductive amination between **142** and **147** and subsequent deprotection then afforded the disaccharide **141.** Screening of compound **141** revealed it to be a stronger inhibitor of glucoamylase compared to isofagomine **25,** suggesting that the second sugar component is important for binding. Additionally, inhibition was also found to be 10-fold greater versus the (1→6) linked disaccharide **48** with glucoamylase.

Thomas and co-workers have also synthesised compounds of this type in an attempt to investigate any contribution of an aglycon moiety to glycosidase affinity (Scheme 18).[45]



Scheme 18

Their synthetic approach involved the regiospecific ring opening and aminocyclisation of *bis*-epoxides derived from natural sugars. For example, from aldonitol **148,** the *bis*-epoxide **149** was synthesised in 4 steps. Reaction of **149** with the amine **150** (derived from galactose) in water at 50 °C yielded an approximate 2:1 mixture of the piperidine and pyrrolidine adducts **151** and **152** respectively. These azadisaccharide products were then deprotected and purified using ion-exchange chromatography. Unfortunately the biological testing of these compounds was not reported.

1.3.2 Homoaza-*O*-disaccharides

Reports describing the synthesis of homo-*O*-linked azadisaccharides are less widespread within the literature compared to their *C*-linked counterparts. The first *O*-linked homoazadisaccharide to be reported was the (1→1) linked azadisaccharide **34** (MDL 25637) (Figure 15) synthesised at the Merrell Dow Research Institute (Indianapolis, IN) by Liu and co-workers.[46] MDL 25637 was designed to be a transition state inhibitor of the α-

glucohydrolase enzyme, sucrase. Studies *in vitro* did show that **34** was a potent competitive inhibitor of intestinal sucrase and α-glucanases and was able to significantly reduce postprandial hyperglycaemia after a sucrose or starch load.[19] Because of this, MDL 25637 **34** was identified as a candidate drug for anti-diabetic therapy. Surprisingly, in spite of this lead, few other compounds of this kind have been reported. Bols and co-workers[47] have synthesised the *O*-linked pyrrolidine **153**, and more recently Martin and co-workers have reported the synthesis of the (1→4) linked homoaza-analogs of both maltose **154**[21] and cellobiose **155**[23] (Figure 15).

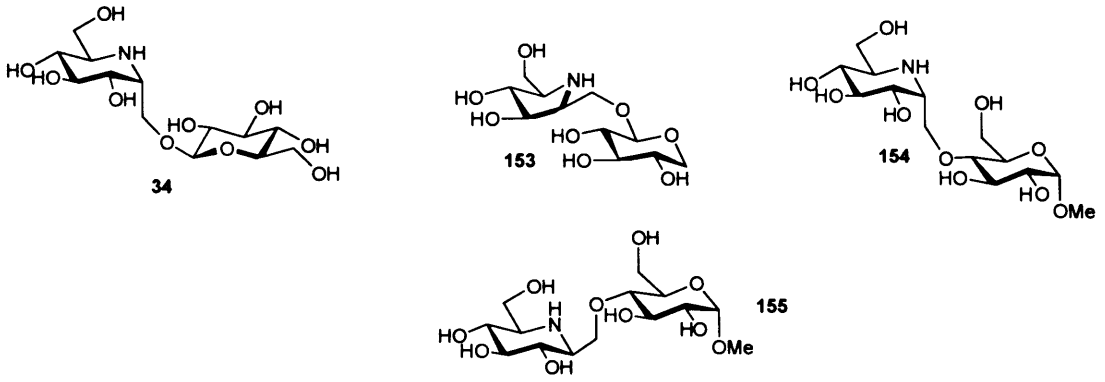
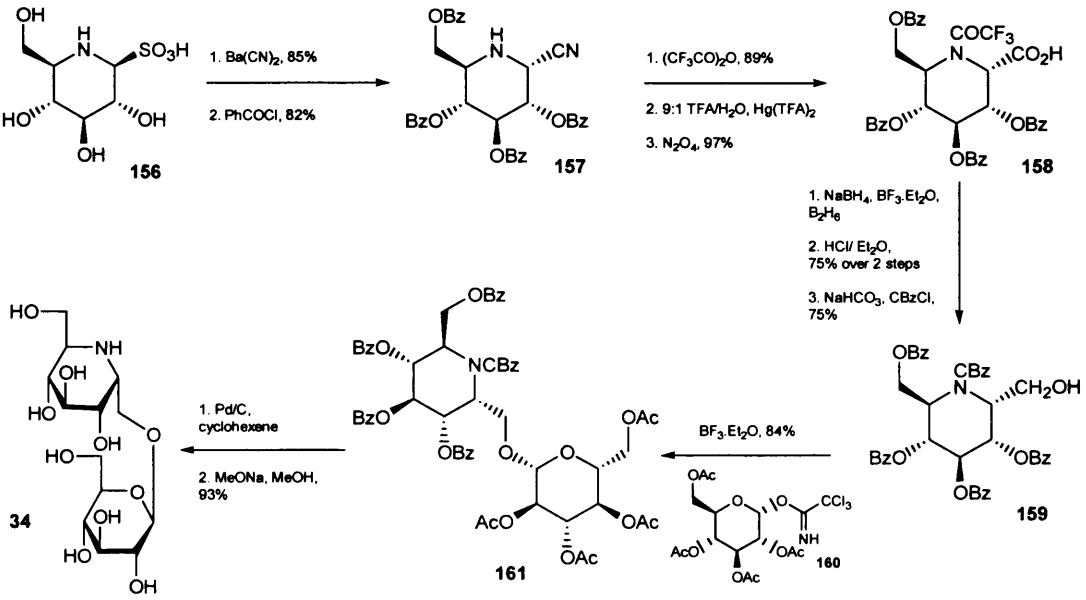


Figure 15

1.3.2.1 Synthesis of (1→1) linked homoaza-O-disaccharides

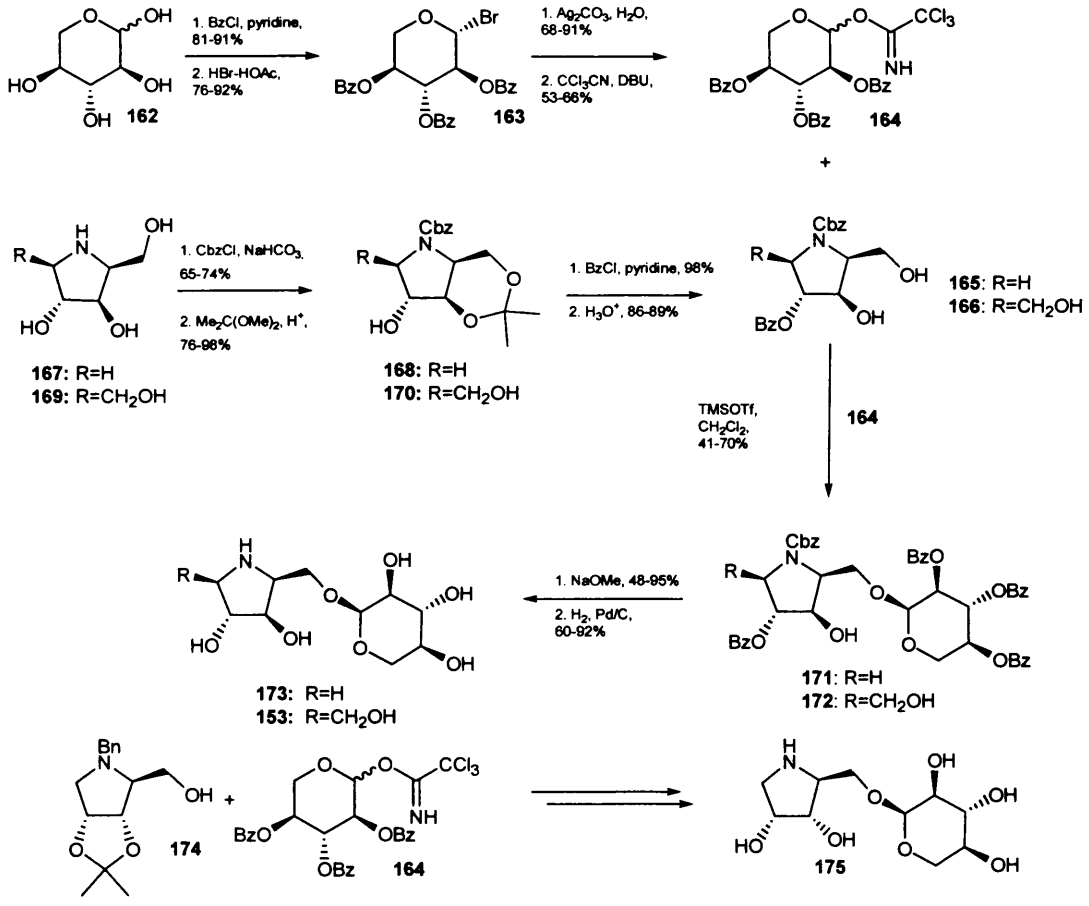
As discussed above, Lui and co-workers established the first synthetic route to a homo-*O*-azadisaccharide to investigate intestinal glucohydrolase activity.[46] The authors used a synthesis employing the readily available bisulfite adduct of nojirimycin **156** as starting material (Scheme 19).



Scheme 19

Conversion of **156** to a nitrile followed by multiple oxygen protection afforded the adduct **157**. The amine was then differentially protected with trifluoroacetic anhydride to allow for subsequent nitrile hydrolysis. Reduction of the resulting carboxylic acid **158** with $NaBH_4$ and $BF_3.Et_2O$ yielded the amino alcohol. Deprotection of the amine and subsequent re-protection as the benzyloxy-carbamate gave the fully protected azasugar **159**. Coupling of **159** and **160** using a Schmidt glycosylation yielded **161** in good yield (84%) and subsequent stepwise deprotection resulted in the target (1→1) linked homoaza-*O*-disaccharide **34** (Scheme 19).

Bols and Lundt have also synthesised (1→1) linked homoaza-*O*-disaccharides using the Schmidt glycosylation (Scheme 20).[47] Using L-xylose **162** as a starting material for the aglycon, complete benzoylation followed by reaction with HBr in acetic acid, afforded the bromide **163** which was subsequently hydrolysed and converted to the trichloroacetimidate **164**. In parallel, the known hydroxypyrrolidines **165** and **166** were prepared and protected to allow regioselective glycosylation.



Scheme 20

Amine **167** was first protected with Cbz and the 1,3-diol as the acetonide to give **168**. Subsequent benzoylation of the free hydroxyl and acetonide hydrolysis then yielded **165**. Following a similar route amine **169** was also converted to the acetonide **170** and then the dibenzoate **166**. Selective glycosidation of **165** and **166** with **164** was then carried out using

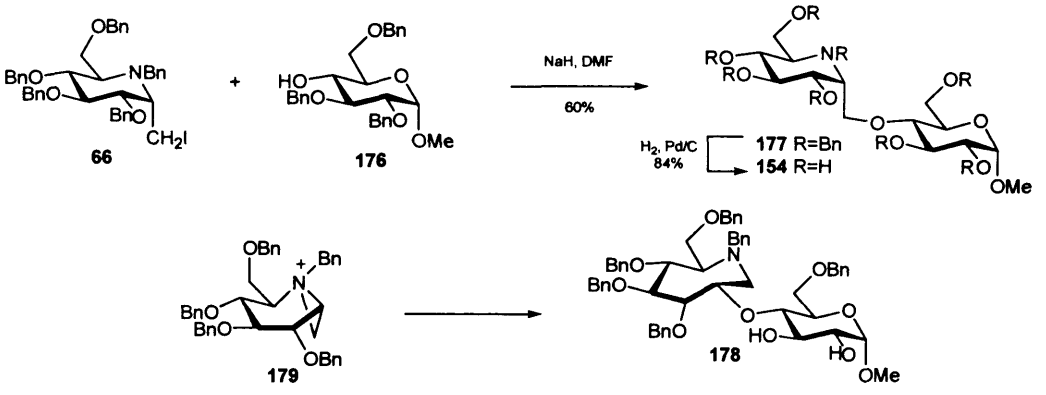
TMSOTf yielding the β-L-xylosides **171** and **172** in good yields. Finally, debenzoylation followed by hydrogenation gave the target compounds **173** and **153**. This methodology was also adapted to the protected pyrrolidine **174** to yield the stereoisomeric disaccharide **175**.

Compounds **173**, **153** and **175** were screened against α-glucosidase (type 1 from bakers yeast) and β-glucosidase (from almonds) however no significant biological activity was observed. The authors suggest the reason for this is due to the inhibitors not fitting into the enzyme active site in the manner they had predicted, and thus were poor transition state analogues.

1.3.2.2 Synthesis of a (1→4) linked α- and β-homoaza-O-disaccharide

Martin and co-workers have also established novel synthetic routes to homoaza-*O*-disaccharides using a simple S_N2 displacement reaction to link the azasugar to the aglycon.[21,23]

Utilising the 1,2,6-trideoxy-2,6-imino-1-iodohepitol derivative **66** that had been used by the same group to synthesise the (1→6) linked aza-*C*-disaccharide **72** (see section 1.3.1.1), the (1→4) linked maltose analogue **154** was produced by reaction with methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **176** followed by routine deprotection (Scheme 21).

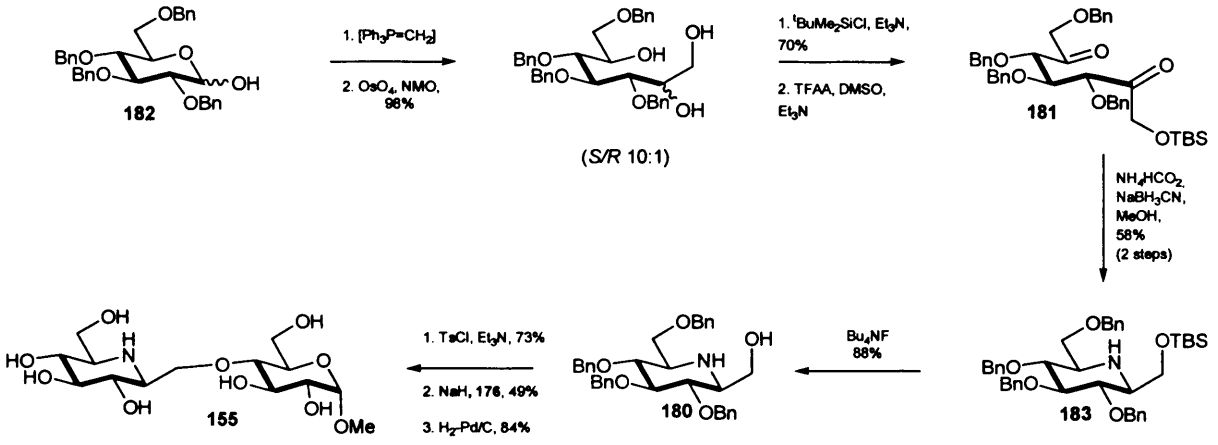


Scheme 21

The nucleophilic displacement reaction not only led to the homodisaccharide **177** but also the azepene derivative **178** (ratio **177** : **178** 1:1), thought to have been produced through the aziridinium cation intermediate **179** (Scheme 21).

To achieve the β-linked analogue the authors developed a route to a β-homonorjirimycin derivative **180** (Scheme 23).[23] The key step in this synthesis was the reductive amination of the D-*xylo* diketosugar **181** (produced from the benzylated glucose derivative **182**) to give exclusively the all-equatorial piperidine derivative **183**. Removal of the silyl group produced **180** which was tosylated and coupled with **176** in a manner similar to before to give the homoaza-analog of methyl α-cellobioside **155**. Unfortunately, although two novel *O*-linked

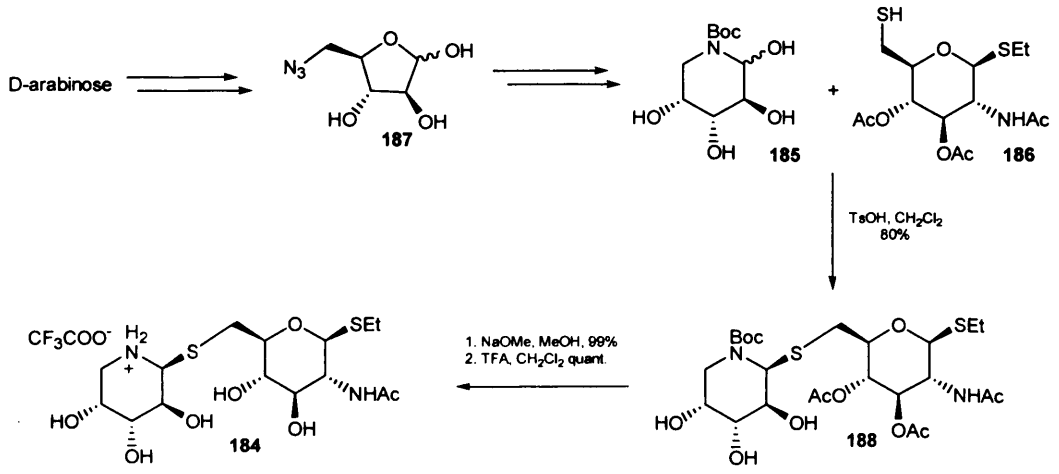
azadisaccharides had been successfully prepared, the biological activities of **154** and **155** were not reported.



Scheme 22

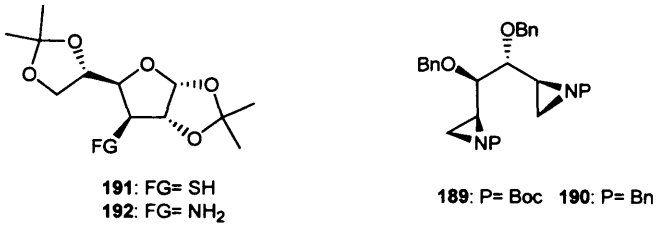
1.3.3 *N*-and *S*-linked azadisaccharides

The number of reports describing the synthesis of nitrogen and sulfur linked azadisaccharides are very few. In 1994 Hashimoto and co-workers reported the first synthesis of a sulfur-linked azadisaccharide **184** (Scheme 23).[48] This was achieved by coupling an azasugar component **185** (synthesised from a D-arabinose) with a thioglycoside **186** in dichloromethane in the presence of a catalyst (*e.g.* TMSOTf or TsOH). To synthesise the azasugar **185**, D-arabinose was firstly converted to the azide **187** in 6 steps. This was then converted to the *N*-Boc aminal **185** in a further 6 steps involving a reductive amination. Condensation of the aminal **185** with the 6-thiosugar **186** in the presence of TsOH yielded the 1,2 *cis*-linked thioglycoside **188** and subsequent deprotection gave the novel azadisaccharide **184**. Unfortunately, the biological activity of this compound was not reported.



Scheme 23

In 1996 Depezay and co-workers[24] also reported the synthesis of *S*- and *N*-linked azadisaccharides. The synthesis was achieved by nucleophilic opening (followed by intramolecular aminocyclisation) of D-mannitol derived *bis*-aziridines **189** and **190** either by 3-deoxy-3-thio-D-glucose **191** or by 3-deoxy-3-amino-D-glucose **192** (Figure 16).

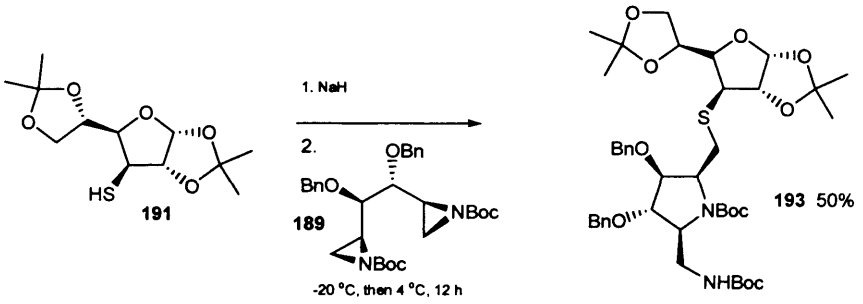


191: FG= SH
192: FG= NH_2

189: P= Boc **190**: P= Bn

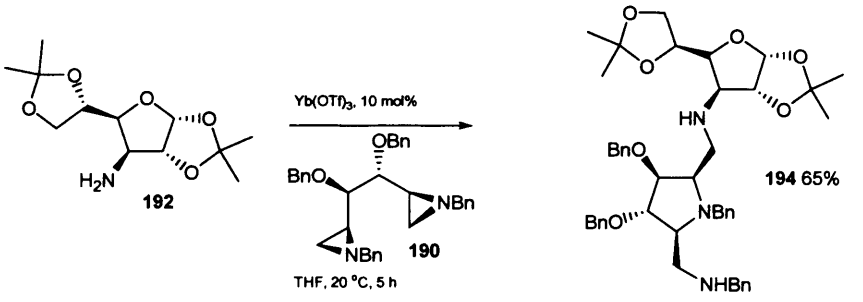
Figure 16

The compounds **191** and **192** were prepared in three steps from diacetone-D-allose. To generate the thiol linked disaccharide **193** the *N*-Boc *bis*-aziridine **189** was reacted with **191**. Opening of the first aziridine ring took place readily at –20 °C followed by slow intramolecular cyclisation to generate the thioglucosyl substituted pyrrolidine **193** in 50% yield together with 30% recovered **189** (Scheme 24).



Scheme 24

The amine-linked disaccharide **194** was synthesised using the same methodology except the *N*-benzyl aziridine **190** together with **192** and ytterbium triflate was employed (Scheme 25).

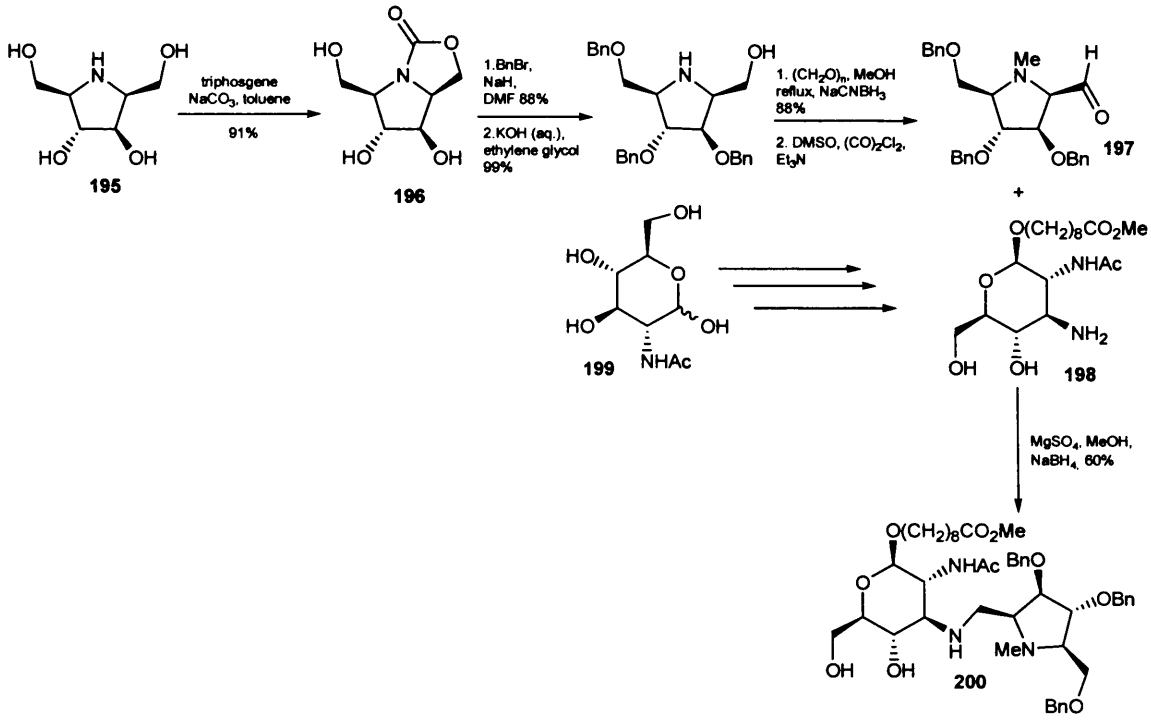


Scheme 25

This report does demonstrate that the nucleophilic opening of *bis*-aziridines with thio- or amino-sugars is an effective route to novel disaccharides, although unfortunately, the deprotection and the biological testing of these compounds were not reported.

An additional example of an *N*-linked azadisaccharide synthesis has been reported by Wong and co-workers.[49] The authors had previously discovered through x-ray

crystallographic analysis that the homoazasugar **195** sits in a half-chair like structure and was an effective inhibitor of α-and β-glucosidases (K_i in the μM range). In view of this, the authors wanted to attach an aglycon moiety to **195**, to investigate whether the azadisaccharide structure would enhance or hinder inhibitory activity.



Scheme 26

Thus, reaction of **195** with triphosgene yielded **196** which was subsequently converted to the aldehyde **197** as shown (*i.e.* **195→197**) (Scheme 26). The aldehyde **197** was then coupled to amine **198** (derived from *N*-acetylglucosamine **199**) *via* a reductive amination to yield the *N*-linked disaccharide **200**. Surprisingly, after accomplishing the synthesis of the disaccharide adduct **200** debenzylation and subsequent biological testing of this compound was not described.

1.3.4 Miscellaneous-linked azadisaccharides

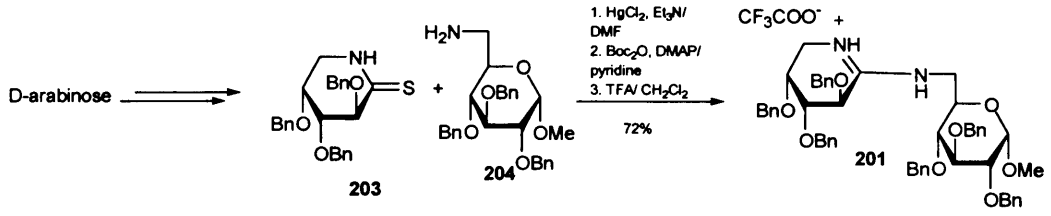
In an attempt to achieve improved transition state mimics, many research groups have investigated the synthesis of disaccharides that incorporate an azasugar analogue more closely resembling the partially planar nature of the transition state. Therefore in addition to *C*-, *O*-, *N*- and *S*-linked azadisaccharides, there are a large number of reports that detail syntheses of disaccharides with alternative linkages. A few of these will now be summarised.

1.3.4.1 Amidine-linked azadisaccharides

As discussed previously, glycoamidines *e.g.* **22** are known to be potent inhibitors of glycosidase enzymes (see section 1.2.2.2). This activity has been ascribed to these compounds supporting a positive charge and sitting in a half chair conformation thought to closely mimic

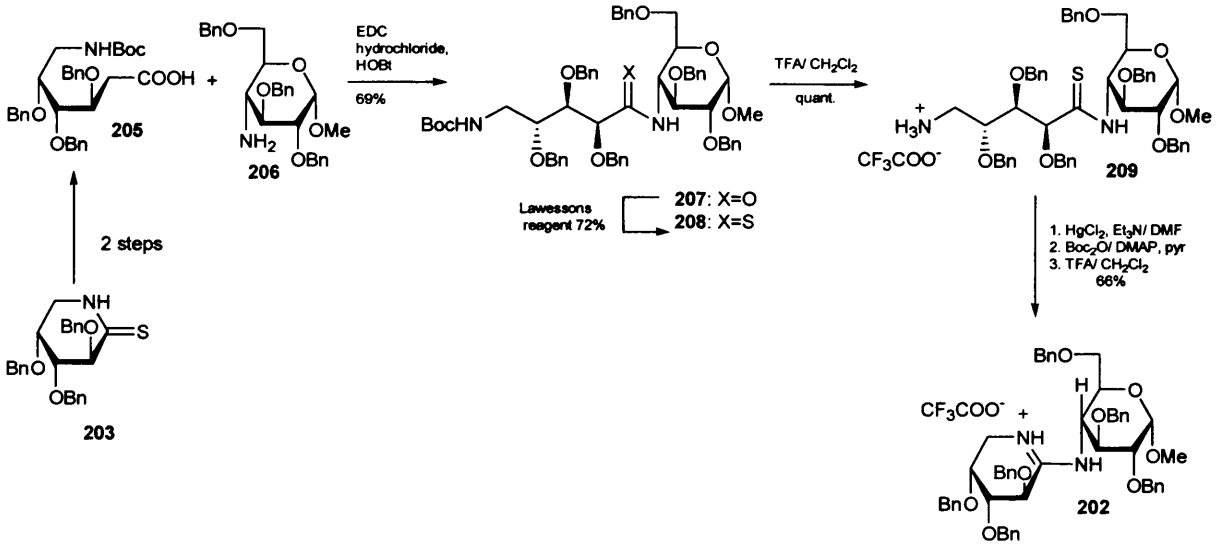
the oxocarbenium ion transition state. Consequently it was proposed[50] that pseudo-oligosaccharides having an interglycosidic amidine linkage would therefore be good candidates as more specific glycosidase inhibitors, due to both the glycosidic linkage and the aglycon moiety being recognised. Hashimoto and co-workers tried to utilise this proposal and synthesised two novel amidine-linked azadisaccharides (Scheme 27).[50] The amidine linkage was constructed *via* both an inter- and intramolecular coupling of a thioamide with an amine in the presence of mercury (II) chloride, yielding two different disaccharides **201** and **202** respectively.

In the intermolecular route the thioamide **203** was synthesised from D-arabinose in 4 steps and was coupled with 6-amino-6-deoxy-D-glucoside **204** using mercury (II) chloride to afford the disaccharide adduct **201** in good yield (72%).



Scheme 27

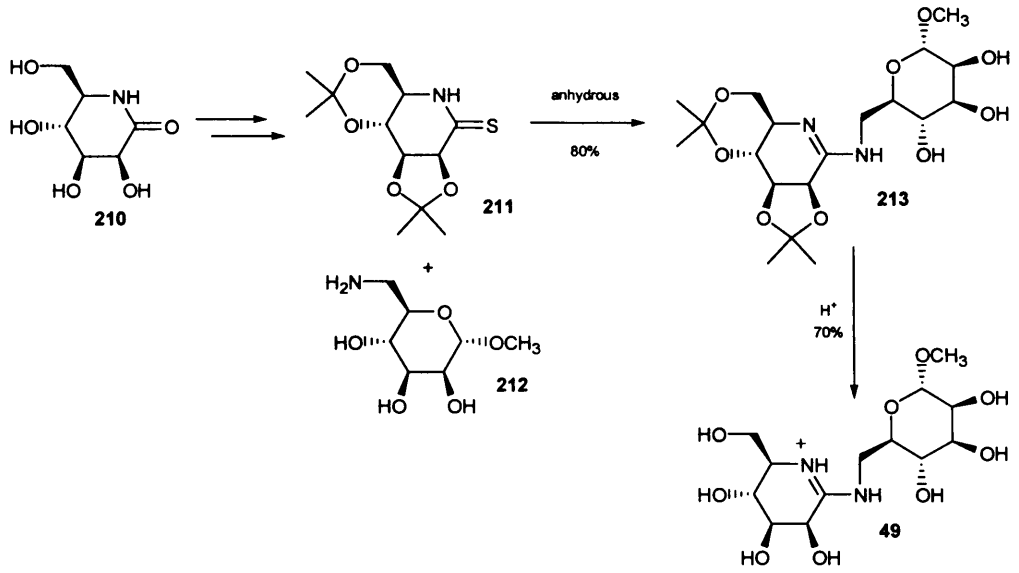
In contrast, the intramolecular approach required the synthesis of 5-*N*-Boc-glyconic acid **205**, which was produced from **203** in two steps (Scheme 28).



Scheme 28

Condensation of **205** with amine **206** using EDC hydrochloride and HOBt yielded the derivative **207**. Thionation using Lawessons reagent gave the thioamide **208** in good yield and subsequent Boc cleavage and cyclisation of the resulting amine **209** using the same conditions described above gave the required azadisaccharide **202** in 66% yield. Whilst this report demonstrated that this methodology could be used to access amidine-linked azadisaccharides, the deprotection and biological testing of both compounds (**201** and **202**) was not reported.

Soon after the report by Hashimoto, a related article by Tellier[26] was published. This outlined a similar approach to the synthesis of an amidine-linked azadisaccharide **49** (Scheme 29) using D-mannolactam **210** to construct the key thioamide **211**. Thus, mannolactam **210** was first protected as the *bis*-acetonide and subsequent thionation using Lawessons reagent gave the key intermediate **211**. Treatment of **211** with the amine **212** under anhydrous conditions yielded the corresponding mannoamidine **213** in good yield (80%) and subsequent acid deprotection yielded azadisaccharide **49** in 70% yield.



Scheme 29

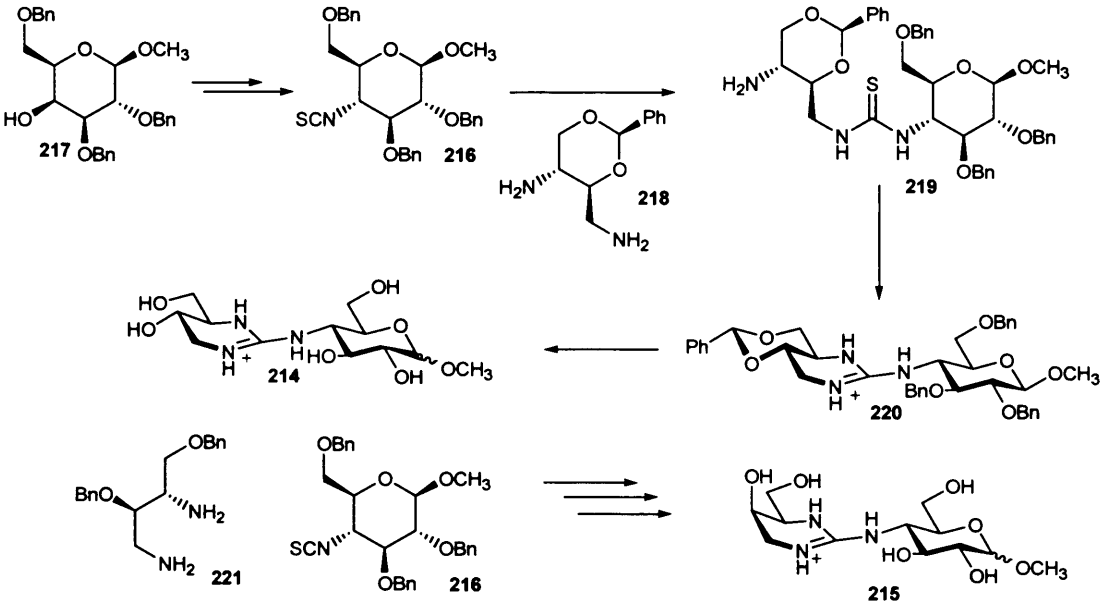
Inhibition studies were conducted with **49** and strong competitive inhibition was observed for Jack bean α-mannosidase (K_i = 2.6μM). This compares with and is similar in magnitude to that reported for deoxymannojrimycin (K_i = 63μM).

1.3.4.2 Disaccharide analogues containing a cyclic guanidinium structure

In an attempt to mimic both the half-chair conformation and the developing positive charge around the anomeric centre in the glycosidase transition state, Lehmann and co-workers[51] synthesised disaccharide analogues containing a cyclic guanidinium linkage *i.e.* **214** and **215** (Scheme 30).

The six membered cyclic guanidine structure was prepared by reacting a 1,3-diamine with an isothiocyanate, and ring closing the resulting thiourea using yellow lead oxide. Thus, for the synthesis of **214**, the key isocyanate **216** was produced from a glucose derivative **217** in 4 steps. Reaction of **216** with the diamine **218** gave the thiourea **219**. Subsequent cyclisation of **219** with yellow lead oxide then gave the derivatised cyclic guanidine **220**. Hydrogenolysis followed by acid hydrolysis gave the glucoside **214** as a mixture of anomers. In a similar manner the disaccharide **215** was synthesised from the diamine **221**. The guanidinium derivatives **214** and **215** were tested against α- and β-glucosidase and α- and β-

galactosidase; however they proved to be ineffective inhibitors. The authors ascribe this poor inhibition to a change in conformation of the guanidinium derivative when an aglycon is attached, and also the absence of hydroxyl groups at C-2 and C-3.



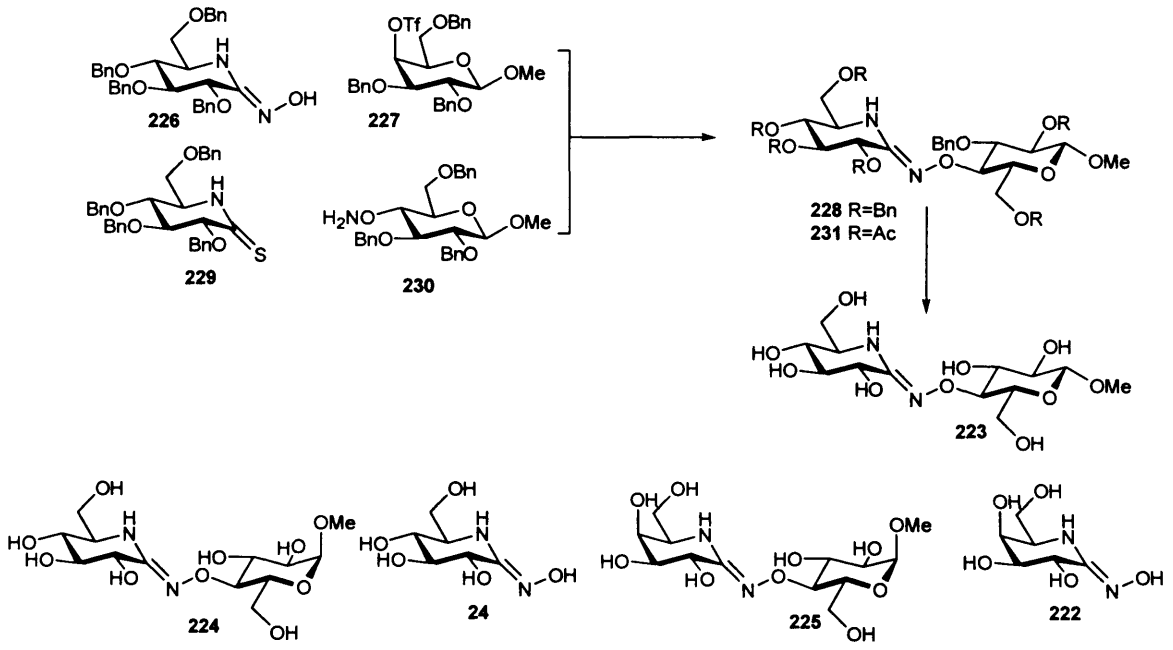
Scheme 30

1.3.4.3 Disaccharide analogues incorporating a lactam oxide

Hydroximinolactams were initially designed as glycosidase inhibitors to try to mimic the flattened chair conformation of the transition state.[13] To investigate whether the introduction of an aglycon onto hydroximinolactams **24** and **222** would increase selectivity and potency of inhibition, Vasella and co-workers synthesised the lactam oxide containing azadisaccharide analogues **223**→**225** (Scheme 31).[52]

Two synthetic approaches to the methyl-α-cellobioside analogue **223** were investigated. One approach involved the alkylation of hydroximinolactam **226** with the triflate **227**. This was achieved using phase-transfer conditions to yield **228** in reasonable yield (59%). Another approach concerned the condensation of thiogluconolactam **229** with the hydroxylamine ether **230**. This condensation was carried out in the presence of $Hg(OAc)_2$ and yielded the adduct **228** in 72% yield. Debenzylation of **228** followed by purification *via* the corresponding acetate **231** gave the disaccharide **223**. These approaches were also attempted for the synthesis of the analogues **224** and **225** however, the *O*-alkylation methodology using alternative triflates was found to be problematic (the authors suspect decomposition of the triflate was occurring). Therefore the second method involving condensation in the presence of $Hg(OAc)_2$ was used to access the disaccharide intermediates. Routine deprotection using

the same conditions used as before yielded the β-cellobioside analogue **224** and the β-lactoside analogue **225**.



Scheme 31

Inhibition studies revealed the cellobioside analogues **223** and **224** were strong inhibitors of β-glucosidase from *C. saccharolyticum* and moderate to weak inhibitors of β-glucosidases from sweet almond, comparable to activity exhibited by the monosaccharide structure **24**. In contrast, **24** showed strong inhibition of the α-glucosidase from brewers yeast, whereas **223** and **224** were found to be weak inhibitors of this enzyme. Similarly the D-galactohydroximo-1,5-lactam **222** was found to be a potent inhibitor of the α-galactosidase from coffee beans and the β-galactosidases from bovine liver and *E. coli*, whereas the galactoside analogue **225** although displaying strong inhibition for β-galactosidases from *E. coli*, only showed weak inhibition for α-galactosidase from coffee beans and β-galactosidase from bovine liver.

1.4 Summary of the current routes in the literature

In an effort to produce more specific, higher affinity glycosidase inhibitors many research groups have investigated the synthesis of azadisaccharides. To stabilise the disaccharide many linkages between the azasugar and aglycon have been explored and a wide range of synthetic methodologies have been used to produce novel sugar mimics.

Within the literature a number of different chemical approaches have been utilised in an attempt to connect the azasugar component/precursor to the natural aglycon mimic. In the construction of *C*-linked azadisaccharides, a Suzuki coupling, a samarium Barbier coupling,

carbonyl addition, a Wittig reaction, cycloaddition of nitrones, an aldol reaction and a Nozaki-Kishi coupling have all been employed to construct the key non-hydrolysable bond. For the synthesis of *O*-, *S*- and *N*-linked compounds fewer approaches have been reported, with most research groups using a Schmidt glycosylation or a nucleophilic substitution in order to link the two monosaccharide components. Finally, using various metal catalysed inter and intramolecular couplings the synthesis of amidine, guanidine and oxime disaccharide analogues has been achieved.

Whilst these chemical approaches to azadisaccharides have been successful and a range of novel compounds have been produced, there is a common thread through many of the syntheses. In a large number of reports the full deprotection of the disaccharide intermediate, a transformation that can cause many problems in many syntheses, is often not discussed. In addition many reports do not give any information regarding the biological activity of the compounds synthesised. This can be quite frustrating, when the initial justification of the publication is to investigate how the attachment of the aglycon affects the inhibitory activity of the initial monosaccharide. Lastly, there is sometimes a general lack of diversity in many of the published syntheses as many research groups construct the azasugar component of the disaccharide from a natural sugar. This consequently does not allow flexibility with regard to azasugar ring size and stereochemistry, unless the reaction sequence is repeated from the beginning using an alternative natural precursor, which is expensive and time consuming.

The understanding of glycosidase inhibition and the factors that contribute to a good monosaccharide inhibitor is well understood. However, with regard to azadisaccharide syntheses, the area is less predictable and although many attempts have been made to design and synthesise potent azadisaccharides, only a few examples exist where this has been successful. Thus, there is a real need for novel, divergent synthetic routes which would allow the rapid preparation of a considerable number of azadisaccharides, where many factors of the compound could be varied at one key point in the synthesis. Screening a range of compounds would allow trends that enhance or hinder inhibition to be identified enabling a greater understanding of glycosidase mechanism and inhibition and potentially facilitate the generation of new, improved inhibitors.

Chapter 2

A Pinacol Coupling Approach to *O*-linked Sugar Mimics

2.0 A Pinacol Coupling Approach to *O*-Linked Sugar Mimics

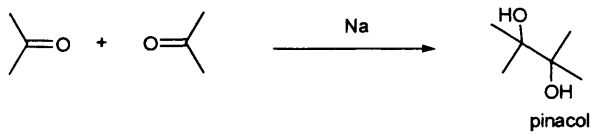
2.1 Introduction

We propose a new, efficient, versatile, and stereoselective synthetic route to novel homoaza-*O*-disaccharides, using a pinacol coupling as the key step. To introduce flexibility into our synthetic route and thus make it more divergent, we plan to construct the azasugar ring using non-carbohydrate starting materials. Once the target compounds have been synthesised, their inhibitory activities will be determined and reported.

2.2 The pinacol coupling

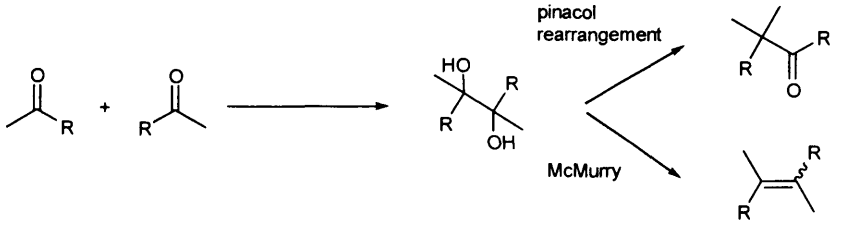
2.2.1 Introduction to the pinacol coupling

The pinacol coupling describes the reductive coupling of two carbonyl compounds to form a 1,2-diol (Scheme 32). The first report of the pinacol coupling was in 1958 by Fittig and Gottingen[53] where the dimerisation of acetone is described using sodium to form the diol (2,3-dimethylbutane-2,3-diol) whose trivial name is pinacol.



Scheme 32

Since this first report considerable effort has gone into the development of milder and more selective methods to achieve this important transformation. Consequently this reaction is still used today as a versatile tool for chemists and it is frequently employed as the key step in the synthesis of natural products and pharmaceuticals. The reason for this can also be attributed to the fact this coupling generates a new C-C bond and two new adjacent stereocentres and also that the product 1,2-diols are also very versatile intermediates in synthesis. For example, 1,2-diols can be used for the preparation of ketones by the pinacol rearrangement or alkenes by the McMurry reaction (Scheme 33).[54]



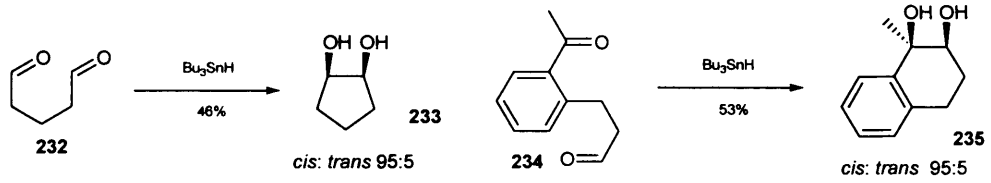
Scheme 33

The intermolecular pinacol coupling is a well-established reaction and many reagents have been developed to cross couple ketones and aldehydes, such as low-valent titanium,[55]

nickel,[56] niobium[57] and vanadium reagents[58] and samarium diiodide.[59] However with regard to the intramolecular pinacol coupling, the reagents most commonly employed are tributyltin hydride,[60] samarium diiodide[61] and low valent titanium species.[62]

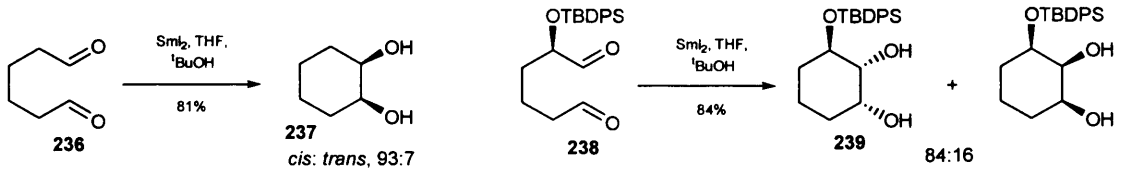
2.2.2 Examples of intramolecular pinacol coupling reactions in the literature

The pinacol cyclisation can lead to the formation of either a *cis*- or *trans*-diol and the selectivity of the reaction can be controlled by the reducing agent, and/or the structure of the carbonyl compound. Intramolecular pinacol couplings have been frequently used in the formation of carbocycles, where diols have been prepared by cyclisation of dicarbonyl compounds and excellent stereoselectivities have been observed. For example, Fu and co-workers[63] reported the use of a tributyltinhydride mediated-pinacol with the 1,5-dialdehyde **232** yielding the *cis*-diol **233** in a reasonable 46% yield with 95% selectivity. In addition, the same authors applied these reaction conditions to the 1,6-dicarbonyl **234** giving the *cis*-1,2-diol **235** in 53% yield and again with greater than 95% selectivity (Scheme 34).



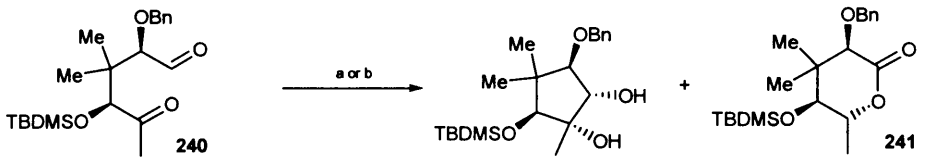
Scheme 34

The use of samarium diiodide has also been demonstrated in the pinacol coupling and excellent selectivities are also observed. For example, Hanessian and co-workers have cyclised the dialdehyde **236** using samarium diiodide to afford the diol **237** in 81% yield with excellent *cis*-selectivity (Scheme 35).[61] Furthermore, it has also been shown the presence of an alkoxy substituent adjacent to the aldehyde *e.g.* **238** causes the major *cis*-diol to have an orientation opposite to that substituent *i.e.* **239** (Scheme 35).



Scheme 35

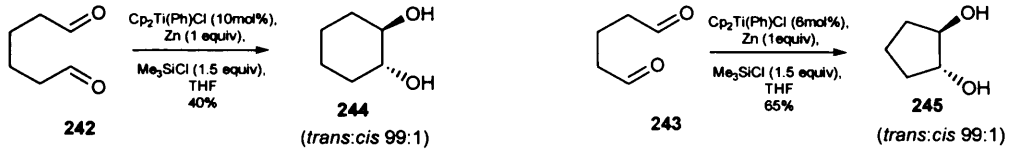
In addition, the samarium diiodide-mediated reaction has also been applied to ketoaldehydes *e.g.* **240** where again good yields and selectivities have been observed (Scheme 36). However, depending on the quality of the samarium reagent used, an intramolecular Tishenko oxidoreduction to form **241** is sometimes a competing reaction (thought to be due to the presence of Sm^{3+}) (Scheme 36).[64]



a. THF, r.t., SmI_2 (1 equiv) prepared freshly in THF (0.1 M) — 80% — 0%
b. THF: hexane (1:1), r.t., SmI_2 in THF (0.1 M) was premixed with MeOH, (99:1) before addition — 18% — 65%

Scheme 36

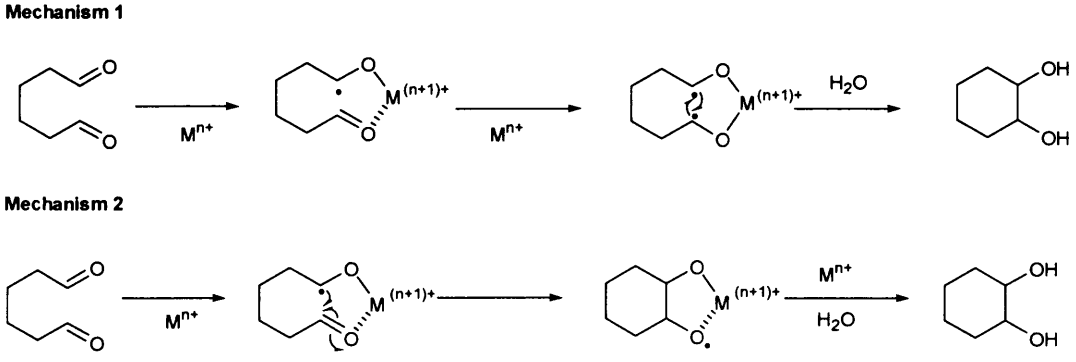
Low valent titanium reagents have also been employed to achieve pinacol cyclisations. For example, Itoh and co-workers[62] developed a Cp_2TiCl-catalysed intramolecular pinacol coupling of dials (*e.g.* **242** and **243**), allowing five and six membered cyclic 1,2-diols (*e.g.* **244** and **245**) to be synthesised in good yields (40-65%) with excellent *trans*-selectivity (99:1 *trans*:*cis*) (Scheme 37).



Scheme 37

2.2.3 Mechanism of the pinacol coupling and origin of stereocontrol

The mechanism of the pinacol coupling is not fully understood, however two possible alternatives have been proposed[54] (Scheme 38). In the first mechanism, a diradical coupling mechanism is thought to occur, whereby both carbonyl functionalities are reduced to their corresponding ketyl radicals and a radical coupling occurs to form the new carbon-carbon bond. In the second mechanism, one of the two carbonyls is preferentially reduced to give a ketyl radical, which then attacks the remaining carbonyl in an *exo*-cyclisation to form the new C-C bond. The resulting oxygen radical, which is then reduced by a second equivalent of M^{n+} gives the required diol after work-up.



Scheme 38

Investigations using a tributyltinhydride mediated pinacol reaction, led to the isolation of the 1,3-dioxa-2-stannolane **246** (Figure 17).[60] This uncovered a clue to the origin of the *cis*-selectivity and consequently a new mechanistic variant of the pinacol reaction was proposed.[60] In this mechanism the reversibility of both the addition of the tributyltin radical to the carbonyl group and the intramolecular radical C-C bond formation is believed to be responsible for the high *cis*-selectivity. In the samarium mediated pinacol coupling, it has been proposed the excellent *cis*-selectivity arises from the formation of an 8 or 9 (depending if a 5 or 6 membered ring is formed) membered Sm^{III} coordinated intermediate **247** (Figure 17) causing both of the developing hydroxyl groups to be formed on the same face of the developing carbocycle.[61] Finally, when the titanium reagent Cp_2TiPh is used in the pinacol coupling, it has been proposed the *trans*-selectivity arises due to the bulky Ti^{IV} fragment surrounded by two cyclopentadienyl and one phenyl ligands not being able to coordinate to the other carbonyl terminus. Therefore, cyclisation is believed to proceed *via* a diradical intermediate such as **248** in which the two bulky Cp_2PhTi moieties occupy axial positions in order to reduce steric repulsion (Figure 17).[62]

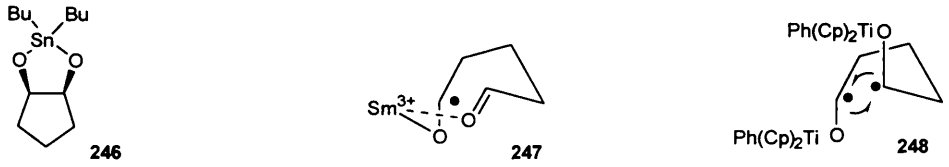
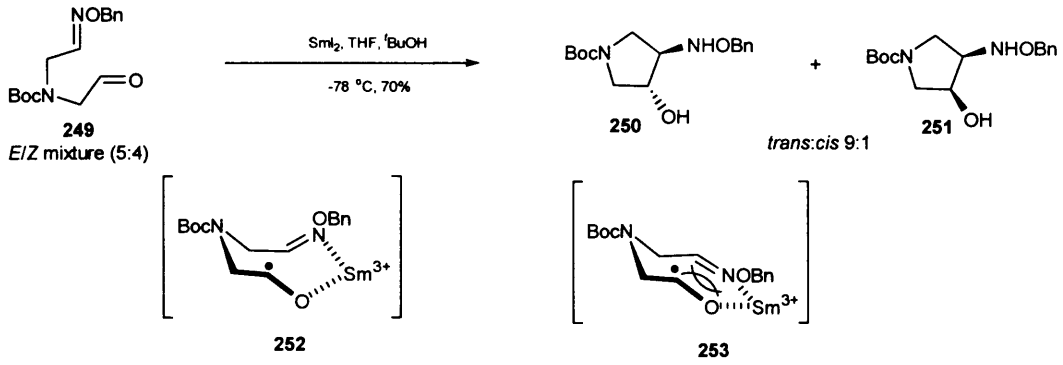


Figure 17

2.2.4 The use of the pinacol coupling in the synthesis of *N*-heterocycles

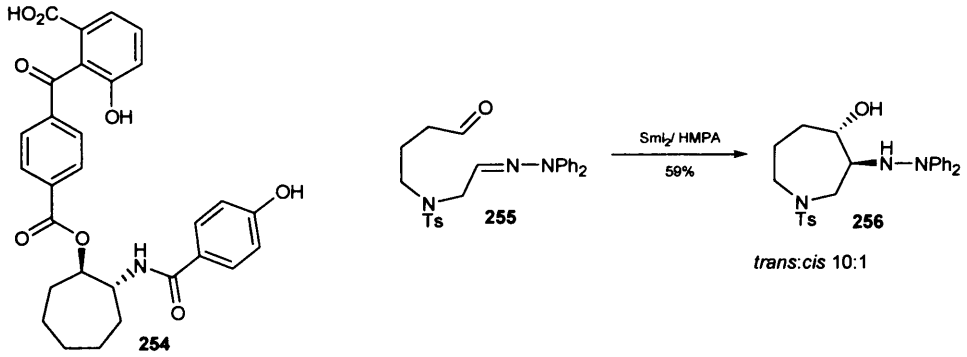
Within the chemical literature, the only reported examples where intramolecular pinacol couplings have been used to synthesise *N*-heterocycles has involved the cyclisations of carbonyloxime and carbonylhydrazone precursors resulting in the formation of *N*-heterocyclic amino alcohols. An example of one of these syntheses has been reported by Naito and co-workers,[65] where the intramolecular radical cyclisation of the carbonyloxime **249** forming the 1,2-amino alcohols **250** and **251** is described using samarium diiodide (Scheme 39).

In contrast to the intramolecular dicarbonyl cyclisations to form carbocyclic diols, treatment of an *E*/*Z* mixture of **249** with SmI_2 generated two cyclised products **250** and **251** in favour of the *trans*-amino alcohol. The preferential formation of the *trans*-adduct is explained by electronic and steric repulsions between the ketyl radical moiety and the oxime ether group in the chair like transition state, hence favouring **252** over **253** (Scheme 39).



Scheme 39

An additional report by Skydstrup and co-workers details the synthesis of the PKC inhibitor balanol **254** (Scheme 40).[66] A samarium mediated intramolecular pinacol coupling was utilised on the carbonyl hydrazone **255** to form the hexahydroazepine adduct **256** with 10:1 *trans*:*cis* selectivity. Interestingly, the catalytic pinacol procedures employing the titanium based reagents Cp_2TiCl_2 or $Cp_2Ti(Ph)Cl/Zn/TMSCl$ were also attempted on the derivative **255** and were found to be ineffective at catalysing ring closure. Additionally this reaction was repeated using 3 equivalents of Cp_2TiPh (prepared from Cp_2TiCl_2 with $^{i}PrMgCl$ and PhMgBr) and again no coupling was observed.



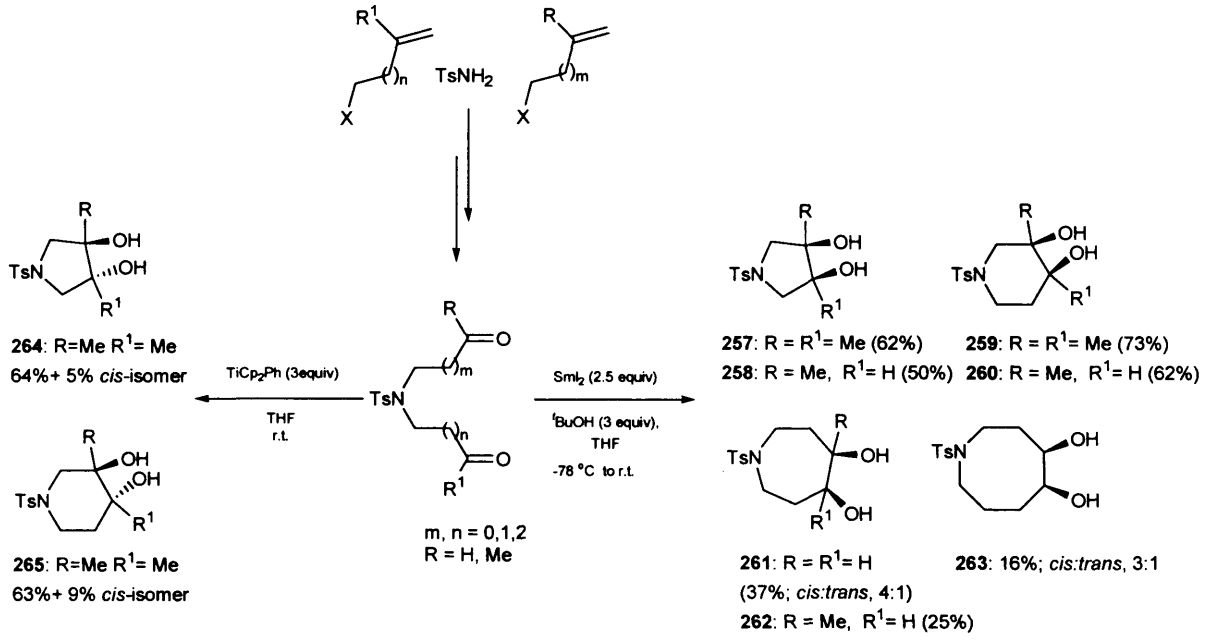
Scheme 40

2.2.5 A pinacol approach to *N*-heterocyclic 1,2-diols - azasugar analogues

Although the intramolecular pinacol coupling has been used widely as a synthetic tool to generate carabocyclic 1,2-diols and *N*-heterocyclic 1,2-aminoalcohols, there have been no reported examples of this reaction being utilised in the formation of *N*-heterocyclic diols.

Thus, an alternative synthetic route to *N*-heterocyclic 1,2-diols utilising both a samarium diiodide and a low valent titanium (Cp_2TiPh) mediated intramolecular pinacol coupling has been developed within the Handa group (Scheme 41).[67] Alkylation of *p*-toluenesulfonamide using a range of allyl-halides followed by ozonolysis (with a reductive work-up) of the resulting dienes, has enabled convenient access to a number of dicarbonyl precursors. Investigations have shown that using a samarium diiodide-mediated pinacol coupling the

synthesis of pyrrolidine and piperidine diols (*e.g.* **257→260**) can be achieved from their corresponding acyclic dicarbonyls, in good yields with complete *cis*-selectivity (in agreement with results obtained with the corresponding carbocyclic systems, discussed above) (Scheme 41).



Scheme 41

The reaction has also been successfully applied to the formation of seven and eight membered heterocycles (*i.e.* **261, 262** and **263**), although lower levels of stereocontrol have been observed with varying amounts of the *trans*-diol being isolated (Scheme 41).

With regard to the titanium-mediated pinacol coupling, investigations have shown that the reaction of the same dicarbonyls with 3 equivalents of Cp_2TiPh (prepared by treatment of Cp_2TiCl_2 with $^{i}PrMgCl$ and then PhMgBr) allows the pyrrolidine and piperidine derived *trans*-diols (*i.e.* **264** and **265**) to be formed in good yields. However, the levels of stereocontrol were found to be not as high as with the corresponding SmI_2-mediated reaction (Scheme 41) and application of the reaction to seven and eight membered rings was also found to be unsuccessful (in agreement with the analogous reaction on the carbonylhydrazone **255** reported by Skydstrup[66] discussed above). Nevertheless, these results have demonstrated the first application of the titanium mediated pinacol coupling to the synthesis of *N*-heterocycles.

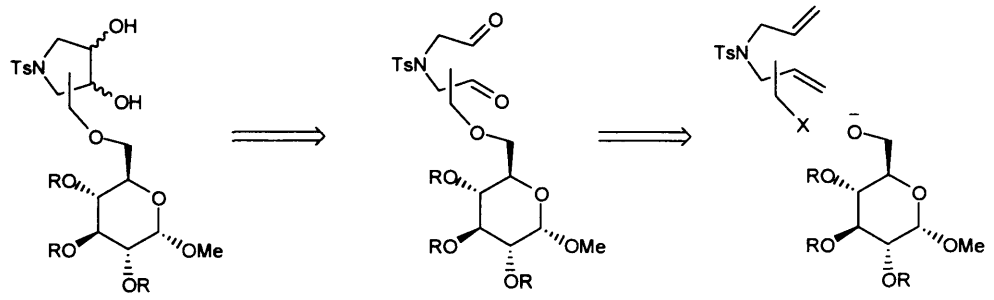
2.2.6 Summary

To summarise, the use of the pinacol coupling in the literature is large and varied and it has been applied as a key step in a number of syntheses. Attention has mainly focused on the use of this reaction to form carbocyclic diols and *N*-heterocyclic amino alcohols. The application of the pinacol coupling to the formation of *N*-heterocyclic 1,2-diols has been

demonstrated by the Handa group and it has been shown to be compatible with a range of ring sizes and substitution patterns. In addition, depending on the reagent used (*i.e.* SmI_2 or $TiCp_2Ph$) the stereochemical outcome of the *N*-heterocyclic 1,2-diol can be effectively controlled. Furthermore, the resulting *N*-heterocyclic intermediates are analogous to 2-deoxyazasugars (described in chapter 1) and thus, this demonstrates a new route to potential glycosidase inhibitors.

2.3 A pinacol approach to azadisaccharides

We propose to use a pinacol coupling approach to synthesise homoaza-*O*-disaccharides. This would illustrate a completely original route to these compounds and allow some interesting sugar mimics to be synthesised. We envisaged a pathway such as one in Scheme 42 would give access to requisite dicarbonyl precursors, which could then undergo a pinacol coupling yielding the desired disaccharide 1,2-diols.



Scheme 42

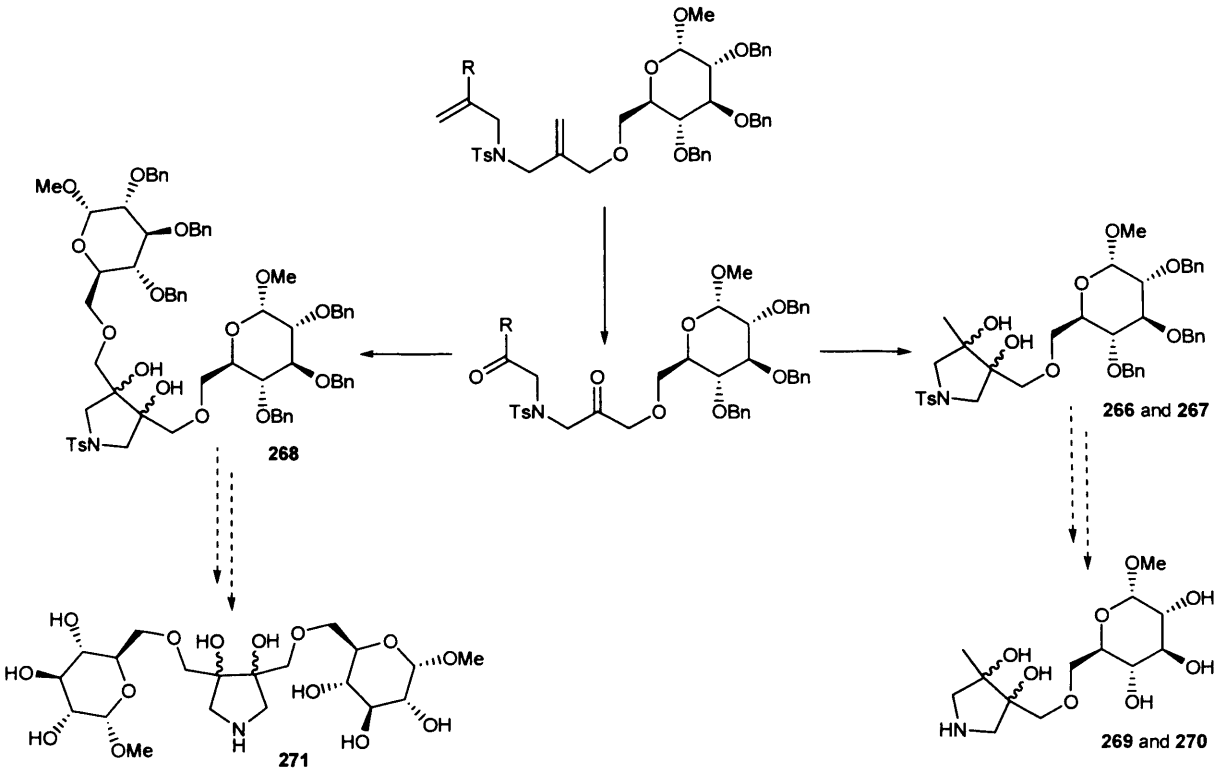
This route would enable the stereochemical outcome of the pinacol cyclisation using heavily substituted dicarbonyl precursors to be investigated and additionally demonstrate the applicability of the reaction. Furthermore, we propose this synthetic pathway to have many advantages over existing routes. Firstly, it utilises cheap and readily available starting materials, allowing a range of dicarbonyl precursors to be synthesised quickly and efficiently. Subjection to the pinacol coupling then gives access to azadisaccharides incorporating azasugars with varying ring sizes and depending on the reagent utilised in the coupling (*i.e.* SmI_2 or Cp_2TiPh) both *trans*-and *cis*-diols can be produced. Furthermore, variation of the aglycon is easily achieved at a centre point in the synthesis.

2.4 Research proposal

2.4.1 Synthesis of a (2→6) linked homo aza-*O*-disaccharide and aza-*O*-trisaccharide

At the outset of the project, investigations into the synthesis of disaccharide intermediates had already been carried out within the group.[68] Thus, the protected azadisaccharides (2 diastereoisomers **266** and **267**) and azatrisaccharide **268** had been

synthesised *via* the route outlined below (Scheme 43). Consequently, the initial aims of the project were to repeat and optimise this synthesis, fully characterise all intermediates and investigate the full deprotection of the key intermediates to form target compounds **269, 270** and **271**. Once this had been achieved, biological screening of the novel compounds was also to be investigated.



Scheme 43

Furthermore, the aim was to also apply this methodology to the synthesis of (1→6) linked homoaza-*O*-disaccharides *e.g.* **272** (Figure 18). Investigations into the synthesis of (1→6) linked systems are discussed in chapter 3.

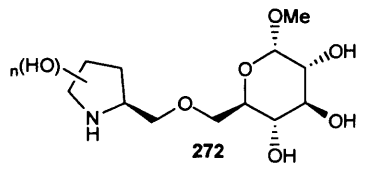


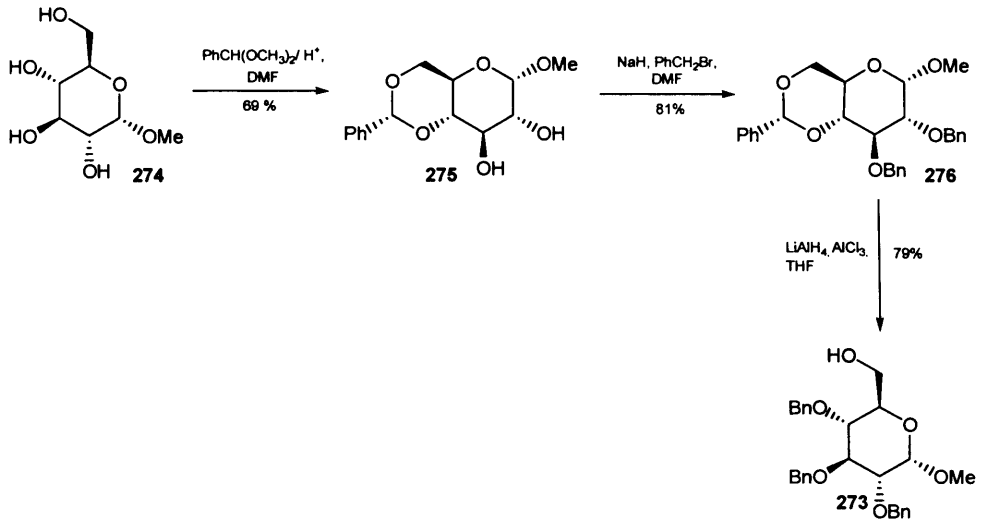
Figure 18

2.5 The synthesis of the (2→6) linked homoaza-*O*-disaccharide and trisaccharide

2.5.1 Preparation of the selectively protected sugar moiety 273

For the synthesis of both the azadisaccharide and azatrisaccharide the glucose derivative **273** (with a free 6-OH group) was required. This was synthesised using the established route shown below (Scheme 44).[68] Methyl α-D-glucopyranoside **274** was first treated with

benzaldehyde dimethylacetal, and a catalytic amount of *p*-toluene sulfonic acid monohydrate in DMF[69] to give the acetal **275** in 69% yield after several rounds of recrystallisation (from 1% pyridine in isopropanol). The partially protected glucose derivative **275** was then further protected using benzyl bromide.[70] In initial reactions the required dibenzylated product **276** was accompanied by a small amount of a monobenzylated sugar; however it was discovered that a longer reaction time (38 h) and 4.5 equivalents of sodium hydride minimised this side product. Due to the complexity of the ^{1}H-NMR, the site of monobenzylation was not determined.



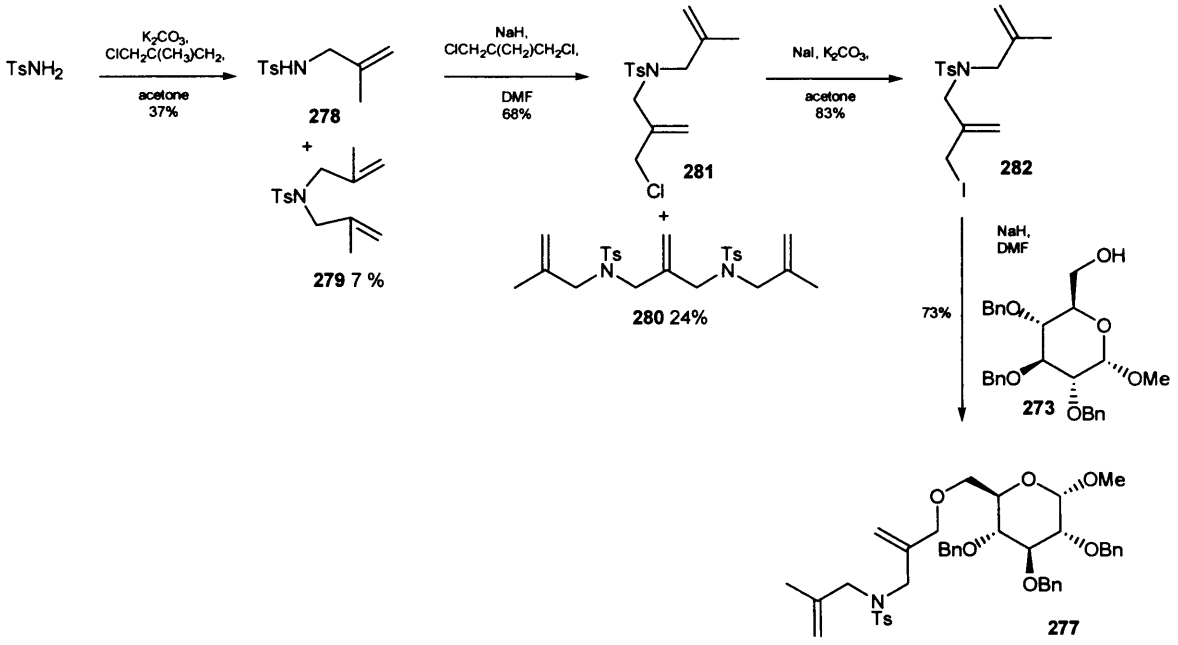
Scheme 44

The acetal group was then selectively cleaved following a procedure reported by Leggetter and co-workers[71] using one equivalent each of $LiAlH_4$ and $AlCl_3$ in THF to generate the 6-hydroxy derivative **273**. Early attempts produced poor yields and required long reaction times but this could be overcome using newly purchased $AlCl_3$ (Lancaster) which allowed the reaction to go to completion more rapidly (approx. 6 hours) and in good yield (79%). Selective cleavage in this reaction (*i.e.* **276→273**) is proposed to occur due to preferred co-ordination of the Lewis acid $AlCl_3$ on the least sterically hindered hydroxy atom (*i.e.* C-6).[71]

2.5.2 The synthesis of the diene precursors

2.5.2.1 Synthesis of the disaccharide diene precursor[68]

With the protected sugar moiety **273** in hand our attentions then turned to the synthesis of the nitrogen containing diene **277** (Scheme 45). Thus, *p*-toluene sulfonamide was monoalkylated using 3-chloro-2-methyl-propene and potassium carbonate. Unfortunately, in our hands this reaction gave **278** in a relatively low 37% yield together with a small amount (7%) of the dialkylated product **279**.

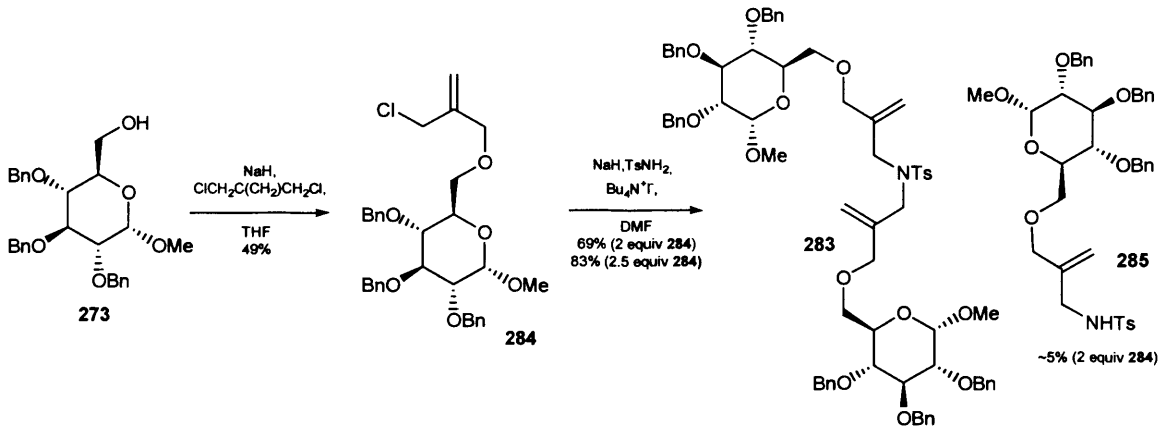


Scheme 45

The sulfonamide **278** was then alkylated with 3-chloro-2-chloromethyl-1-propene and to minimise the production of the side product **280** (resulting from reaction of the product **281** with the anion of **278**) an excess of the dichloride (1.8 equivalents) was used. Pleasingly, the chloride **281** was obtained in good yield (68%) although this was still accompanied by the side product **280** (24%). The chloride **281** was then converted to the corresponding iodide **282** to enable a more effective S_N2 reaction to be undertaken with the sugar moiety **273**. This was achieved using the Finkelstein reaction and the iodide **282** was obtained in a pleasing 83% yield. Once the two coupling partners had been synthesised, the protected sugar moiety **273** was attached *via* its primary hydroxyl to the diene **282** using sodium hydride in THF to yield the required diene precursor **277** in an excellent 73% yield.

2.5.2.2 Synthesis of the trisaccharide diene precursor [68]

Adaptation of the previously described synthetic route allows the preparation of a novel (2→6) linked aza-*O*-trisaccharide. To our knowledge there are no reports in the literature detailing the synthesis of such compounds and hence this demonstrates the production of a new class of sugar mimic. The diene intermediate **283** required for the synthesis of the trisaccharide is symmetrical and therefore a more concise route was followed as shown (Scheme 46).



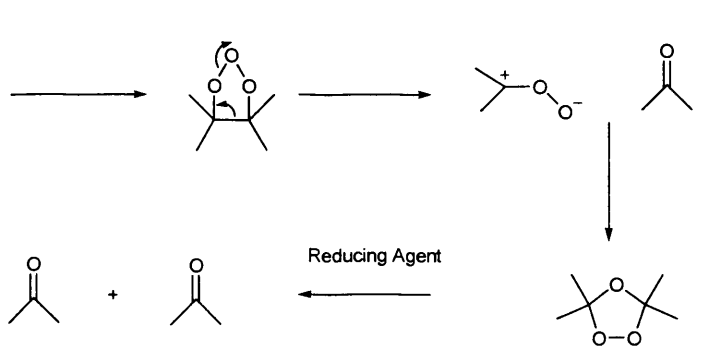
Scheme 46

Firstly, the glucose derivative **273** was reacted with 3-chloro-2-chloromethylpropane using sodium hydride in THF to give the chloride **284** in 49% yield (70% based on recovered starting material **273**). The chloride **284** was then treated with *p*-toluenesulfonamide (0.5 equivalents) in the presence of sodium hydride and tetra butyl ammonium iodide (for *in situ* iodination). Our initial attempt at this reaction yielded the required diene **283** (69%) together with a minor amount (~5%) of the monoalkylated product **285**. Attempts to remove this side product from the diene **283** proved to be problematic, both at this stage and at the subsequent diketone stage of the synthetic pathway (see below). Therefore in an effort to minimise the formation of **285** the reaction was repeated using an increased amount (2.5 equivalents) of the chloride **284** with respect to *p*-toluenesulfonamide. Gratifyingly this proved successful and the diene **283** was produced with a minimal amount of **285** (<5% by ^{1}H-NMR) in an excellent 83% yield.

2.5.3 Ozonolysis of the dienes 277 and 283

2.5.3.1 The ozonolysis reaction

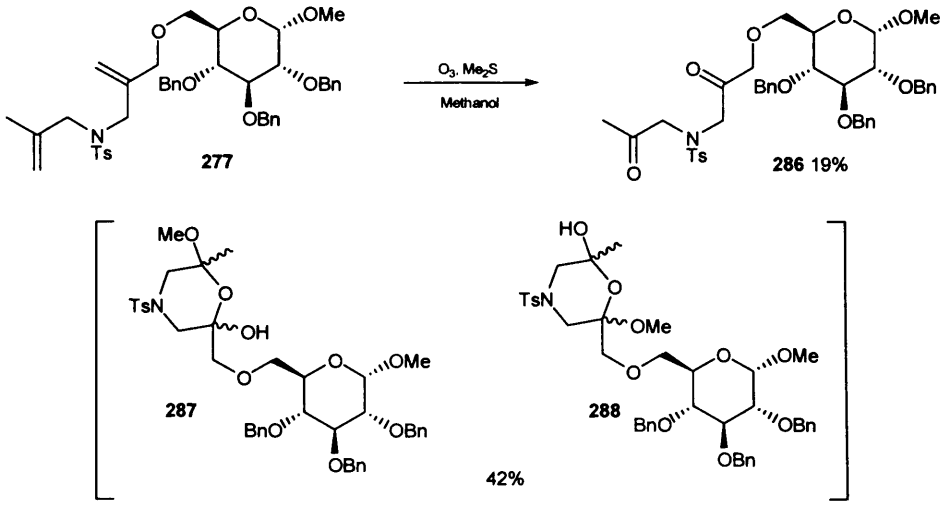
With the key dienes **277** and **283** in hand our attentions turned to the synthesis of the requisite dicarbonyls. The use of ozone is an effective method of oxidative alkene cleavage to give carbonyl species. The accepted mechanism identified by Criegee and co-workers[72] (of carbonyl formation) is illustrated below (Scheme 47).



Scheme 47

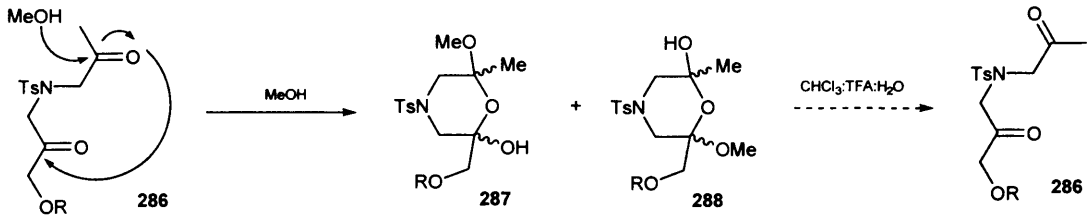
2.5.3.2 Ozonolysis of the disaccharide diene precursor 281[68]

Diene **277** underwent ozonolysis in methanol using dimethylsulfide as a reducing agent to give (after purification by column chromatography) the dicarbonyl **286** (19%) (Scheme 48).



Scheme 48

The relatively poor yield of dicarbonyl **286** can be explained by the isolation of a significant amount (42%) of side products from the reaction. Whilst not completely identified, these side products were thought to be the cyclic ketals **287** and **288** (from mass spectrometry and ^{1}H- and ^{13}C-NMR analysis) formed as a result of attack of methanol followed by an intramolecular cyclisation (Scheme 49).

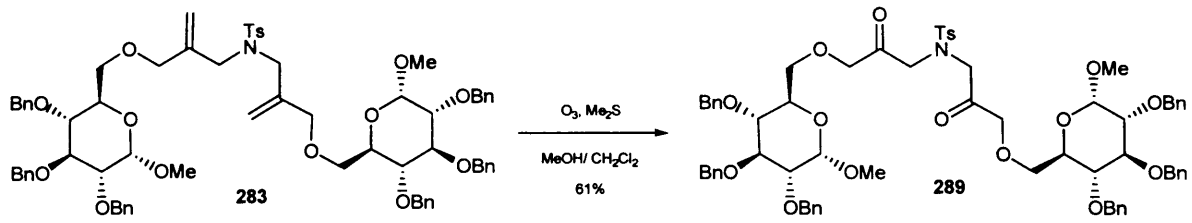


Scheme 49

Efforts were made to convert the suspected ketal mixture back to the dicarbonyl **286** by heating **287** and **288** in a 6:3:3 solution of $CHCl_3$, TFA and H_2O (Scheme 49). This method had previously been successfully employed on 'simpler' cyclic ketals within the group. However, with the disaccharide system this proved to be unsuccessful as from ^{1}H-NMR analysis of the resulting material, decomposition was evident. Repeating the hydrolysis using a 3:0.5:1.5 mixture of the above components (*i.e.* a reduction in acid concentration), also yielded the same outcome and no identifiable diketone **286** was isolated.

2.5.3.3 Ozonolysis of the trisaccharide diene precursor

Following the same procedure described for the disaccharide precursor **277**, ozonolysis of trisaccharide diene precursor **283** was undertaken in a 50:50 methanol-dichloromethane solution (due to the insolubility of the diene precursor solely in methanol) and yielded dicarbonyl **289** in a good 61% yield (Scheme 50). Fortunately, in contrast to the ozonolysis of **277**, no cyclic acetal was isolated. The reason for this is unknown although it could possibly be explained by two factors. Firstly, because the ozonolysis reaction was carried out in a mixture of methanol and dichloromethane, this may have considerably reduced the probability of attack by methanol on the dicarbonyl **289**. Secondly, compound **289** is extremely hindered, and steric factors may have additionally disfavoured nucleophilic attack.



Scheme 50

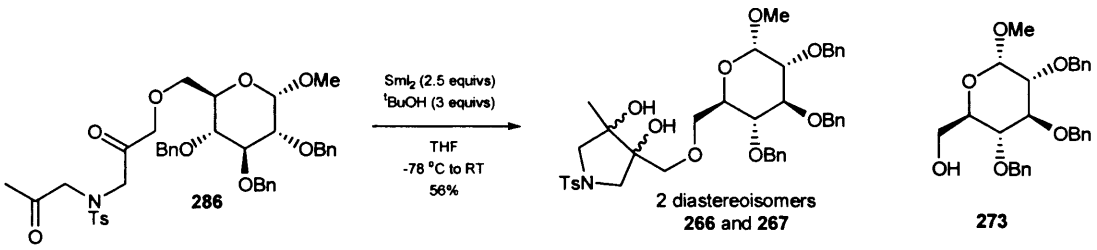
2.5.4 Pinacol coupling of the dicarbonyls 286 and 289

With the dicarbonyls **286** and **289** in hand, the pinacol coupling was subsequently investigated. Due to the more straightforward nature of the reaction procedure, the samarium diiodide-mediated pinacol coupling was attempted first. To enable the metal to perform its role as a reductant, the samarium must remain in its reduced +2 state. Consequently, the reaction was carried out under argon using a strict protocol. Investigations within the group[73] had shown that the use of commercially available samarium diiodide gave unpredictable results. Thus, SmI_2 (2.5 equivalents) was made *in situ* from samarium metal and CH_2I_2 in THF and used straight away as a dark blue solution. Additionally ^{t}BuOH was added as a proton source to allow the formation of the diol product.

2.5.4.1 Pinacol coupling of the disaccharide dicarbonyl

The disaccharide dicarbonyl precursor **286** underwent a samarium diiodide-mediated pinacol coupling using the standard conditions developed within the group.[68,73] After conducting the reaction at -78 °C the reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Work-up followed by ^{13}C- and ^{1}H-NMR analysis of the resulting crude material signified the formation of two major products in a 1:1 ratio. Purification by flash column chromatography enabled these to be separated (product 1 (22%),

product 2 (20%), mixed fractions (14%): total yield (56%)) (Scheme 51). A small amount of the sugar moiety **273** was also thought to have been produced in the reaction (from analysis by TLC), although this was not isolated. Analysis of the two major products by mass spectrometry showed them to have the same molecular ion ($M+NH_4^+$ = 765) and detailed examinations of 1D and 2D ^1H- and ^{13}C-NMR additionally confirmed them be the 1,2-diols **266** and **267**.*



Scheme 51

2.5.4.2 Determination of diol stereochemistry

To investigate the stereochemistry of the newly created diol nOe investigations were carried out. As the pyrrolidine methyl group gave an obvious 3H singlet at ~1.15ppm in both isomers **266** and **267**, this was irradiated in nOe decoupling experiments. Irradiation of this signal in diastereoisomer 1 **266** (higher R_f isomer) induced nOe enhancements to the C-2 (pyrrolidine numbering) proton resonances appearing as a multiplet (AB quartet overlapping at 3.20-3.32ppm) (12.7%) and to the C-4 CH_2O resonance (appearing as a singlet at 3.44ppm) (8.1%) (Figure 19). Irradiation of the methyl group in diastereoisomer 2 **267** (lower R_f isomer) induced similar nOe enhancements (C-2 resonances appearing as a multiplet (AB quartet overlapping at 3.20-3.30ppm) (8.8%) and the C-4 CH_2O resonance (appearing as an AB quartet at 3.48ppm) (8.8%)).

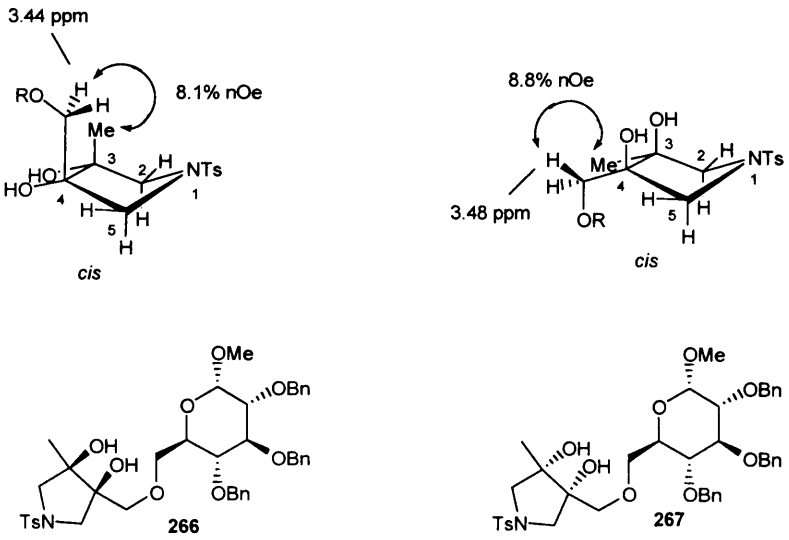


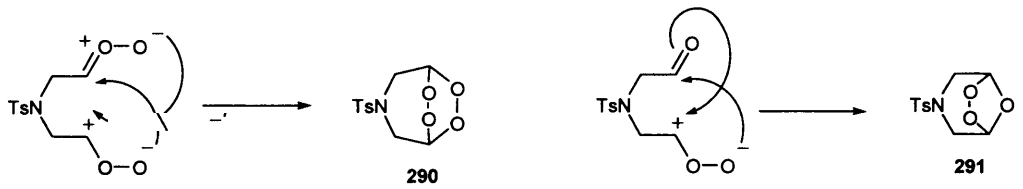
Figure 19

* In agreement with results obtained by S. Reakes

The large nOe interactions between the C-3 methyl and C-4 hydroxymethyl protons strongly suggest both isomers are the *cis*-1,2-diols **266** and **267** shown in Figure 19.† This *cis*-selectivity for the samarium diiodide-mediated pinacol reaction is as expected. From these results alone it was not possible to determine the absolute stereochemistry of each isomer, (*i.e.* which corresponds to **266** or **267**), although investigations into this area have been undertaken and are described in section 2.5.8.2.

2.5.4.3 Optimisation of the ozonolysis step

As the yield of the dicarbonyl **286** isolated from the ozonolysis step was so low, this made the overall synthesis somewhat inefficient and consequently optimisation of this step was needed. Investigations by M. S. Kachla in related work had demonstrated that dicarbonyl precursors formed by an ozonolysis reaction in dichloromethane (*vs.* methanol used previously) could be taken into the pinacol coupling as crude mixtures. This still generated the required diols in good yields and circumvented the problem of cyclic acetal formation.[73] Methanol had previously been used as a solvent in the ozonolysis step to prevent potentially explosive cyclic peroxides and/or ozonides *e.g.* **290** and **291** forming through 1,3-intramolecular additions (Scheme 52). However, investigations by M. S. Kachla, determined that the Sm^{2+} used in the pinacol coupling reduces the oxygen-oxygen bond in these (or related) compounds generating the required dicarbonyl, which then undergoes the pinacol coupling *in situ* to form the corresponding diol.[73]



Scheme 52

In view of these findings ozonolysis of the diene **277** was performed in dichloromethane and the resulting crude dicarbonyl **286** taken directly into the pinacol coupling using an excess of samarium diiodide (5 equivalents, to make sure total reduction of the cyclic peroxides/ozonides is achieved). This proved very successful and purification by column chromatography yielded the required diols **266** and **267** in a 49% yield overall from **277**. Pleasingly, comparing this to our initial attempts (8% conversion over two steps), the yield had been greatly improved. Furthermore, together with the expected diols **266** and **267** we also isolated the free sugar moiety **273** in significant amounts (17%) from this reaction (this was suspected to have been formed in the previous pinacol reaction, although was not

† In agreement with results obtained by S. Reakes

isolated). The presence of **273** suggested cleavage of the α-ether linkage in the dicarbonyl **286** was taking place and cleavage of C-heteroatom bonds α to carbonyl groups by SmI_2 is a known reaction although not usually observed in the intramolecular pinacol reactions of protected α-hydroxy dicarbonyl systems.[74,75] To investigate whether this side reaction was due to the use of the excess (5 equivalents) of SmI_2 used in the transformation the diketone **286** was purified and isolated (in 65% yield) after ozonolysis in dichloromethane. Interestingly we also isolated 19% of the suspected ozonide **292** (mass spectrometry showed the molecular ion $M+NH_4^+$ 809 and ^{13}C-NMR indicated the presence of only one C=O and a CH_2 at 92.94 ppm and a quaternary C at 107.67 ppm) (Figure 20).

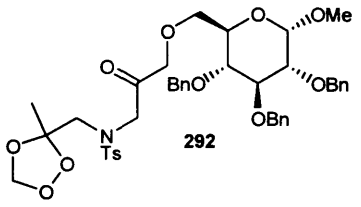
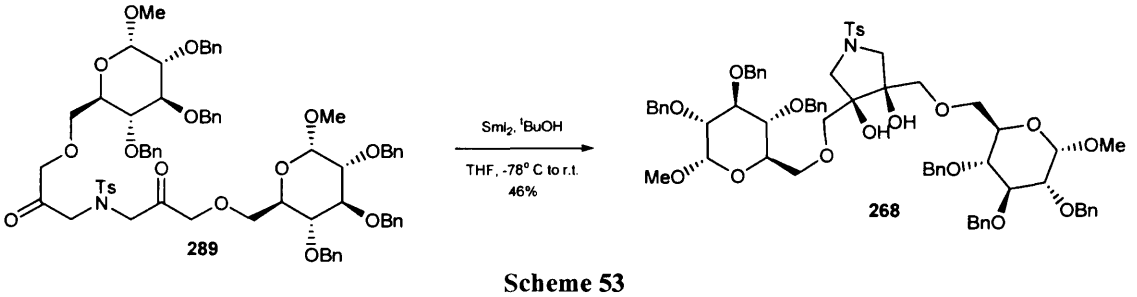


Figure 20

Reaction of pure **286** with 2.5 equivalents of SmI_2 subsequently afforded both diastereoisomers of the cyclic diol (**266** and **267**) in 58% yield (35% yield from **277**) and **273** was again isolated although in a smaller amount (6%).

2.5.4.4 Pinacol coupling of the trisaccharide dicarbonyl

The isolated trisaccharide dicarbonyl **289** underwent a pinacol coupling reaction (using 2.5 equivalents of SmI_2 as explained for the disaccharide) to produce the expected diol **268** (46% yield). Again, as with the disaccharide mimic, a small amount (11%) of the sugar moiety **273** was produced. Although no cyclic acetal was formed in the ozonolysis of the diketone **289**, in an attempt to improve the yield of the diol over two steps, the ozonolysis reaction was repeated in 100% dichloromethane (*vs.* 50:50 methanol-dichloromethane used previously) and the crude diketone subjected to the pinacol coupling (using 5 equivalents of SmI_2). Gratifyingly, this was successful and **268** was obtained in 40% yield from **283** (compared to 28% over two steps) but again the cleaved sugar moiety **273** was also recovered (12%) (Scheme 53).

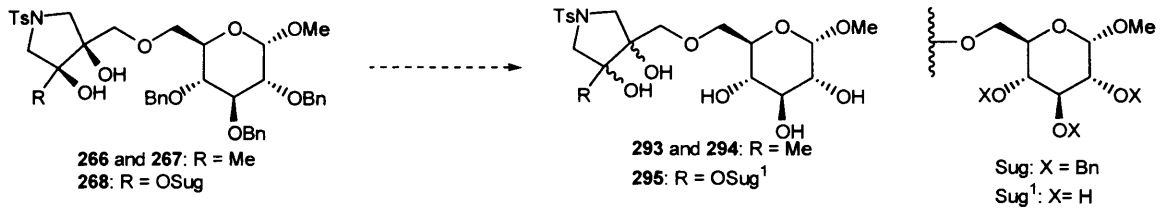


Scheme 53

Determination of the diol stereochemistry in the azatrisaccharide adduct **268** was much more difficult as nOe experiments could not be undertaken due to the symmetrical nature of the molecule. Additionally, the material was not crystalline, so x-ray diffraction analysis could not be used. The isolation of only one product from the pinacol reaction does support **268** being the *cis*-diol, as we would have expected the formation of two diastereoisomers if the *trans*-diol had been favoured. Investigations into assigning the stereochemistry of the diol were attempted and are discussed in section 2.5.8.4.

2.5.5 Benzyl deprotection

With the pinacol adducts **266**, **267** and **268** in hand, our attentions were subsequently focused on removing the benzyl protecting groups. The standard method of accomplishing this transformation is by hydrogenolysis using H_2 in combination with a catalyst *e.g.* Pd/C or $Pd(OH)_2$, [76] although *in situ* hydrogen transfer methods using ammonium formate with Pd/C or cyclohexene with $Pd(OH)_2$ are also commonly reported. [77]



Scheme 54

2.5.5.1 Benzyl deprotection of the disaccharide

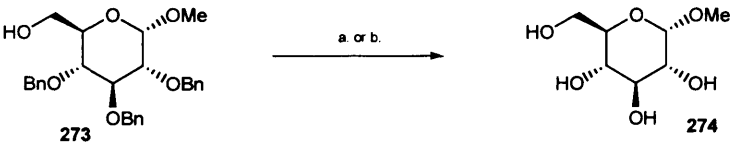
Initially, deprotection was attempted following a standard procedure using 10% palladium on charcoal in methanol under a hydrogen atmosphere.[76] After several attempts we discovered the reaction was very difficult to push to completion and a mixture of products always resulted. We suspected catalyst poisoning was occurring and consequently in succeeding reactions when no further change was apparent (analysis by TLC), the catalyst was filtered and without isolation of material, the reaction repeated using fresh Pd/C. Unfortunately, this proved unsuccessful and the fully deprotected compound **293** was never isolated. We therefore attempted using high-pressure to achieve complete deprotection. Thus, stirring the reaction mixture for 3.5 hours at a hydrogen pressure of 60 psi, induced a minimal reaction (analysis by TLC). Disappointingly, filtering of the catalyst and repeating the reaction for a further 5 hours at a pressure of 150 psi, produced no further reaction.

Hydrogen transfer methods were then investigated as it is reported these are less prone to catalyst deactivation.[77] A procedure reported by Beig and co-workers was first followed using ammonium formate and Pd/C in methanol.[77] After several attempts at this reaction, complete deprotection was achieved by employing a similar approach as before. Thus,

filtering the reaction mixture and repeating the reaction with fresh catalyst approximately 2-3 times enabled isolation of the pentol **293** to be achieved. Disappointingly, because of the larger amounts of catalyst needed using this procedure, filtration of the reaction mixtures proved very difficult and often the isolated yields of **293** were very low (~30 % crude yield).

An alternative method using 20% $Pd(OH)_2$ and cyclohexene[78] was then explored. Due to the valuable nature of the disaccharide intermediate **266**, a test reaction was first undertaken on the sugar moiety **273**. Pleasingly, after two hours the reaction was successful and the deprotected sugar **274** was isolated in good yield (99%) (Scheme 55). Unfortunately, when these conditions were applied to the deprotection of the disaccharide adduct **266**, the reaction was unsuccessful and no identifiable products by ^{1}H-NMR were isolated. On the basis of this result, we therefore strongly suspected an inorganic contaminant, possibly from the ozonolysis reaction or the pinacol coupling step was causing catalyst deactivation.

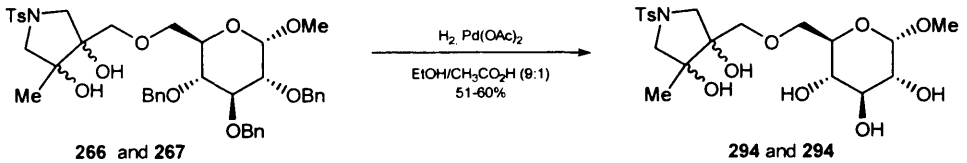
A comprehensive literature search uncovered a report by Fairbanks and co-workers in which $Pd(OAc)_2$ in ethanol and acetic acid under a hydrogen atmosphere (balloon) had been used to achieve multiple benzyl deprotections.[79] To test these conditions this procedure was firstly attempted on the sugar moiety **273**. Fortunately, the reaction went to completion in 3 hours and **274** was isolated in good yield (crude yield ~100%) (Scheme 55).



a. cyclohexene, $Pd(OH)_2$, EtOH, 99% crude yield
b. H_2, $Pd(OAc)_2$, EtOH/CH_3CO_2H (9:1), >100% crude yield

Scheme 55

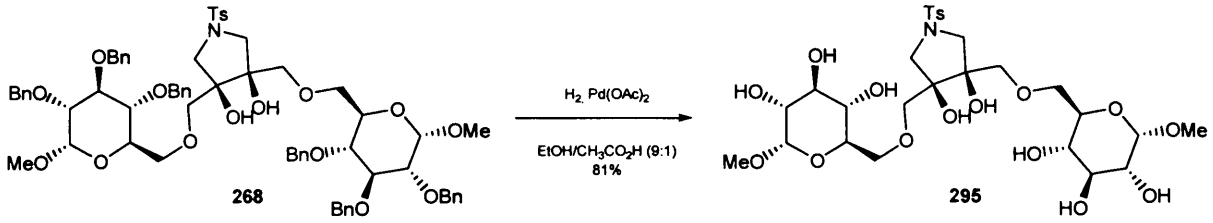
Thus, the same conditions were applied to one of the disaccharide diastereoisomers **266**. Pleasingly, the reaction was successful, as after stirring for only 3 hours, the reaction had gone to completion. Due to the homogenous nature of the reaction, filtration of the mixture was less problematic and an excellent crude yield was obtained (>100%). Purification by flash column chromatography subsequently afforded the deprotected disaccharide **293** in good yield (51%) (Scheme 56). This method was also repeatable and hence deprotection of the second isomer **267** was also achieved yielding **294** in good yield (60%).



Scheme 56

2.5.5.2 Benzyl deprotection of the trisaccharide

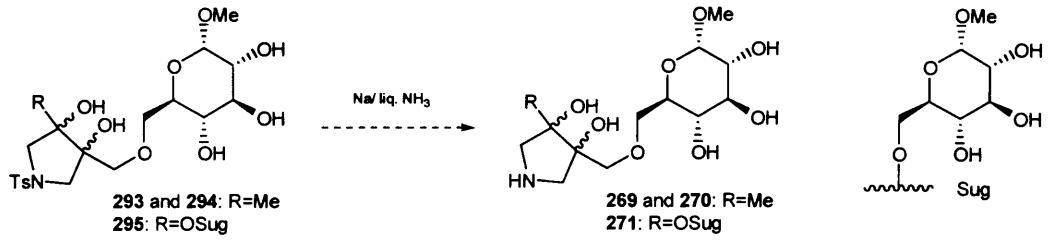
The same benzyl deprotection procedure discussed above was then applied to the trisaccharide intermediate **268** (Scheme 57). Fortunately the reaction again proved successful and the reaction went to completion after 3 hours. Purification by flash column chromatography subsequently gave the octol **295** in excellent yield (81%).



Scheme 57

2.5.6 Removal of the tosyl group

With the benzyl deprotected adducts in hand, removal of the tosyl group was next investigated. Reagents commonly employed to carry out this transformation include, sodium in liquid ammonia,[80] HBr in acetic acid,[81] HBr and phosphorous,[82] electrolysis[83] and sodium napthalenide.[84] Within the group M. S. Kachla had already demonstrated tosyl deprotection in related compounds using Birch-type conditions (sodium in liquid ammonia)[73] and hence this method was investigated for the oligosaccharide adducts **293, 294** and **295** (Scheme 58).



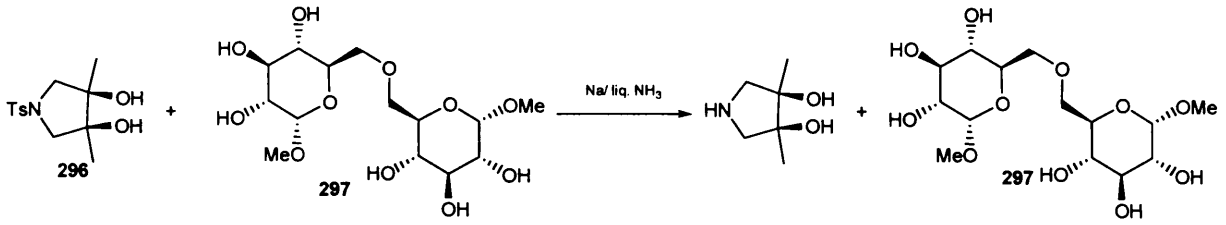
Scheme 58

2.5.6.1 Tosyl deprotection of the disaccharides 293 and 294

Group 1 metals such as sodium or lithium readily give up their outer-shell electrons as they dissolve in solvents such as liquid ammonia or ethanol. Electrons are the simplest reducing agents and will reduce any functional group with a low-energy π^* orbital in which the electron can go (*e.g.* NSO_2 of the tosyl group). In a typical reaction the substrate is dissolved in liquid ammonia and sodium metal is added portion-wise. A blue colour is observed which is indicative of the sodium ionising and the electrons being solvated with ammonia. Over time the blue colour fades as the ammonia is reduced to NH_2^- and hydrogen

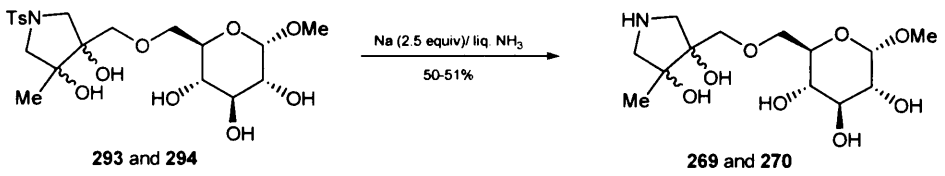
gas. Therefore sodium is added to the solution until blue colour remains indicating the completion of the reaction.[80]

To gauge the stability of the ether linkage in the oligosaccharide intermediates **293, 294** and **295** a test reaction was first carried out using **296** (donated by M. S. Kachla) together with the synthesised disaccharide **297** (Scheme 59).



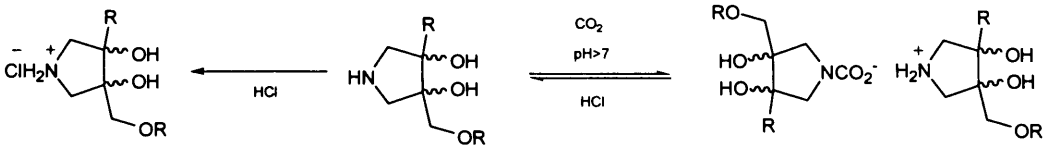
Scheme 59

Using a dry ice condenser, **296** and **297** were dissolved in a minimal amount of liquid ammonia. Sodium was then added to the mixture until a blue colour persisted. Subsequent work-up followed by mass spectrometry and ^{1}H-NMR analysis of the crude mixture revealed the tosyl deprotection had been successful and fortunately, the disaccharide **297** was still intact. This procedure was subsequently repeated on the disaccharide adduct **293** although unfortunately, ^{1}H-NMR analysis of the resulting crude material indicated total decomposition had occurred. In the procedure a large amount of sodium (more than for **296**) was needed for a blue colour to persist, so we suspected further reactions had occurred in addition to tosyl group removal. The reaction was therefore repeated using a weighed amount of sodium (2.5 equivalents) and although the blue colour did not persist the reaction was worked-up. Pleasingly, ^{1}H-NMR analysis of the crude material from the reaction indicated the reaction had been successful and isolation of the final product **269** was then investigated. Purification using a DOWEX (50WX2-200 regenerated from 1M HCl and washed to pH6 with H_2O) column was first attempted and although the isolation of **269** was achieved, this was accompanied with a large amount of unknown inorganic material. Therefore tosyl deprotection was repeated and purification was attempted using flash column chromatography on silica using basified (NH_3) methanol as eluent. Pleasingly, this proved to be successful and the fully deprotected disaccharide **269** was isolated in 51% yield (Scheme 60). Application of the same tosyl deprotection reaction protocol to the second isomer **294** subsequently gave access to the disaccharide **270** in a reasonable 50% yield (Scheme 60).



Scheme 60

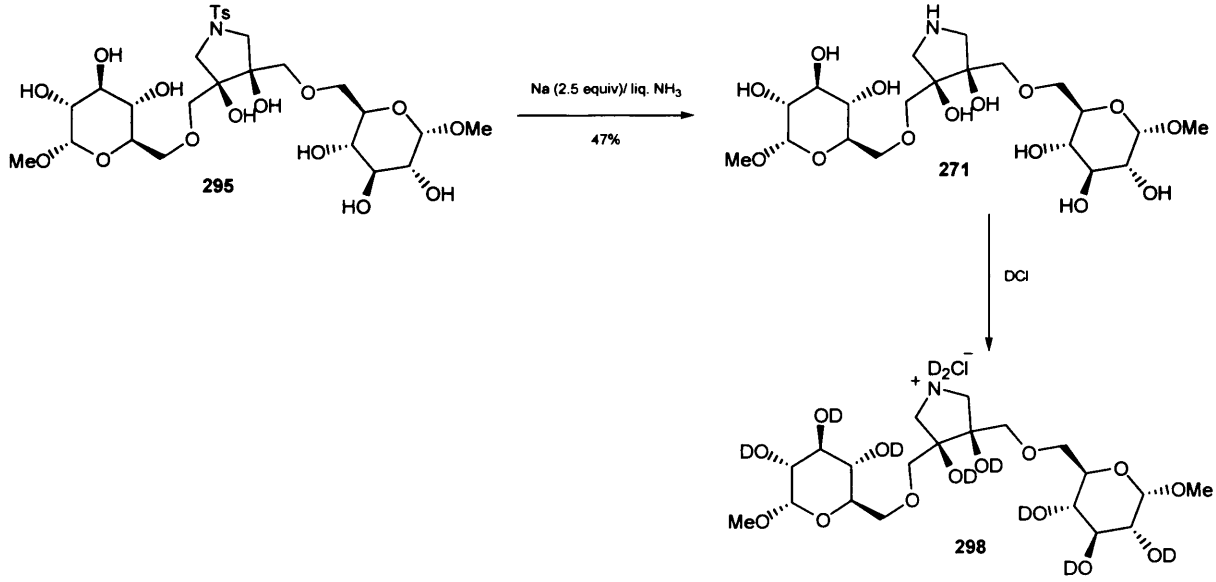
The stability of these compounds then became questionable when ^{1}H- and ^{13}C-NMR analysis of the pure compounds in D_2O revealed the presence of two products (doubling up of signals). Additionally an unknown signal at ~162.8 ppm in the ^{13}C-NMR of both compounds was also present. From these observations we suspected the amines were undergoing a reaction with CO_2 present in D_2O in the NMR tube. This is a commonly reported reaction, although it can be reversed by acidification of the reaction mixture as the resulting carbamic acids are only stable in basic or neutral conditions (Scheme 61).[85] Subsequent acidification by the addition of DCl to the D_2O in the NMR tube confirmed this and subsequent ^{1}H- and ^{13}C-NMRs of the DCl salts of **269** and **270** showed the disappearance of the signal at ~162.8 ppm in the ^{13}C-NMR and the presence of only one product.



Scheme 61

2.5.6.2 Tosyl deprotection of the trisaccharide 295

Tosyl deprotection using sodium in liquid ammonia was also successfully applied to the trisaccharide **295** (Scheme 62) to yield the amine **271** in 45% yield. This was immediately acidified (in the same manner described for the disaccharides **269** and **270**) to give the DCl salt **298** (Scheme 62).



Scheme 62

Whilst the ^{13}C-NMR of this compound showed no C=O signal, doubling up of signals was apparent, a phenomenon seen also in the earlier trisaccharide intermediates **268** and **295** (Schemes 57 and 62). From analysis by TLC and COSY experiments of these compounds, we

were confident only one product was present in each case and therefore suspected this to be due to the diastereotopic nature of the molecule once the two new chiral centres have been formed in the pinacol coupling step. For example, if asked to differentiate between the two carbons on the azasugar ring (*e.g.* one X and one Y), this can be displayed in two ways (Figure 21). Due to the fact these compounds are not identical these two carbons are diastereotopic and consequently give rise to two different signals in the ^{13}C-NMR.

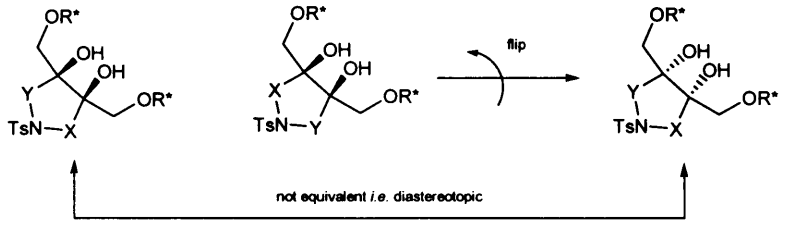


Figure 21

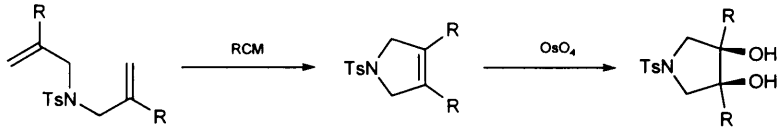
2.5.7 Summary

To summarise, the synthesis of the fully deprotected disaccharides **269** and **270** and trisaccharide **271** has been accomplished. The existing routes established to the disaccharide intermediates **266** and **267** and the trisaccharide intermediate **268** have been repeated and most steps have been successfully optimised. After extensive investigations, removal of the benzyl groups and tosyl groups in the intermediates has been achieved and the fully deprotected compounds **269**, **270** and **271** have been fully characterised.

In an attempt to verify the *cis*-stereochemistry in the diol intermediates, and to enable the synthesis of derivatives that would potentially shed light on the absolute stereochemistry of these compounds, investigations into an alternative non-ambiguous route to the diols **266**, **267** and **268** were also carried out.

2.5.8 Alternative synthesis of the pinacol intermediates 266 and 267 and 268, investigations into relative and absolute stereochemistry

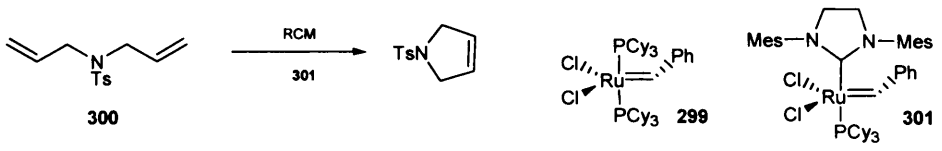
The stereoselective pinacol coupling reactions of dicarbonyl species to synthesise *N*-heterocyclic 1,2-diols complements an alternative route to these compounds based on a ring closing metathesis and dihydroxylation approach (Scheme 63) (a full detailed discussion of this methodology and its application in synthesis is given in chapter 3). We therefore decided to employ this strategy to synthesise **266** and **267** and **268** to firstly demonstrate this route to the compounds and to also confirm the *cis*-selectivity of the pinacol coupling.



Scheme 63

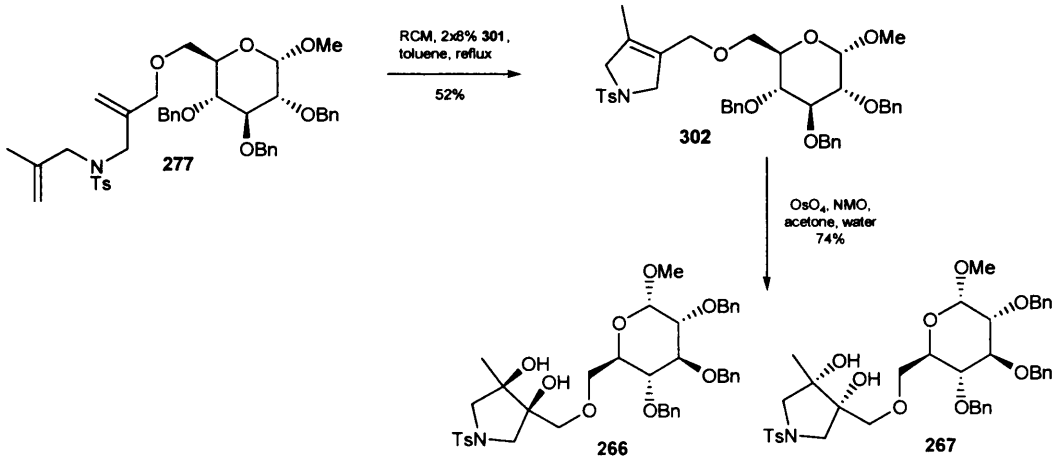
2.5.8.1 Use of ring closing metathesis (RCM) and dihydroxylation to verify the relative cis-stereochemistry in the disaccharides 266 and 267

Ring closing metathesis (RCM) using the first generation Grubbs catalyst **299** (Scheme 64) is a very versatile technique for the formation of five to seven membered carbocycles and heterocycles.[86] However, although this catalyst is increasingly used in organic synthesis, its use is generally confined to the metathesis of unsubstituted dienes *e.g.* **300** (Scheme 64).[87] Nowadays, the second generation Grubbs catalyst **301** overcomes this difficulty and the metathesis of substituted dienes to form tri- and tetra-substituted alkenes has recently been reported.[86, 88]



Scheme 64

At the outset of this project, the second generation Grubbs catalyst was not commercially available, however towards the latter stages of our work this catalyst could be purchased and hence, ring closing metathesis of the diene **277** was attempted (Scheme 65).[88] Initial heating of the diene in refluxing dichloromethane using 2% of catalyst gave only a minimal reaction after 24 hours (<10% conversion by TLC). The reaction was subsequently repeated in refluxing toluene using 8% catalyst although after 24 hours the same outcome was apparent. However, the addition of a fresh portion of catalyst (8%) and further heating did increase the conversion (~50% by TLC) and after work-up and purification by flash column chromatography, the alkene **302** was isolated in 52% yield. Alkene **302** then underwent dihydroxylation using OsO_4 with NMO in acetone and water (Scheme 65).[89]



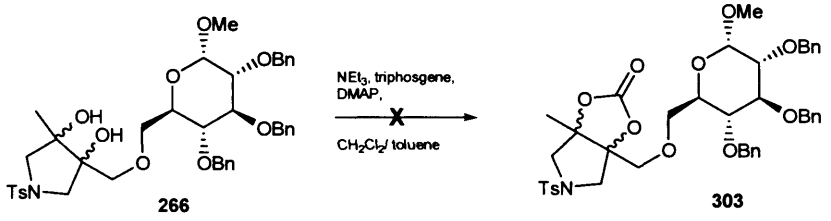
Scheme 65

This proceeded smoothly and after stirring at room temperature for 20 hours an approximate 1:1 mixture of products was formed. Purification by chromatography subsequently yielded the

diols **266** and **267** in 74% yield. Gratifyingly, comparison of ^{1}H- and ^{13}C-NMR data of these compounds to the diols synthesised *via* the pinacol coupling confirmed these compounds to be identical hence confirming the *cis*-selectivity of the aforementioned pinacol reaction.

2.5.8.2 Investigations into the absolute stereochemistry of the disaccharides 266 and 267

Disappointingly, all the diol compounds that were synthesised during our route proved to be non-crystalline and consequently we were not able to use x-ray crystallographic analysis to determine the absolute stereochemistry of the diols **266** and **267**. Efforts were therefore made to synthesise crystalline derivatives of these compounds in an attempt to use this technique. Initially, the diol **266** was reacted with triphosgene in an attempt to synthesise the carbonate **303** (Scheme 66).[90] Unfortunately this transformation proved problematic, presumably due to the steric bulk of the molecule, and no derivatised product could be formed even after refluxing in toluene for 2 days.



Scheme 66

In view of this, the synthesis of the di-carbamate **304** was attempted from the diol **266** using CCl_3CONCO followed by K_2CO_3 in MeOH (Figure 22).[91] Unfortunately, an inseparable mixture of products was produced from the reaction and hence isolation of **304** proved difficult. From mass spectrometry we suspected the mono-carbamates **305** and **306** had been produced. Using the same method, the synthesis of **307** from **293** was also attempted (Figure 22). Disappointingly a mixture of inseparable products also resulted (resulting from incomplete derivatisation) and no crystalline material was isolated from the mixture.

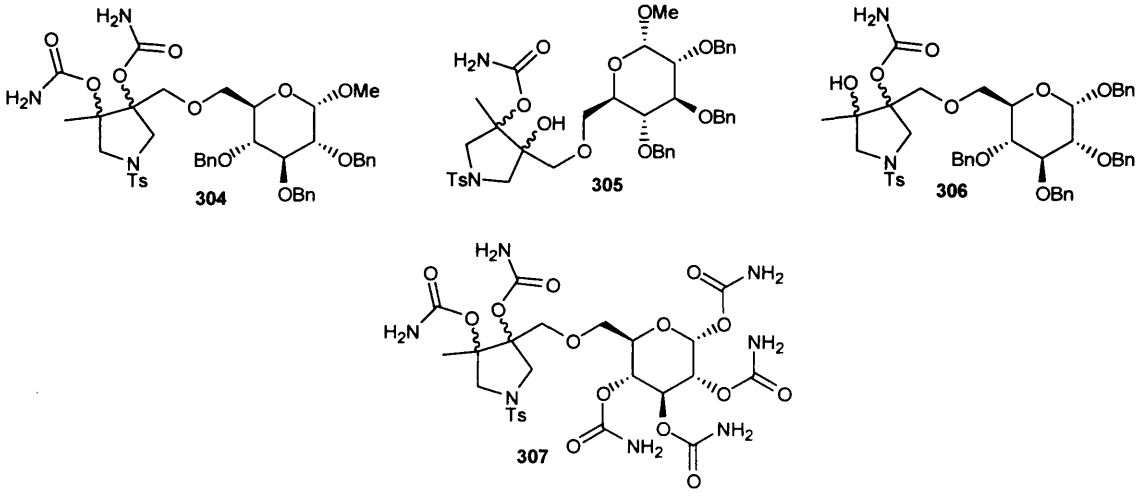


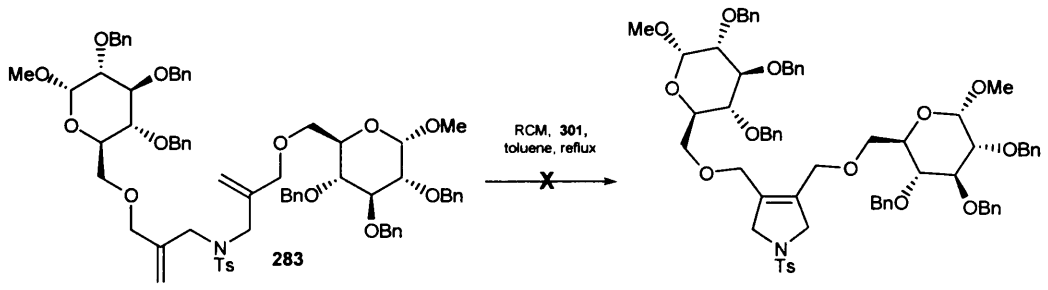
Figure 22

2.5.8.3 Summary

To summarise, RCM and *syn*-selective dihydroxylation (OsO_4-NMO) has been used to complement the synthesis of the pinacol diols **266** and **267**. Due to the unambiguous dihydroxylation reaction, this synthesis has confirmed the relative diol stereochemistry of these compounds and hence the *cis*-selectivity of the pinacol coupling. Unfortunately, attempts to synthesise crystalline derivatives of these intermediates has proved to be unsuccessful. Therefore the absolute stereochemistry of these compounds is still unknown, and hopefully future work in this area will address this.

2.5.8.4 Use of RCM and dihydroxylation to confirm the relative cis stereochemistry of the trisaccharide

Because nOe investigations could not be used to confirm the *cis*-diol stereochemistry in the trisaccharide intermediate **268**, RCM on the diene **283** was attempted (with the intention of dihydroxylating the resulting alkene) to try and achieve this. Disappointingly RCM of the diene **283** proved very problematic as after several attempts only starting material was recovered (Scheme 67). We suspected this low reactivity to be either due to the steric bulk of the substrate or the coordination of the catalyst to the ether functional groups within the molecule. The use of $Ti(O^iPr)_4$ has been reported to suppress coordination of the Grubbs catalyst in substrates containing an ester functional group[92] and thus, $Ti(O^iPr)_4$ was added to the reaction mixture in a subsequent attempt. Unfortunately this proved unsuccessful as even after heating at 90 °C for 3 days no alkene formation was observed. This was unfortunate as the diols **268**, **295** and **271** were additionally non-crystalline and so x-ray crystallographic analysis could not be used to verify the *cis*-stereochemistry. Furthermore stocks of the trisaccharide diol products had also depleted and so the synthesis of any potential crystalline derivatives could not be investigated.



Scheme 67

Nonetheless, on the basis of the complete *cis*-selectivity observed for the formation of the disaccharide and the fact the pinacol coupling of the diketone **283** yielded only one product

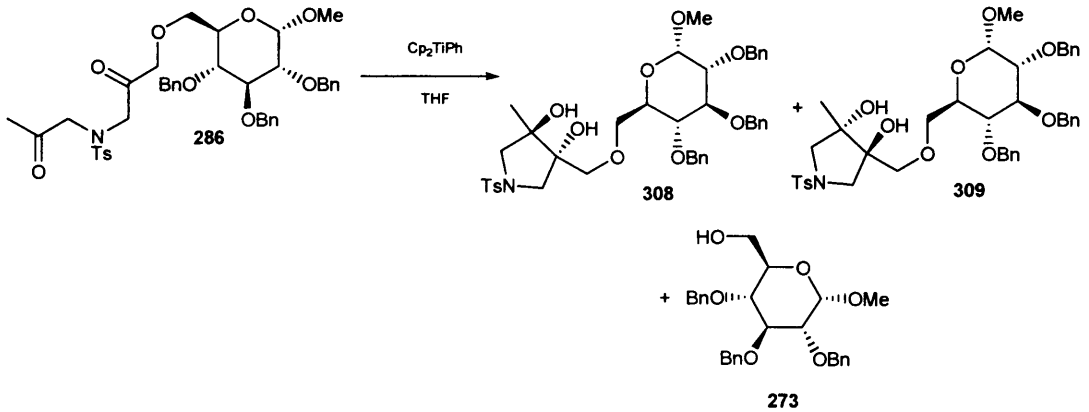
268, we strongly suspect the 1,2-diol in the trisaccharide to be *cis*. However, this does need to be confirmed and hopefully future work will address this.

2.5.9 Summary

Although complete proof of the relative *cis*-stereochemistry in the trisaccharide adduct **268** has not been achieved, results from the azadisaccharides **266** and **267** suggest the samarium-mediated pinacol coupling has been completely *cis*-selective. Thus, in an attempt to access the corresponding *trans*-isomers our attentions were then focused on the analogous titanium-mediated pinacol reaction.

2.5.10 The titanium mediated pinacol coupling

The diketone **286** was subjected to the titanium pinacol coupling protocol (established by M. S. Kachla[73]) using Cp_2TiPh. In the procedure Cp_2TiPh was prepared by treatment of Cp_2TiCl_2 with $^{i}PrMgCl$ then PhMgBr in THF under an argon atmosphere. A degassed solution of the diketone **286** substrate was subsequently added to the resultant dark-green Cp_2TiPh solution (Scheme 68). After stirring the reaction mixture for 24 hours at room temperature work-up yielded a complicated mixture of products. Attempted purification using column chromatography failed to separate these although a substantial amount of the cleaved sugar moiety **273** was isolated (29%). Thus, the reaction sequence was repeated, however in an effort to prevent sugar cleavage the reaction was worked-up after only 2 hours. Subsequent purification by chromatography again yielded a substantial amount of the sugar moiety **273** (24%) and 33% yield of two compounds thought to be the diastereoisomers **308** and **309** (mass spectrometry showed a substantial peak at 765 ($M+NH_4^+$) and ^{13}C-NMR showed indicative signals of the diol products).



Scheme 68

Due to the complicated mixture of products it was difficult to determine whether any of the *cis*-diol had been formed and unfortunately, due to the large amount of titanium by-products, complete separation of the two isomers was never achieved. Additional attempts at this

reaction were undertaken, although we found the procedure to be very sensitive and sometimes several attempts were needed to generate reagent quality Cp_2TiPh. Consequently no improvement to the yield or isolation of the diol products was achieved.

2.6 Summary and conclusions

A flexible and efficient route to two new diastereomeric (2→6) linked azadisaccharides **269** and **270** and a novel (2→6) linked azatrisaccharide **271** has been demonstrated. Using very simple starting materials the diene precursors were efficiently synthesised and subsequent ozonolysis allowed access to the requisite diketones. We have shown the samarium diiodide-mediated pinacol coupling to be completely *cis*-selective in forming the disaccharide diols **266** and **267** and preliminary results suggest this is also true for the trisaccharide **268**. Deprotection protocols of the *cis*-diol products have been established enabling access to the target aza-oligosaccharide products **269**, **270** and **271**. Unfortunately attempts to determine the absolute stereochemistry of the disaccharide has proved unsuccessful and hopefully future work will address this.

Preliminary investigations into the titanium mediated pinacol coupling have revealed this reaction to be successful in generating *trans*-diol products **308** and **309**. Unfortunately, because of the large amounts of titanium by-products produced in the reaction, isolation of the diol products was not achieved and the selectivity of diol formation determined.

We have also demonstrated the complementary use of ring closing metathesis and dihydroxylation to generate *cis*-diols **266** and **267**. However, although the *cis*-diols can be generated using this approach, access to the corresponding *trans*-diols does require the use of additional steps. Thus, the pinacol coupling is consequently still a competitive synthetic tool in the synthesis of both *cis*- and *trans*-1,2 -cyclic diols.

Compounds **269**, **270**, and **271** have been tested (in collaboration) against a range of glycosidase enzymes and the results are discussed in chapter 5.

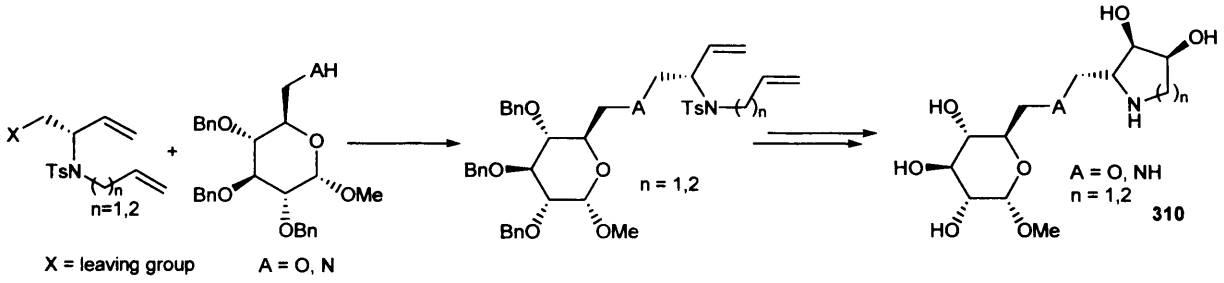
Chapter 3

Synthesis of (1→6) Linked Homoaza-*O*- and *N*-Disaccharides

3.0 Synthesis of (1→6) Linked Homoaza-*O*- and *N*-Disaccharides

3.1 Introduction

In view of the successful synthesis of the (2→6) linked sugar mimics (chapter 2), we wished to apply a similar approach to synthesise a number of novel (1→6) linked azadisaccharides *e.g.* **310** (general structure) (Scheme 69). These would represent more accurate sugar mimics as rarely in nature are disaccharides linked at a hydroxylated carbon (*i.e.* as in the (2→6) linked compounds). We envisaged an approach such as the one outlined in Scheme 69, would give efficient access to the (1→6) linked target compounds.

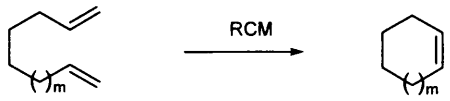


Scheme 69

Because this proposed route would involve the synthesis of an unsubstituted diene, we wanted to investigate both a pinacol coupling and a ring closing metathesis-dihydroxylation route to these target compounds. Additionally, to demonstrate flexibility we aimed to synthesise *O*- and *N*-linked azadisaccharides containing both pyrrolidine and piperidine rings. Lastly, to determine the biological activity of these (1→6) linked systems, we aimed to screen the final compounds against a range of commercially available glycosidases.

3.1.1 Introduction to ring closing metathesis

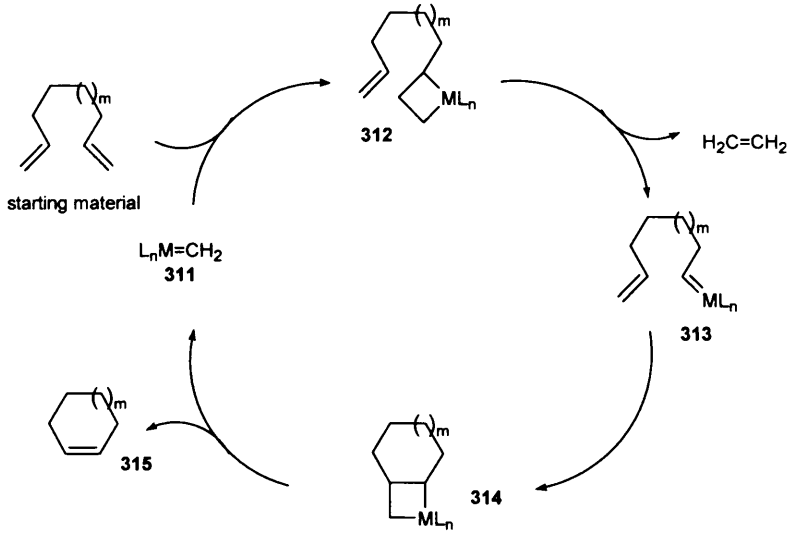
Olefin metathesis, a process by which alkylidene groups on alkenes are exchanged, was first reported in 1955 by Anderson and Merckling (Scheme 70).[93]



Scheme 70

Whilst displaying a widespread use in industry as a method for producing higher olefins and polymers, this reaction has only in recent times become more generally applicable. The use of this reaction as a valuable tool within synthetic chemistry has been accelerated by the discovery of well-defined and functional group-tolerant catalysts.[93]

The general mechanism for RCM is displayed in Scheme 71. Firstly, the carbene complex **311** adds to the starting diene in a [2+2] cycloaddition to give a four membered metallocycle **312**. The same reaction then occurs again in reverse to form a new carbene complex **313** with the expulsion of ethene. Next, an intramolecular [2+2] cycloaddition closes the ring and produces a second metalla-cyclobutane **314**, which undergoes a retro-cycloaddition to regenerate the carbene complex **311** and produce the product **315**. The carbene complex then attacks another molecule of diene and the cycle is repeated.[94]



Scheme 71

At the outset of this project, there were two types of RCM catalyst in frequent use, the molybdenum-based complex **316**, developed by Schrock and co-workers[95], and the ruthenium-based complexes *e.g.* **317** and in particular **299** developed by Grubbs and co-workers (Figure 23). [96]

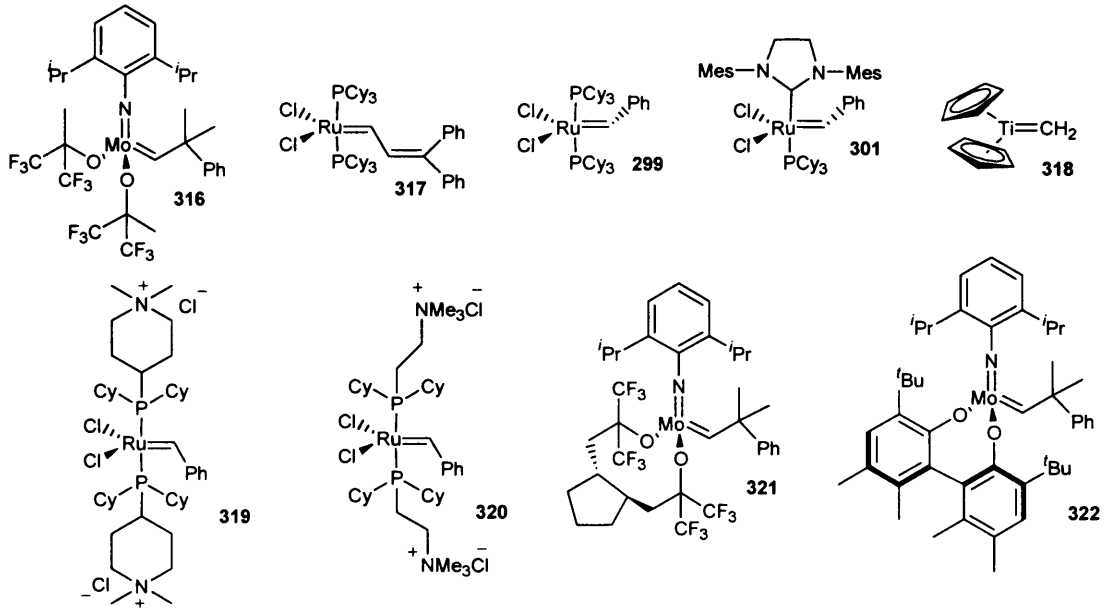


Figure 23

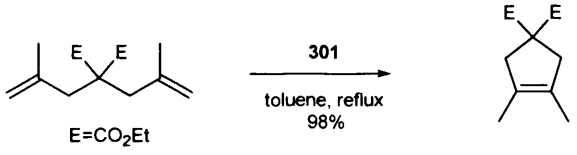
There were additionally a number of other catalysts that found occasional use in RCM reactions such as the titanium carbene **318**,[97] the water-soluble ruthenium based complexes **319** and **320** (developed by Grubbs),[98] and the chiral molybdenum carbenes **321** and **322** (Figure 23).[99] Nowadays the second generation Grubbs catalyst **301** (briefly discussed in Chapter 2, section 2.5.8.1) (Figure 23) takes precedent over all of these complexes and thus, is the most commonly used initiator for RCM.

3.1.2 Scope and functional tolerance

The use of RCM reactions as a key synthetic step has been well reported in the literature in the last 10 years, and trends in the scope and functional tolerance of the mainstream catalysts have been identified and extensively reviewed by Phillips and Abell.[93] Both the molybdenum-based complex **316** and the ruthenium complex **299** are capable of catalysing the formation of five to eight membered rings. In general, the ruthenium based complex **299** has been shown to be less active than **316** with respect to the formation of tri-substituted alkenes and is incapable of cyclisations that form tetra-substituted alkenes. In contrast the molybdenum-based complex **316** is capable of catalysing the formation of both tri- and tetra-substituted bonds. However whereas the ruthenium based catalyst **299** is tolerant to oxygen and moisture, catalyst **316** has the major disadvantage of being particularly unstable under these conditions.

Both catalysts are remarkably tolerant to a range of functional groups. Within simple systems catalyst **316** and **299** will tolerate ketones, esters, amides, epoxides, acetals, silyl ethers, some amines and sulfides. The ruthenium catalyst **299**, can also tolerate substrates containing free alcohols. However, problems have been encountered when this catalyst has been used in substrates containing functional groups that can coordinate to the metal centre (*e.g.* esters), although this problem has now been largely overcome by the addition of a Lewis acid *e.g.* $Ti(O^iPr)_4$ to these reactions (briefly discussed in chapter 2).[100]

The second generation Grubbs catalyst **301** combines the qualities of both catalysts **316** and **299**; higher thermal stability, wider functional group tolerance and lower sensitivity to double bond substitution. For example, catalyst **301** is able to catalyse RCM to give di-, tri-, or tetra-substituted cycloolefins[94] (Scheme 72) and hence, this catalyst is currently the most commonly applied in RCM reactions.



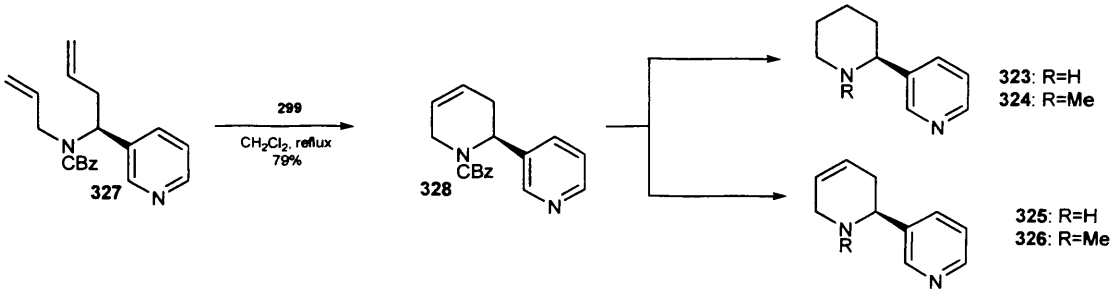
Scheme 72

3.1.3 Applications of RCM in the formation of *N*-heterocycles

There are a large number of examples of RCM involving substrates in which the diene linker contains a nitrogen atom. Cyclisation of these compounds gives access to a number of useful classes of *N*-heterocycles such as pyrrolidines, pyrrolidinones, piperidines and piperidinone systems, which are important intermediates in the synthesis of biologically active compounds such as azasugars and alkaloids.[93]

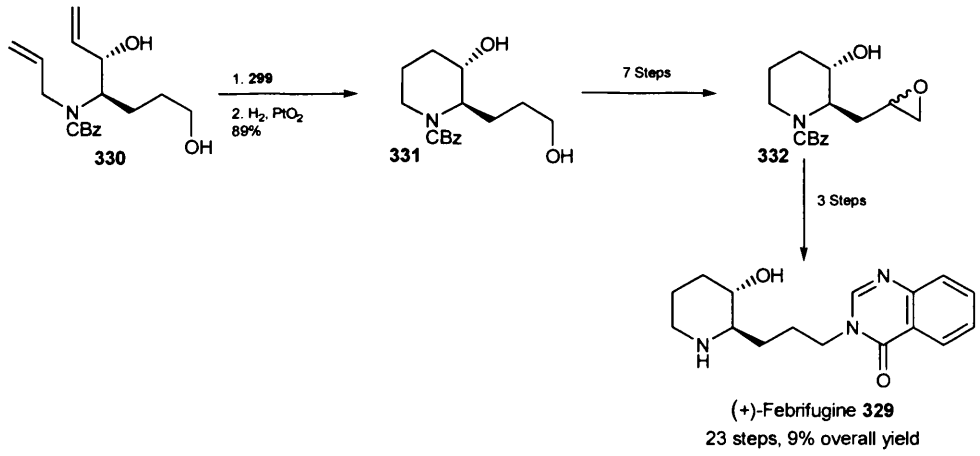
3.1.3.1 Use of RCM in the synthesis of alkaloids

RCM has been widely applied to the synthesis of alkaloids. As part of a programme directed towards the preparation of nicotine analogues for the assessment of their structure activity relationships, Lebreton and co-workers synthesised four chiral piperidine alkaloids **323**→**326** employing RCM (using Grubbs catalyst **299**) on the key intermediate **327**.[101] Subsequent transformation of the resulting alkene **328** gave the target compounds, (*S*)-anabasine **323**, (*S*)-*N*-methylanabasine **324**, (*S*)-anatabine **325** and (*S*)-*N*-methylanatabine **326** (Scheme 73).



Scheme 73

Ogasawara and co-workers have reported a route to the anti-malarial agent (+)-februfugine **329** using RCM to construct the piperidine moiety (Scheme 74).[102]



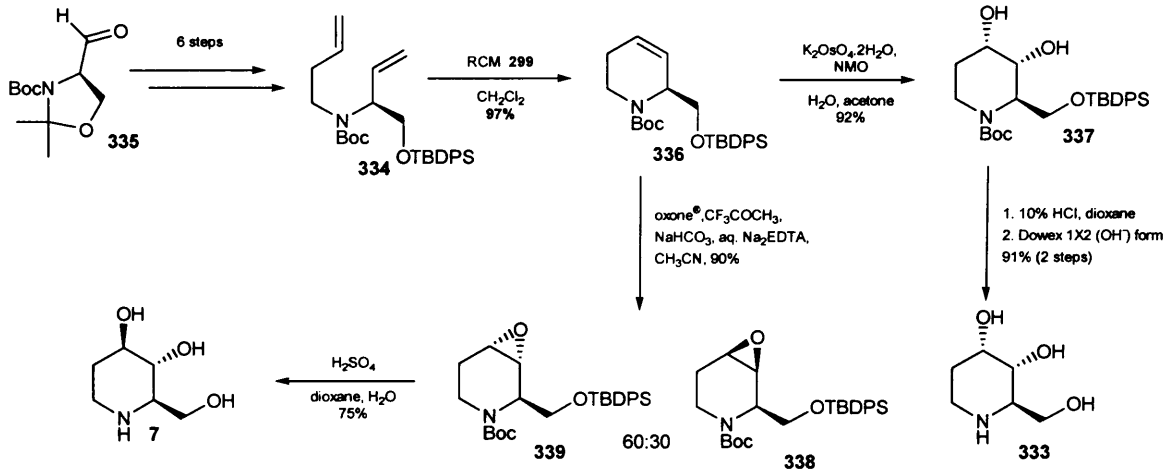
Scheme 74

Thus, the diene intermediate **330** underwent RCM (using Grubbs catalyst **299**) to give a dedihydropiperidine, which was subsequently hydrogenated to give the piperidinediol **331** in

89% yield over two steps (Scheme 74). Connection of the 4-quinozoline to the piperidine moiety **331** was achieved in 10 steps *via* the epoxide **332** to give (+)-frebrifugine **329** in 9% overall yield over 23 steps.

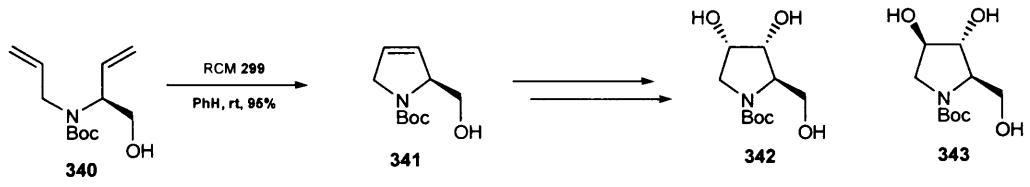
3.1.3.2 Use of RCM in the synthesis of azasugars

RCM has been commonly employed in the synthesis of azasugars and their derivatives. For example, Takahata and co-workers have synthesised 3-*epi*-fagomine **333** and fagomine **7** using this reaction (Scheme 75).[103] The diene **334** (synthesised in 6 steps from the Garner's aldehyde **335**) underwent olefin metathesis using Grubbs catalyst **299** to yield the key alkene **336** in 97% yield. Alkene **336** was then dihydroxylated using modified UpJohn conditions (occurring at the less hindered face *anti* to the siloxymethyl substituent) to furnish the diol **337** in 92% yield. Cleavage of the protecting groups under acidic conditions followed by treatment with ion-exchange resin then gave 3-*epi*-fagomine **333**. In contrast, epoxidation of the alkene **336** with trifluoromethyldioxirane (generated *in situ* from oxone® and 1,1,1-trifluoroacetone) gave both the *syn*- and *anti*-epoxides **338** and **339** respectively. Concomitant acidic hydrolysis of the *anti*-epoxide **339** and the protecting groups subsequently provided fagomine **7**.



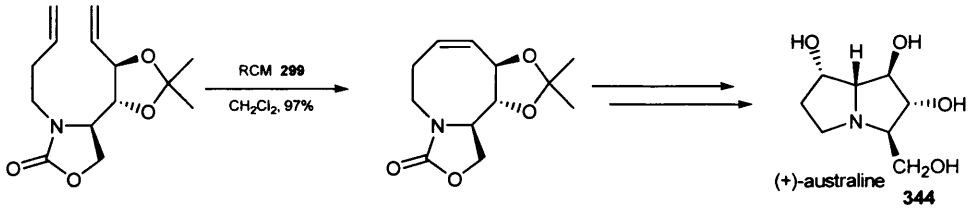
Scheme 75

A flexible synthesis of azasugars and homoazasugars has also been reported by Blechert and Huwe (Scheme 76).[104] RCM of a vinyl glycine methyl ester derived diene **340**, followed by stereoselective functionalisation of the alkene in the product **341** allowed the desired sugar derivatives **342** and **343** to be synthesised in good yields.



Scheme 76

Additionally, an elegant synthesis of (+) australine **344**, utilising RCM as the key step to form an eight membered ring, has been reported by White and co-workers (Scheme 77).[105]



Scheme 77

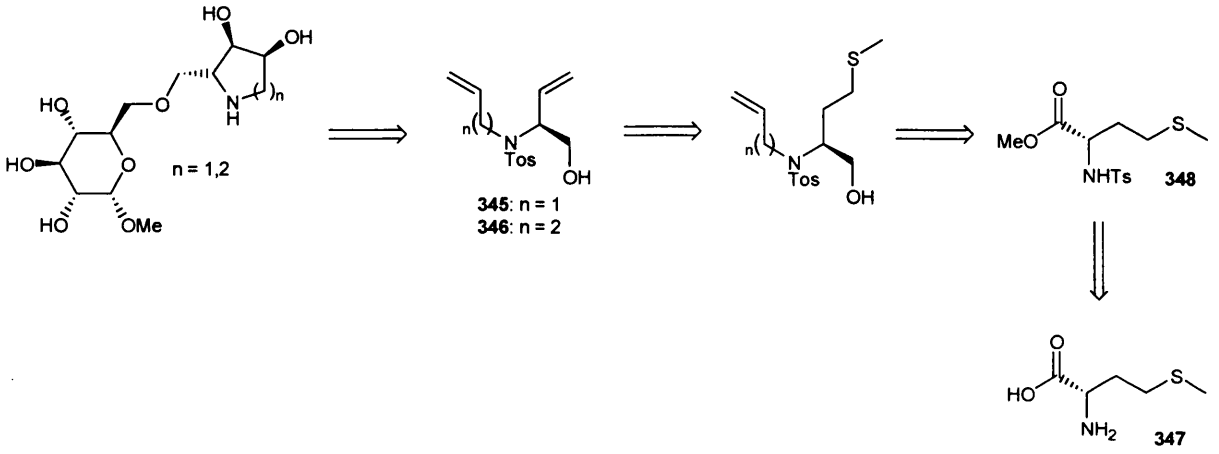
3.2 Investigations into a RCM-dihydroxylation approach to azadisaccharides

Apart from the use of the second generation Grubbs catalyst in the synthesis of the (2→6) linked azadisaccharides **266** and **267** (see chapter 2, section 2.5.8.1), to our knowledge there have been no reported examples of a RCM-dihydroxylation approach to the synthesis of azadisaccharides. Consequently, this approach, along with the established pinacol methodology, was investigated to synthesise our target (1→6) linked aza-*O*- and aza-*N*-disaccharides.

3.3 The synthesis of homoaza-*O*-disaccharides

3.3.1 Synthetic Approach

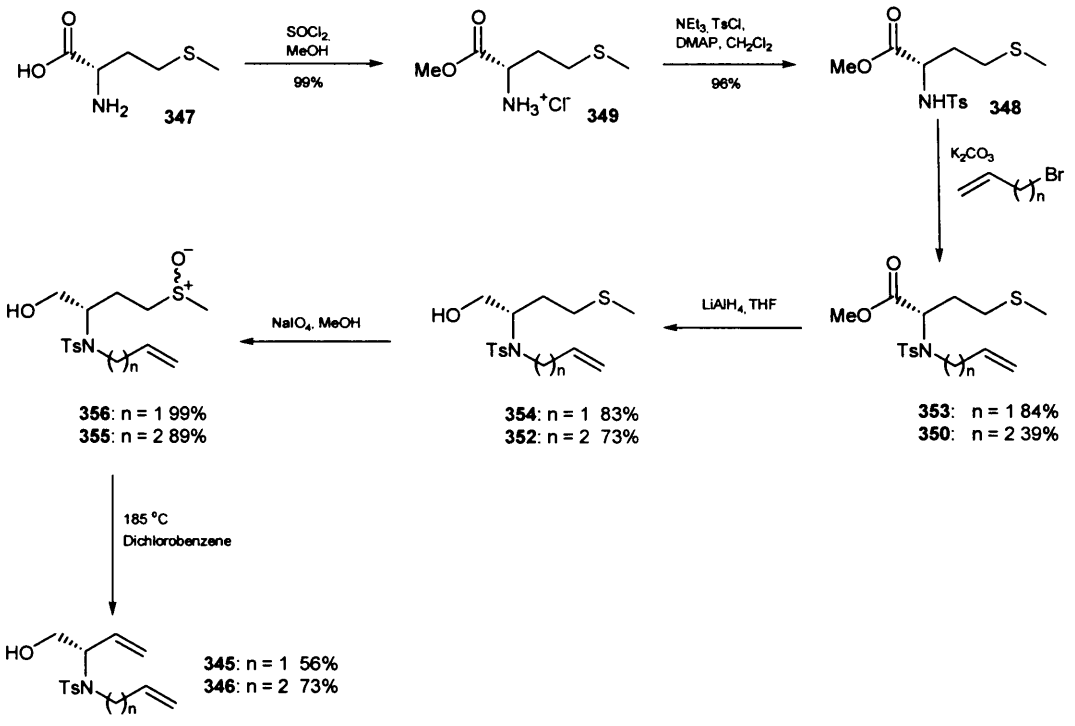
As discussed above (Scheme 69), we envisaged dienes such as **345** and **346** to be key intermediates in the synthesis of our target compounds (Scheme 78). We proposed to synthesise these dienes following the RSA shown in Scheme 78 using a modified version of a route reported by Ohfune and co-workers.[106] This route utilises L-methionine **347** as a cheap chiral source, and allows variability of azasugar ring size and substitution pattern (depending on the substrate used in alkylation of the sulfonamide protected amine **348**).



Scheme 78

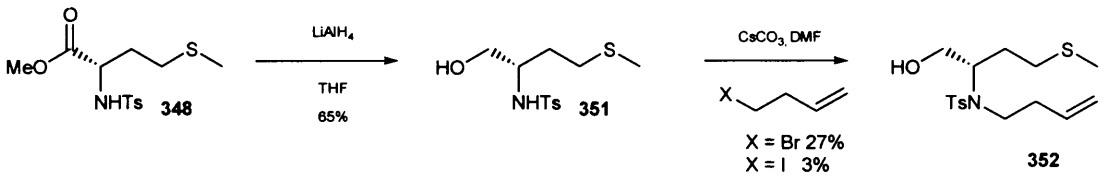
3.3.2 Synthesis of the dienes[107]

The dienes **345** and **346** were synthesised using the route outlined in Scheme 79. L-methionine **347** was firstly esterified using thionyl chloride in methanol to afford the methyl ester **349** in an excellent 99% yield. Tosylation of the free amine **349** using triethylamine, tosyl chloride and DMAP in dichloromethane then gave the sulfonamide **348** (96%). At this point in the synthesis variability of the azasugar ring size was investigated and to generate both piperidine and pyrrolidine systems, alkylation of the sulfonamide **348** using bromobutene and allyl bromide was attempted.



Scheme 79

Alkylation of **348** with bromobutene was initially investigated using sodium hydride in DMF however, disappointingly only a poor 22% yield of the alkylated product **350** was obtained. Replacing sodium hydride with potassium carbonate improved the yield of **350** (39%; 78% based on recovered starting material **348**), although this was far from satisfactory and consequently, alternative methods of achieving alkylation were investigated. Following a report by Abiko and co-workers,[108] alkylation of the alcohol **351** (formed by reduction of the ester **348** using $LiAlH_4$) using caesium carbonate in DMF was attempted (Scheme 80). Unfortunately using this procedure, no improvement was observed and the alkylated product **352** was only isolated in 27% yield. In an effort to improve this conversion, iodobutene (synthesised from bromobutene in 32% yield using a procedure reported by Falb and co-workers[109]) was utilised in the reaction, yet disappointingly, **352** was isolated in a very poor yield (~3%).



Scheme 80

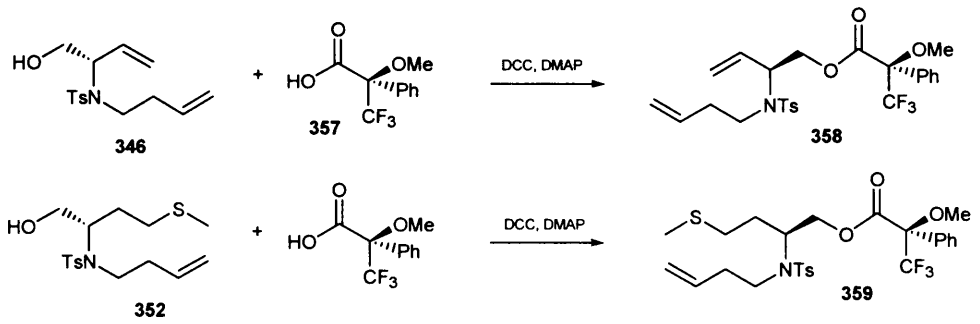
Unfortunately no further improvement of this reaction was achieved. We suspected the inefficiency of this alkylation was due to a competing elimination reaction and although conversions of **348**→**350** were poor, the yields based on recovered starting material **348** were satisfactory. Therefore we decided the use of this reaction in the synthetic route was warranted and hence no further investigations were carried out.

To produce the pyrrolidine precursor, **348** was alkylated using allyl bromide and K_2CO_3 in DMF (Scheme 79).[110] Unsurprisingly, this reaction was found to be much more efficient than the corresponding reaction with bromobutene and **353** was obtained in 84% yield with no purification being required.

With the alkylated precursors in hand, the methyl esters **350** and **353** were reduced using lithium aluminium hydride in THF and the resulting alcohols **352** and **354** oxidised to the sulfoxides **355** and **356** (produced as a mixture of diastereoisomers in both cases) using sodium periodate in methanol (Scheme 79). The sulfoxides **355** and **356** finally underwent β-hydride elimination (heating at 185 °C in dichlorobenzene for 1.5 hours) to give the required dienes **346** and **345** in good yield (Scheme 79).

3.3.3 Racemisation of the chiral centre?

Since we had used basic conditions (NaH/K_2CO_3) and high temperatures (185 °C in dichlorobenzene) in the synthesis of dienes **345** and **346** we were concerned racemisation of the chiral centre may have occurred. To investigate this possibility we decided to derivatise the alcohols **346** and **352** as their corresponding Mosher's esters (Scheme 81).



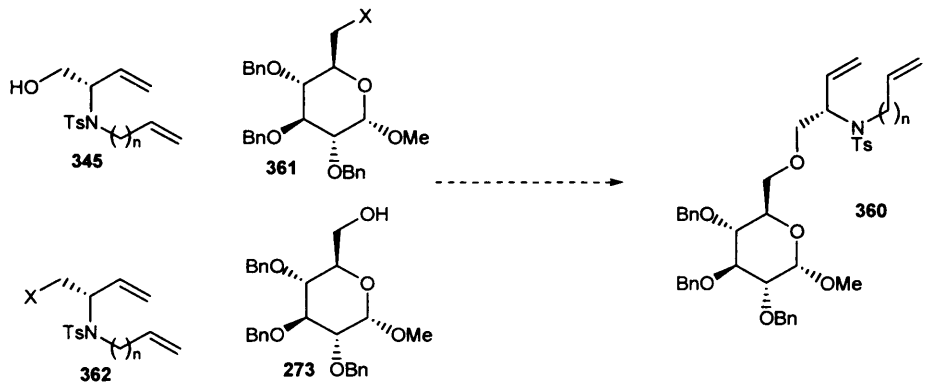
Scheme 81

The racemic standards of **346** and **352** were synthesised following the same the synthetic route shown in Scheme 79 starting with racemic DL-methionine. Compounds **346** and **352** (and racemic **346** and **352**) were then derivatised using the carboxylic acid **357**,

DCC, and a catalytic amount of DMAP in dichloromethane[111] to give the esters **358** and **359** (Scheme 81). Subsequent analysis and comparisons of crude samples of **358** and **359** to their racemic standards using ^{19}F reduced window NMR experiments (to determine the ratios of the CF_3 signals) indicated there had been minimal racemisation (<1%) demonstrating neither the alkylation step (using K_2CO_3 or NaH) or the elimination step (**356**→**345**) were of concern and the chiral integrity of the diene had been retained.

3.3.4 The etherification coupling

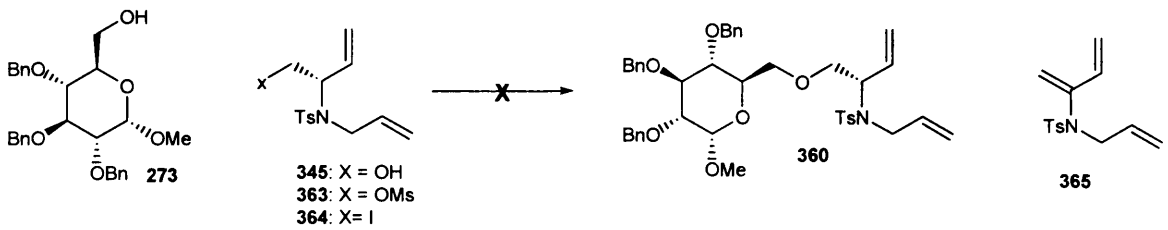
With the dienes **345** and **346** in hand, we turned our attentions to coupling these to the sugar moiety **273** (the synthesis of **273** was described in chapter 2, section 2.5.1) to synthesise the diene precursor **360**. It was envisaged this could be achieved by a 'simple' nucleophilic displacement reaction of a suitable leaving group (X in **361** or **362**) by an oxygen anion as shown in Scheme 82.



Scheme 82

3.3.4.1 Initial attempts

We believed the displacement of a leaving group on the diene *e.g.* **362** by the aglycon **273** would generate a more flexible synthetic pathway, allowing the aglycon, or the site of attachment on the aglycon to be varied in subsequent syntheses (*i.e.* without having to consider inversion of stereochemistry at the reacting centre). Therefore the mesylate **363** and the iodide **364** were synthesised from the alcohol **345** (Scheme 83).



Scheme 83

Synthesis of the mesylate **363** was achieved in an excellent 84% yield using standard conditions (MsCl, NEt_3, CH_2Cl_2)[112], however further reaction of **363** to form the iodide **364** (using NaI in acetone) proved to be more problematic (we suspected the mesylate **363** was

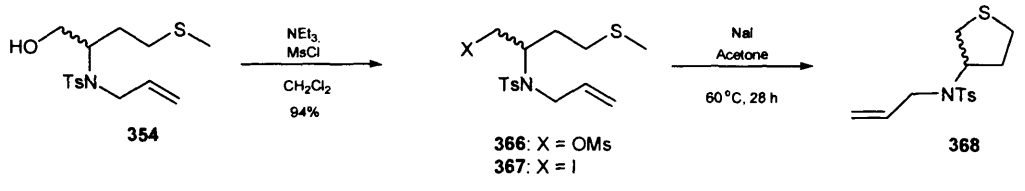
unreactive towards nucleophilic displacement and/or the resulting iodide **364** was unstable) and hence, **364** was only obtained in a disappointing 29% yield.

Unfortunately efforts to react either **363** or **364** with the alkoxide anion of **273** (generated using NaH in DMF and THF) proved unsuccessful and none of the target product **360** was observed (Scheme 83). With both the iodide **364** and the mesylate **363** we suspected elimination of the leaving group had occurred, as the triene **365** was isolated in small amounts (~10%) from the reaction mixtures. This result is perhaps not surprising if the allylic anion resulting from removal of the α-proton in **363** or **364** and the resulting stable, conjugated triene **365** are considered. Unfortunately, we could find no literature precedent for nucleophilic displacement reactions using oxygen anions in similar systems. The displacement of homoallylic halides with oxygen nucleophiles has been reported, although using only phenolic anions, generated using weaker bases such as K_2CO_3.[113] In view of this, attempts were made to achieve the desired reaction of **363** or **364** with **273** using milder basic conditions (AgO_2, $AgCO_3$), however no reaction resulted even at high temperatures. Microwave conditions were also investigated, although only decomposition of starting materials was observed in this reaction

3.3.4.2 Reducing the acidity of the α-proton

In an effort to minimise possible elimination reactions and promote nucleophilic substitution, the mesylate **366** was formed from the alcohol **354** (Scheme 84). So as to not 'waste' valuable chiral material, the synthesis of these derivatives was carried out using racemic intermediates. We suspected the α-proton in **366** would be less acidic than in the corresponding dienes **363** and **364** and thus may reduce the rate of elimination.

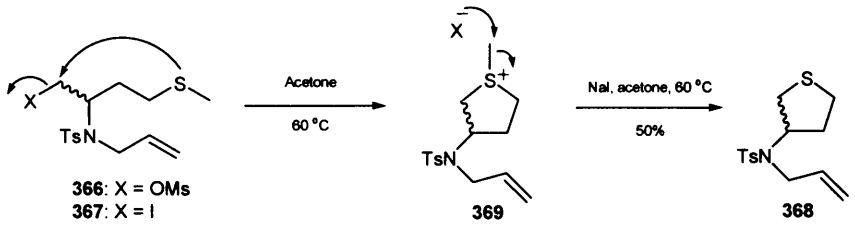
Disappointingly, reaction of **366** with the anion of the alcohol **273** under the conditions described previously (NaH in DMF or THF) generated none of the target ether and we again suspected elimination had occurred. To generate a better leaving group in the etherification step, efforts were next made to convert the mesylate **366** into the corresponding iodide **367** (Scheme 84).



Scheme 84

Interestingly, instead of forming the iodide **367**, the cyclic sulfide **368** was isolated in 56% yield from the reaction mixture (Scheme 84). Sulfide **368** presumably arises by the mechanism shown below (Scheme 85) whereby the mesylate **366** or the iodide **367** (formed *in*

situ) is displaced intramolecularly by the nucleophilic sulphur atom to give the sulfonium intermediate **369** which undergoes a subsequent demethylation.

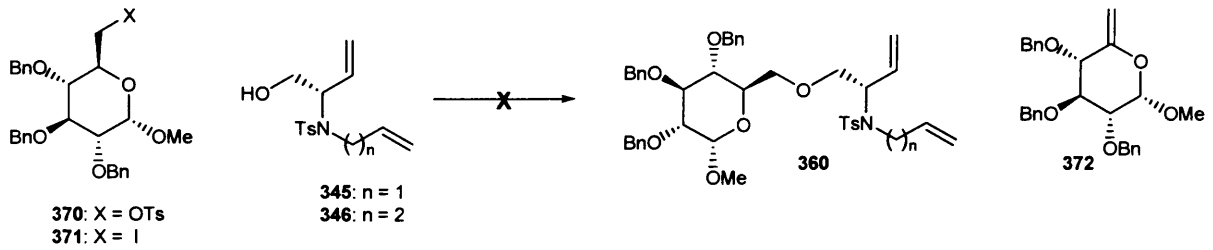


Scheme 85

To test this hypothesis the mesylate **366** was heated (60 °C) alone in acetone for 60 hours. On cooling, a white solid precipitated, which was assigned as the salt **369** (X=OMs) (by mass spectrometry and ^{1}H- and ^{13}C-NMR analysis). Isolation of **369** can be explained by the low nucleophilicity of the mesylate anion compared to iodide and consequently, when **369** was heated in acetone with NaI the sulfide **368** was formed in reasonable yield (50%).

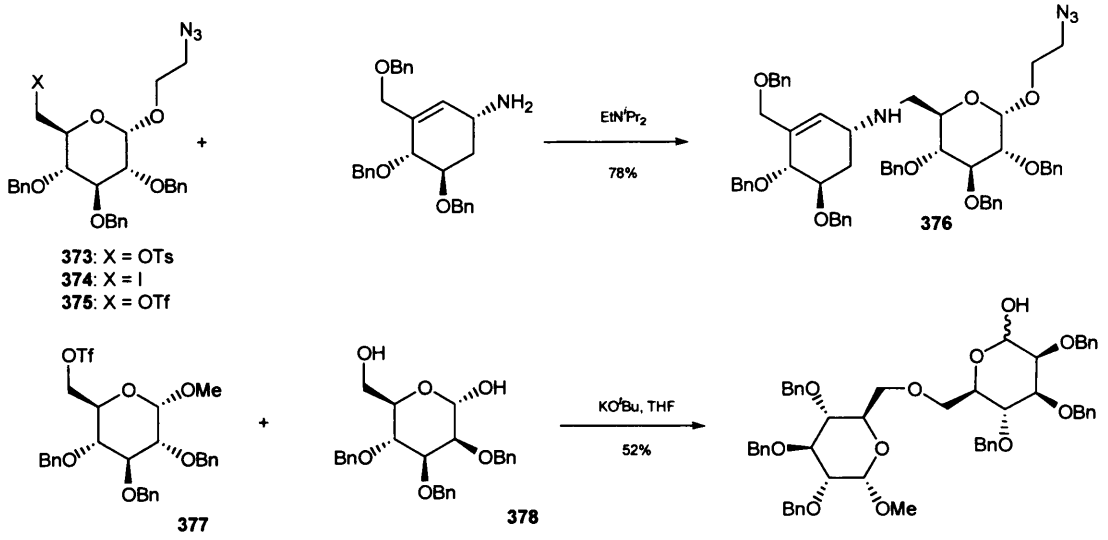
3.3.4.3 Reversing the polarity of the two reacting moieties

In view of the failure to produce the ether linkage from the reactions above we decided to attempt the coupling by reversing the polarity of the two reacting moieties. Thus, the 6-OH group of the sugar moiety **273** was converted to the tosylate **370** and the iodide **371**. Attempts to etherify either **370** or **371** with alcohols **345** and **346** using sodium hydride in DMF however, resulted in no ether formation and only small amounts of the alkene **372** were isolated (Scheme 86).



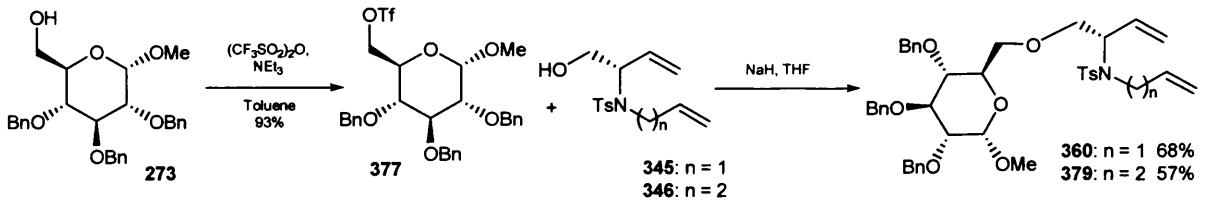
Scheme 86

These findings compare to results obtained by Bols and co-workers where the displacement of the similar tosylate **373** and iodide **374** with bulky sugar derived amines has been unsuccessfully attempted (although no elimination of **373** or **374** was observed) (Scheme 87).[114] The authors concluded the tosylate and iodide were not good enough leaving groups to achieve nucleophilic substitution and consequently the triflate **375** was synthesised (Scheme 87). Using the triflate **375** the desired disaccharide **376** was synthesised and no elimination was observed. In addition, a literature search also revealed a report by Schmidt and co-workers[115] in which the triflate **377** (Scheme 87) had been used as a coupling partner with the alcohol **378**.



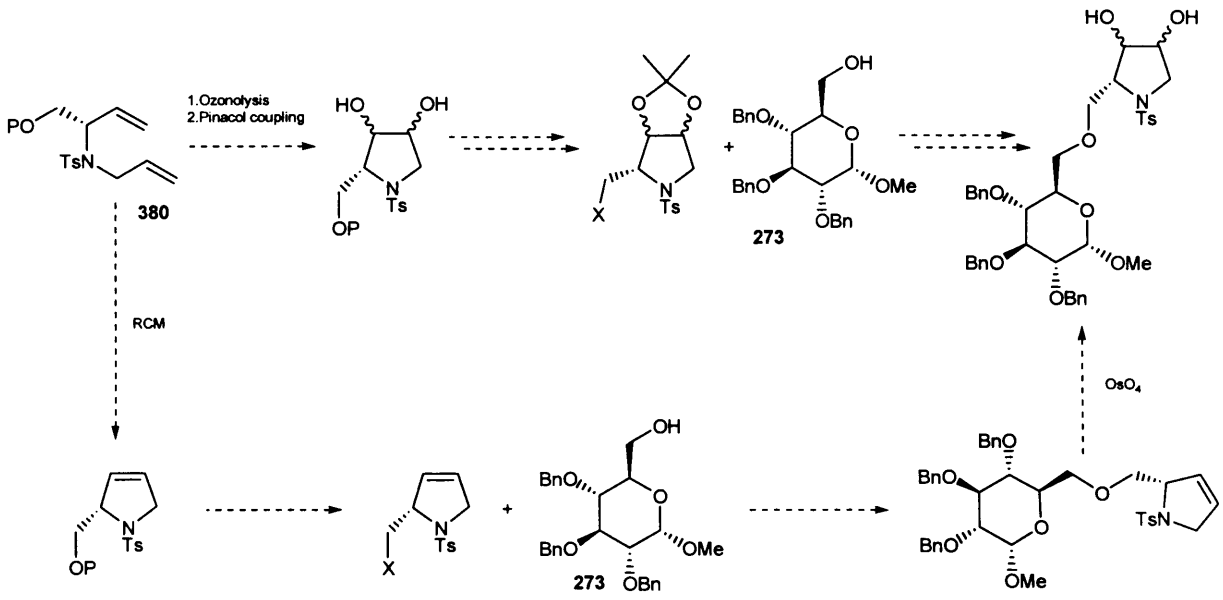
Scheme 87

In view of these findings we set out to synthesise the triflate **377** (Scheme 88). Following the procedure reported by Schmidt,[115] alcohol **273** was reacted with NEt_3 and triflic anhydride under an argon atmosphere. After 30 minutes analysis by TLC indicated the reaction was complete and following a quick work-up the triflate **377** was isolated in an excellent 93% yield. Pleasingly, utilising **377** in the etherification reaction together with the alcohol **345** (using NaH in THF) gave the required ether **360** in a gratifyingly 68% yield after purification (column chromatography) (Scheme 88). Application of this reaction with the diene **346** additionally allowed the formation of the piperidine precursor **379** also in good yield (57%) (Scheme 88).



Scheme 88

With the key diene precursors in hand, we then turned our attentions to investigating both an ozonolysis reaction followed by pinacol coupling, and a RCM to achieve cyclisation. We decided to initially explore these two approaches using the 'simpler' diene system **380** (Scheme 89). We had synthesised this to originally investigate an alternative approach to the target azadisaccharides (Scheme 89). Thus, before the successful etherification reaction, described above, had been achieved we had postulated that cyclisation of **380** (*via* RCM or pinacol reaction) could then be followed by ether formation as shown. However, in light of the success in the formation of the diene ethers **360** and **379**, we now saw this new route as an opportunity to allow prior investigation into the applicability of the pinacol reaction and RCM on a similar system before these reactions were attempted on the dienes **360** and **379**.

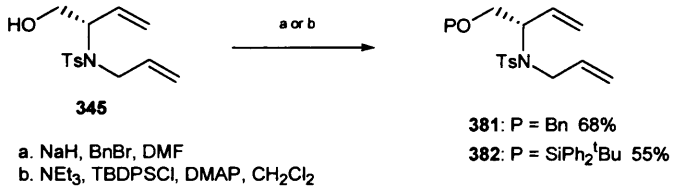


Scheme 89

3.3.5 Comparison of RCM *vs.* pinacol coupling with the diene 380

3.3.5.1 Investigations using the pinacol coupling

To investigate the pinacol coupling, the alcohol **345** was protected both as the benzyl and TBDPS ethers **381** and **382** respectively (Scheme 90). Benzyl protection was achieved using benzyl bromide in DMF to give **381** in 68% yield[116] and using triethylamine, ^{t}BuPh$_2$SiCl and a catalytic amount of DMAP in dichloromethane, **382** was synthesised in 55% yield.[117]

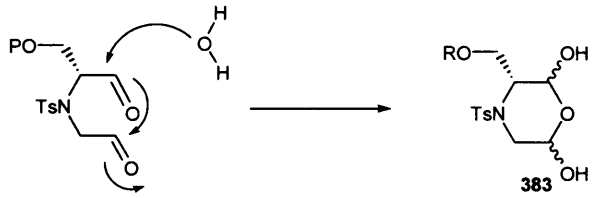


Scheme 90

3.3.5.2 Ozonolysis and pinacol coupling of the dienes 381 and 382

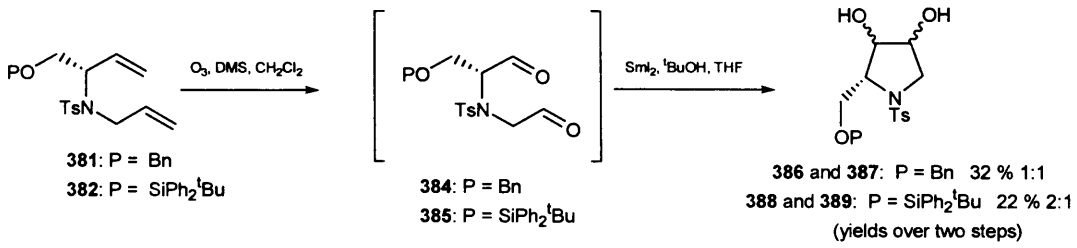
Following the same method discussed previously (chapter 2, section 2.5.3), the dienes **381** and **382** underwent ozonolysis in dichloromethane. No attempt was made to purify the resulting dialdehydes, as we were aware that dialdehydes similar to **381** and **382** commonly hydrate to form cyclic species such as **383** and hence are very unstable (Scheme 91).[73] Studies have shown cyclic hydrates such as **383** to be stable towards samarium diiodide (used in the

subsequent pinacol coupling step), however their formation significantly reduces the yield of the target diol product.[73]



Scheme 91

From ^{1}H- and ^{13}C-NMR analysis of the crude material from the ozonolysis reactions of **381** and **382**, we were confident the dialdehydes **384** and **385** had formed and were the major products, although there was evidence (mass spectrometry, ^{1}H-NMR) of hydrated material in minor amounts.



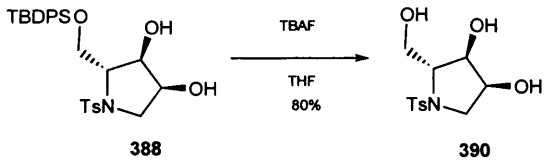
Scheme 92

The crude dialdehydes **384** and **385** were then subjected to a pinacol coupling (using the conditions described in chapter 2, section 2.5.4) to give the diols **386** and **387** and **388** and **389** respectively (Scheme 92). Analysis of the crude reaction mixtures by TLC indicated two diastereoisomers had been generated from both the benzyl and silyl protected derivatives. Due to the complicated nature of the crude reaction mixtures, the approximate ratios of diastereoisomers were determined using ^{13}C-NMR. Whilst the benzyl protected derivative yielded a 1:1 mixture of **386** and **387**, we suspected the silyl derivative had afforded an undefined 2:1 ratio of isomers **388** and **389**. Although the benzyl derivative **384** gave the diols in greater yield we found the silyl derivative **385** produced a cleaner reaction that was much easier to purify (especially with regards to separation of the two diastereoisomers) by flash column chromatography and hence further investigations into the stereochemical outcome of this reaction were carried out.

3.3.5.3 Determination of the stereoselectivity of the pinacol coupling of 385

Analysis of the COSY and NOESY spectra of the major isomer generated from the pinacol reaction of the silyl-protected dialdehyde **385,** suggested both hydroxyl groups were *cis* (as expected for the SmI_2 mediated pinacol) and were *anti* to the silyl ether substituent. In an effort to confirm this, **388** was treated with TBAF in THF to give the triol **390**, a known

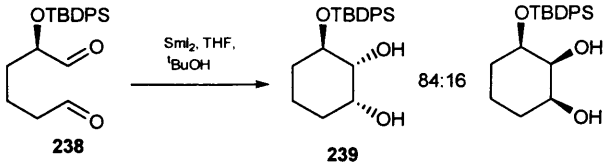
literature compound for which full ^{13}C-NMR data has been published.[118] Subsequent comparisons of ^{13}C-NMR data confirmed **390** to be identical to the literature compound thus, revealing the compound to be the isomer shown (Scheme 93).



388 **390**

Scheme 93

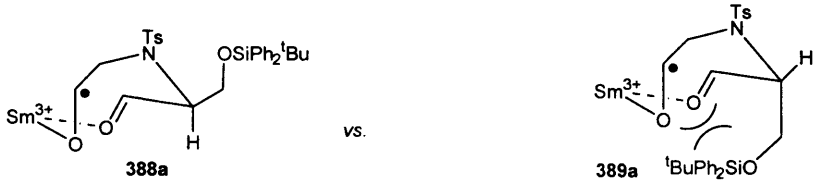
To our knowledge there have been no reports of a samarium diiodide mediated pinacol cyclisation to form the analogous carbocyclic α-hydroxymethyl substituted 1,2-diol and therefore we are unable to put our results into direct context. However, a samarium diiodide mediated pinacol coupling has been utilised on the α-substituted dialdehyde **238** (discussed previously, chapter 2, section 2.2.2) forming the 6-membered triol **239** (favouring diol formation *anti* to the substituent 84:16)[61] (Scheme 94) and M. S. Kachla has observed lower selectivities (of approximately 2:1) in the formation of corresponding α-substituted *N*-heterocyclic diols.[73] Thus it may be that inclusion of the *N*-sulfonamide substituent in these systems results in lower stereoselectivity of the *anti*-isomer although it is impossible to draw definite conclusions for these initial results.



238 **239**

Scheme 94

Although we have only observed modest selectivity in the formation of the diol **388**, we propose the formation of diastereoisomer **388** is preferred due to the presence of unfavourable steric interactions in the formation of the minor isomer **389** (*i.e.* intermediate **388a** *vs.* **389a**) (Figure 24).



388a **389a**

Figure 24

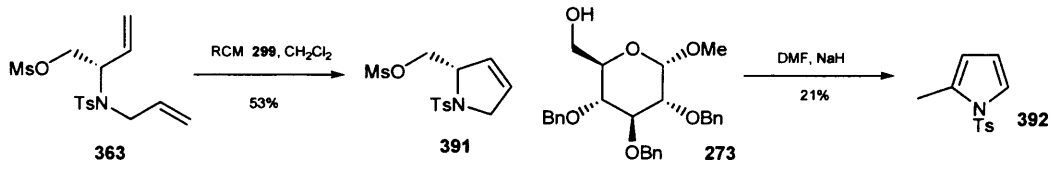
3.3.5.4 Summary of the pinacol approach

To summarise, the pinacol coupling has been used to synthesise *N*-heterocyclic triols and depending on the nature of the protecting group, the diastereoselectivity of the reaction can be somewhat controlled. Disappointingly, because of the poor yields observed using this

approach, we found a large amount of the diene (**381** or **382**) was needed to generate enough diol to enable further investigations of the planned synthesis. Consequently, we could not warrant the use of this reaction to access our target compounds and hence investigations using RCM were undertaken.

3.3.5.5 Investigations using a RCM-dihydroxylation approach

Referring back to the synthetic route shown in Scheme 89, to enable attachment of the sugar moiety **273**, we needed to generate a cyclic alkene incorporating a leaving group. Thus, an RCM reaction was attempted on the mesylate **363** (prepared earlier; see section 3.3.4.1). Reaction of **363** with 4mol% of the first generation Grubbs catalyst **299** in refluxing dichloromethane (45 °C) [119] gave, after 4 hours, a major product (analysis by TLC and mass spectrometry) although the reaction had not gone to completion. In an attempt to increase the conversion of the mesylate **363** to the alkene **391**, a further portion of catalyst (4 mol%) was added. Unfortunately, although the proportion of product **391** did increase (indicated by TLC) the reaction did not go to completion and after purification by column chromatography the mesylate **391** was isolated in a reasonable 63% yield, together with recovered starting material **363** (89% based on recovered **363**). Attempts were then made to displace the mesylate **391** using the anion of the sugar alcohol **273**.



Scheme 95

Reaction of the mesylate **391** with **273** in DMF using NaH did yield a major product after 2 hours. Disappointingly, this was identified as the pyrrole **392** (Scheme 95) formed from elimination of the mesylate and subsequent alkene migration and unfortunately none of the desired product was isolated. In view of this, no further exploration of this route was undertaken.

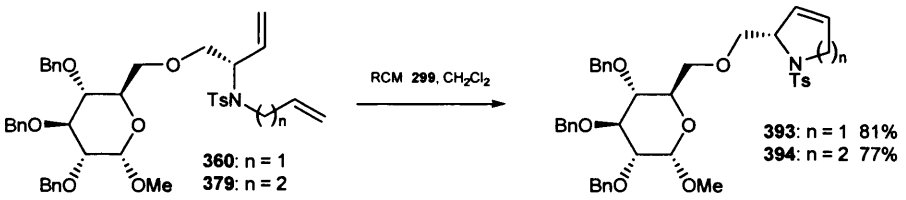
3.3.5.6 Summary of the RCM approach

RCM has been effectively utilised on the mesylate **363** to access the cyclised alkene **391**. Unfortunately, attempts to displace the mesylate **391** proved to be unsuccessful, as only the pyrrole **392**, resulting from elimination, was isolated. However, because the sugar moiety is already attached in the dienes **360** and **379** (Scheme 88), we foresaw an RCM approach to be most applicable to the synthesis of our target azadisaccharides from the dienes **360** and **379**.

3.3.6 Synthesis of two (1→6) linked homoaza-*O*-disaccharides

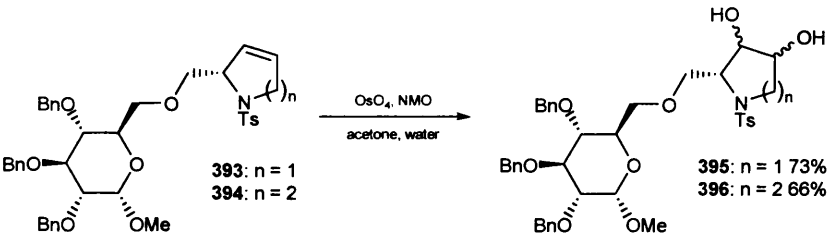
3.3.6.1 RCM and dihydroxylation of the dienes 360 and 379

The dienes **360** and **379** underwent RCM using 5 mol% Grubbs catalyst **299** in dichloromethane (Scheme 96).



Scheme 96

After heating for 3 hours at 45 °C, analysis by TLC (of both reactions) indicated the disappearance of starting material and the formation of a major product. After work up and purification by column chromatography, the required alkenes **393** and **394** were isolated in 81% and 77% yield respectively (Scheme 96). With the alkenes in hand, our attentions then turned to achieving dihydroxylation. In an attempt to isolate the *cis*-diol, the diene **393** was treated with OsO_4 and NMO in an acetone-water mixture (UpJohn conditions).[104] After 18 hours the reaction was carefully worked-up and purification by flash column chromatography gave the diol **395** in 73% yield (Scheme 97).



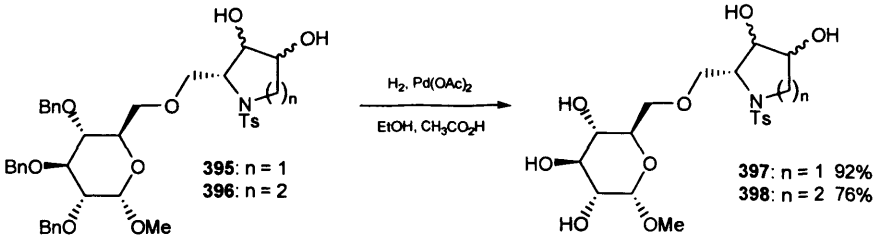
Scheme 97

Following a similar approach, **394** was also dihydroxylated to give the piperidine diol **396** in good yield (66%) (Scheme 97). Only one diol product was isolated in both the pyrrolidine and piperidine systems, signifying that dihydroxylation had been completely stereoselective. Unfortunately at this stage of the synthesis, confirmation of stereochemistry in both compounds **395** and **396** could not be achieved (compounds were not crystalline and ^{1}H-NMR was too complicated to undertake nOe studies), and consequently deprotection of the disaccharide intermediates was next investigated.

3.3.6.2 Deprotection of the disaccharide intermediates

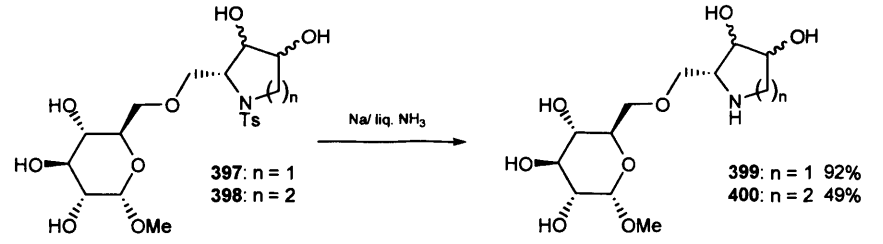
Conditions for benzyl group deprotection had already been successfully demonstrated on the pinacol disaccharide intermediates described previously (see chapter 2, section 2.5.5)

and consequently, these were also applied to the (1→6) linked compounds **395** and **396** (Scheme 98). Thus, the pyrrolidine disaccharide **395** was treated with $Pd(OAc)_2$ in ethanol and acetic acid under a hydrogen atmosphere (balloon) to give the pentol **397** in an excellent 92% isolated yield after chromatography. Using a similar approach, the piperidine disaccharide **396** was also deprotected to give the pentol **398** in a good 76% yield.



Scheme 98

Conditions for tosyl group removal had also been successfully demonstrated previously (see chapter 2, section 2.5.6) and hence, these were applied to the (1→6) linked compounds **397** and **398** (Scheme 99). The pyrrolidine disaccharide **397** was thus reacted with sodium (2.5 equivalents) in liquid ammonia. Pleasingly, analysis by TLC, ^{1}H-NMR and mass spectrometry of the resulting crude material from the reaction, indicated deprotection had been successful and subsequent purification by flash column chromatography yielded the target (1→6) linked homoaza-*O*-disaccharide **399** in an excellent 92% yield. Once more, using similar conditions the piperidine adduct **398** was also deprotected, although disappointingly, the target (1→6) linked homoaza-*O*-disaccharide **400** was only isolated in 49% yield. Fortunately, we observed no reaction of these secondary amines with CO_2 (as with the (2→6) linked compounds) and therefore full analysis could be achieved with the free amines **399** and **400**.



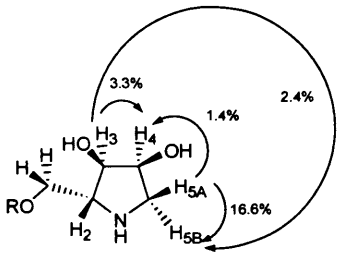
Scheme 99

3.3.6.3 Determination of stereochemistry

Unfortunately, none of the diol derivatives we had synthesised were crystalline, and consequently x-ray crystallographic analysis could not be used to determine the stereoselectivity of the dihydroxylation step in both the pyrrolidine and piperidine systems. Due to steric factors and reports in the literature detailing dihydroxylations of similar

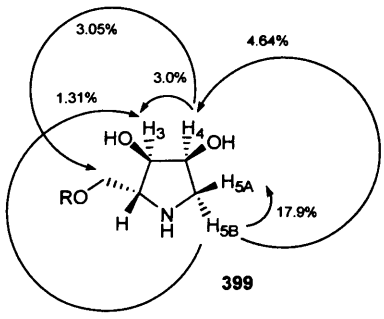
precursors[103,104] we suspected in both cases dihydroxylation had occurred *anti* to the C-2 substituent. Therefore, in an attempt to prove this prediction, a series of nOe experiments were carried out.

For the pyrrolidine adduct, nOe experiments were run on the fully deprotected derivative **399** (Figure 25). Thus irradiation of H_{5A} revealed an expected large (16.6%) enhancement of the H_{5B} resonance and a small (1.4%) enhancement of the H_4 resonance. Interestingly no enhancement of the H_2 resonance was observed. Irradiation of the H_{5B} signal induced a medium (4.6%) enhancement of the H_4 resonance and a small enhancement (1.3%) of the H_3 resonance suggesting H_3, H_4 and H_{5B} are on the same face of the pyrrolidine ring. This was backed up by a medium (3.0%) signal enhancement of the H_3 resonance respectively when H_4 was irradiated. Additionally a medium (3.05%) signal enhancement of the CH_2O resonances was also seen when H_4 was irradiated, strongly suggesting the methylene substituent was on the same face as the H_4 hydrogen. Most importantly medium enhancements (3.3% and 2.4%) of the H_4 and H_{5B} resonances respectively were seen when H_3 was irradiated. Whilst not conclusive, these results do fit with the azadisaccharide **399** being the suspected (2*R*, 3*S*, 4*R*) isomer, *i.e.* dihydroxylation had gone *anti* to the C-2 substituent.



399

nOe enhancements observed on irradiation of H_{5A} and H_3



399

nOe enhancements observed on irradiation of H_4 and H_{5B}

Figure 25

With the piperidine azadisaccharide, similar NOESY experiments were carried out on the tosyl protected derivative **398** (Figure 26). From an initial NOESY spectrum one of the H_5 protons did not show an nOe to one of the H_6 protons, suggesting that these were in a diaxial-like orientation (assigned H_{5A} and H_{6A}) (Figure 26). Subsequent irradiation of H_{6A} revealed a medium nOe enhancement (3.4%) of the H_{5B} resonance and no enhancement of the H_{5A} resonance. A medium nOe enhancement (3.7%) was also observed of the H_4 resonance when H_{6A} was irradiated signifying that these protons were on the same face of the piperidine ring (*i.e.* a 1,3-diaxial interaction) (Figure 26). In addition a medium nOe enhancement (3.3%) of the H_3 resonance and a medium (4.3%) enhancement of the CH_2O resonances were observed when H_2 was irradiated (Figure 26). These results suggest the piperidine **398** is sat in a chair-

like conformation in which the bulky C-2 substituent is axial and both hydroxyl groups are *anti* to this substituent (*i.e.* the (2*R*, 3*S*, 4*R*) isomer as shown in Figure 26)

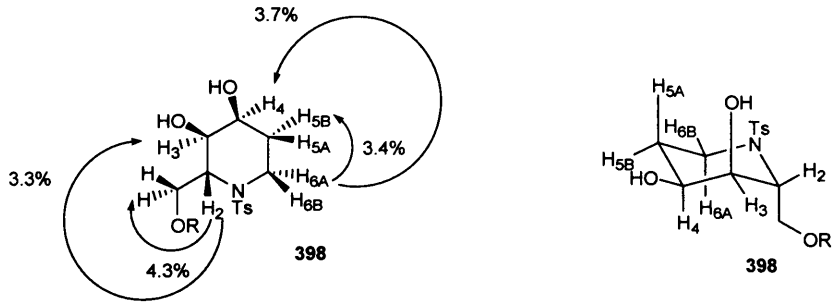


Figure 26

These findings are in agreement with results obtained by Laschat and co-workers[120] where x-ray crystallographic data had been obtained for the *N*-tosylated piperidine compound **401** (Figure 27). This shows that the piperidine **401** is sat in exactly the same conformation as **398** (suggested from the results we have obtained from NOESY and nOe experiments). It has been proposed that in these *N*-tosylated piperidine systems this is the lower energy conformer (C-2 substituent being in an axial position) as this avoids a 1,3-allylic strain type interaction between the C-2 substituent and the oxygen atoms on the sulfonamide (Figure 27).[121]

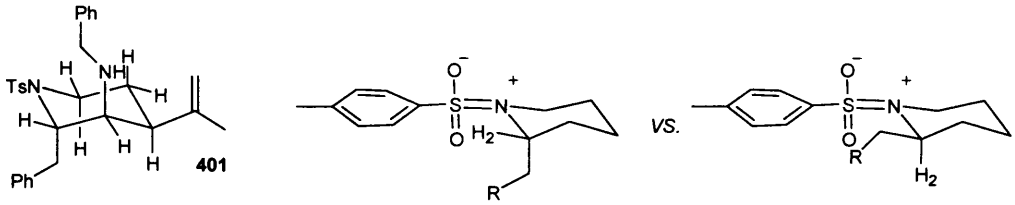


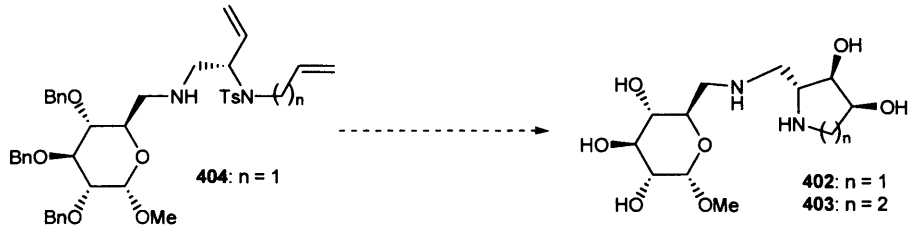
Figure 27

3.3.6.4 Summary

To summarise, the synthesis of two novel (1→6) linked homoaza-*O*-disaccharides **399** and **400** has been achieved (Scheme 99, pg 83). Using a synthetic route starting from L-methionine **347** diene precursors **345** and **346** were efficiently synthesised and no racemisation was detected. Initial difficulties in coupling the sugar moiety to the diene allowed the prior investigation of the pinacol and RCM methodology on a similar substrate and subsequently due to the difficulties encountered with the pinacol route, RCM was utilised to form the cyclic alkenes **393** and **394**. Stereoselective dihydroxylation (*anti* to the C-2 substituent) followed by routine deprotection then gave access to the target pyrrolidine and piperidine azadisaccharides **399** and **400** respectively in good yields. These compounds have been screened against a range of glycosidases to determine if they are biologically active, however the results from these investigations are discussed in chapter 5.

3.4 Synthesis of (1→6) linked homoaza-*N*-disaccharides

Due to the limited number of reports detailing the synthesis of amine-linked azadisaccharides, we decided to investigate the synthesis of the azadisaccharides **402** and **403** (Scheme 100).



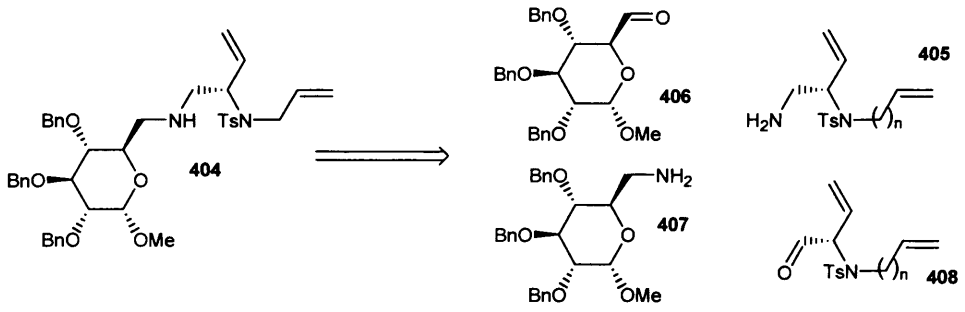
Scheme 100

3.4.1 Synthetic approach

We envisaged two approaches to these target compounds, the first a reductive amination approach and the second using a nucleophilic substitution.

3.4.1.1 The reductive amination approach

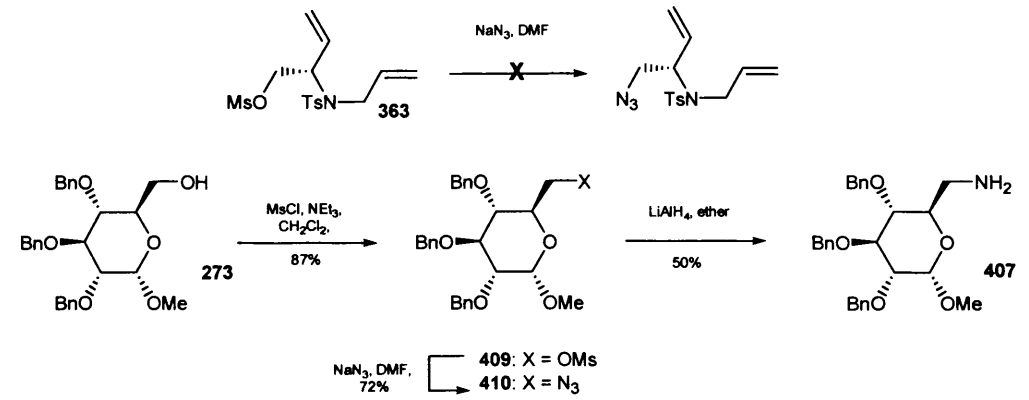
To achieve the synthesis of the diene **404** (Scheme 100) we foresaw two methods of achieving reductive amination. These involved either synthesising the diene amine derivative **405** and coupling with the sugar moiety aldehyde **406** or vice versa (*i.e.* **407** and **408**) (Scheme 101).



Scheme 101

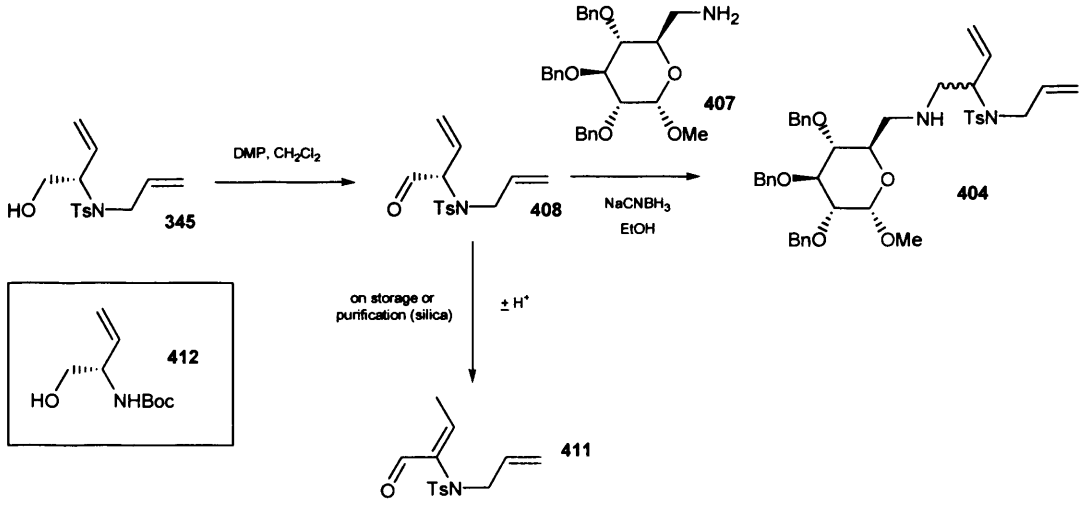
Investigating the first of these approaches, we attempted to introduce the amine functionality into the diene by displacement of the mesylate **363** (prepared earlier; see section 3.3.4.1) using NaN_3 in DMF (Scheme 102). Unfortunately this reaction proved to be unsuccessful and an unidentifiable mixture of products was isolated. In view of this and the general difficulty we had observed in attempting nucleophilic displacement with the diene system, we decided to explore the second disconnection (*i.e.* using **407** and **408**). The synthesis of the amine derivative **407** was undertaken using the alcohol **273** following a procedure by Bols and co-workers.[114] Synthesis of a mesylate **409** was first achieved using MsCl, NEt_3 in CH_2Cl_2 in good yield (87%) (Scheme 102). Nucleophilic displacement of the

mesylate **409** using NaN_3 in DMF afforded the azide **410** in 72% yield, which then underwent reduction using $LiAlH_4$ in diethyl ether following a procedure by Kobayahsi and co-workers[122] to give the amine **407** in a reasonable 50% yield (Scheme 102).



Scheme 102

We then turned our attention to synthesising the aldehyde **408** (Scheme 103). Thus, alcohol **345** was oxidised using the Dess-Martin periodinane[123] (synthesised in-house) however, due to the unstable nature of the aldehyde **408**, we found the reaction and the isolation of **408** to be very problematic. After extensive optimisation, we found using 3 equivalents of Dess-Martin periodinane gave good conversions of alcohol **345** to the aldehyde **408** yet, if the reaction mixture was left for long periods (*i.e* overnight) or purification was attempted (using flash column chromatography on silica), varying amounts of the more conjugated aldehyde **411** was observed (Scheme 103). This observation has also been reported by Taylor and co-workers in the oxidation of the *N*-Boc protected homoallylic alcohol **412** (Scheme 103).[123] Consequently, aldehyde **408** was used as a crude mixture in the subsequent reductive amination.



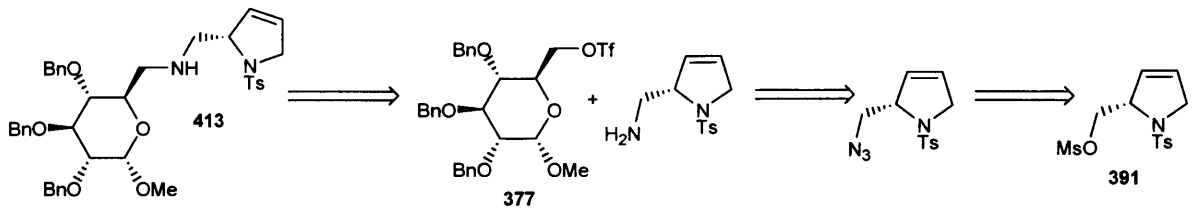
Scheme 103

Reductive amination was attempted with the amine **407** and crude aldehyde **408** using $NaCNBH_3$ in ethanol. After leaving the reaction for 18 hours, analysis by TLC and mass spectrometry indicated formation of a product with the correct molecular weight, however

attempts to purify and isolate the diene **404** proved to be very difficult as a complicated mixture of products had resulted. Material suspected to be **404** was isolated, however analysis by ^{1}H-NMR suggested this was impure as a complex set of signals were observed. Because of this, we suspected the aldehyde had undergone racemisation, leading to a possible mixture of two diastereoisomers and therefore an alternative approach to the *N*-linked intermediate **404** was explored.

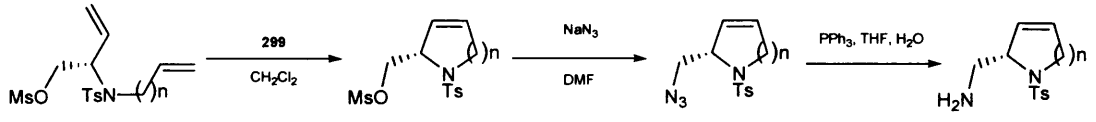
3.4.1.2 The nucleophilic substitution approach

In view of the successful synthesis of the *O*-linked diene **360** using the sugar derived triflate **377** (see section 3.3.4.3), we envisaged a similar approach could be used to form the disaccharide **413** as shown in Scheme 104. We considered the nucleophilic displacement of the pyrrolidine mesylate **391** (synthesised previously, section 3.3.5.5) by sodium azide to be a viable route to a suitable amine coupling partner. Whilst previous attempts to displace mesylate **391** using the sugar moiety anion **273** resulted in formation of the pyrrole **392** (see section 3.3.5.5), we suspected a non-basic small nucleophile such as azide would not initiate such elimination.



Scheme 104

Mesylate **391** was reacted with sodium azide in DMF (Scheme 105). Pleasingly, this reaction was successful, and after heating for only 4 hours the azide **414** was isolated in 84% yield. Reduction of the azide **414** was then achieved following the Staudinger reduction reported by Knouzi and co-workers[124] using triphenylphosphine in THF and water to give the amine **415** in an excellent 90% yield (Scheme 105). Initial attempts to achieve this transformation were carried out using $LiAlH_4$ however, due to the water-soluble nature of the amine **415**, purification proved to be very difficult and low yields of the amine **415** resulted.



363: n = 1
416: n = 2

391: n = 1 63%
n = 2 60%

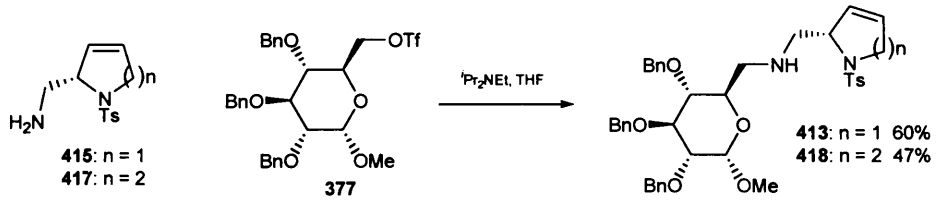
414: n = 1 77%
n = 2 70%

415: n = 1 90%
417: n = 2 94%

Scheme 105

To generate the piperidine amine, the same sequence of reactions was applied to the diene mesylate **416** to give the amine **417** in good yield (Scheme 105).

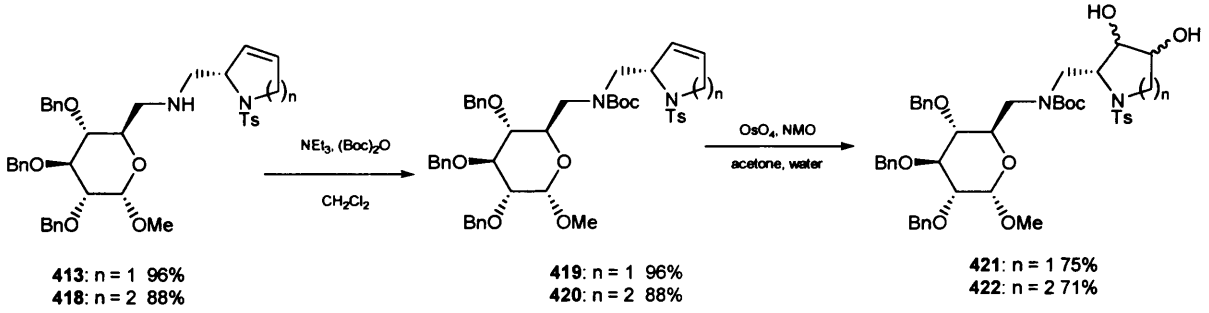
With the amines in hand we then turned our attention to reacting them with the triflate **377** to generate the key precursors **413** and **418**. As discussed previously (section 3.3.4.3) Bols and co-workers had reported the successful coupling of bulky sugar derived amines with the triflate **373** and thus, following this procedure[114] amines **415** and **417** were reacted with **377** to give the desired products **413** and **418** in reasonable yields (60% and 47% respectively) (Scheme 106).



Scheme 106

3.4.1.3 Dihydroxylation and deprotection

Dihydroxylation was initially attempted on the pyrrolidine derivative **413** using OsO_4 and NMO in acetone and water. Unfortunately, this reaction gave an unidentifiable mixture of products and no dihydroxylation was observed. We suspected the amine **413** may have co-ordinated to osmium and caused inhibition of the catalyst.[125] Therefore, the secondary amines **413** and **418** were protected using Boc anhydride and NEt_3 in dichloromethane to yield the Boc derivatives **419** and **420** in good yield (96% and 88% respectively) (Scheme 107).



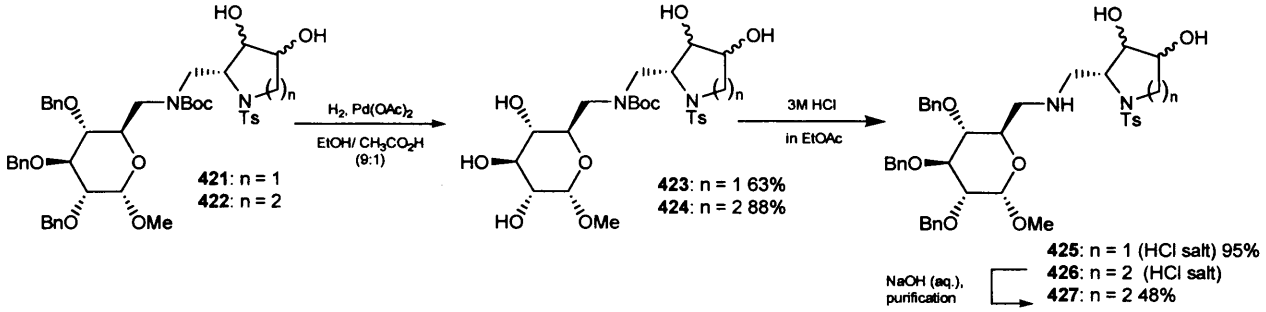
Scheme 107

The presence of the Boc group in these compounds and in subsequent intermediates caused analysis by NMR to be very difficult as broad signals in the ^{1}H-NMR and doubling up of signals in the ^{13}C-NMR were commonly seen. This was attributed to the presence of rotamers caused by the restricted rotation of the Boc carbamate. Variable temperature NMR was largely used to overcome this problem, and thus in all compounds NMR data was sufficiently assigned.

Both the *N*-Boc pyrrolidine and piperidine alkenes **419** and **420** underwent dihydroxylation to yield the diols **421** and **422** in 75% and 71% yields respectively. As with the *O*-linked compounds, dihydroxylation was found to be completely stereoselective and only one product was isolated in both reactions. Unfortunately at this stage in the synthesis,

conformation of the stereoselectivity of the dihydroxylation step could not be achieved (compounds were not crystalline and ^{1}H-NMR was too complicated to undertake accurate nOe studies), and consequently deprotection of the disaccharide intermediates was investigated.

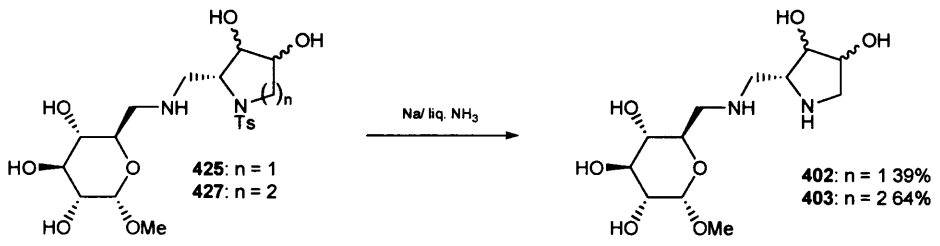
Benzyl deprotection was carried out on the pyrrolidine and piperidines **421** and **422** respectively using H_2 (balloon) and $Pd(OAc)_2$ in ethanol/acetic acid, to yield the pentols **423** and **424** in reasonable yields (63% and 88% yield respectively) (Scheme 108). Following a standard procedure **423** and **424** then underwent Boc deprotection using 3M HCl in ethyl acetate (generated *in situ* from ethanol and acetyl chloride) yielding the amines **425** and **426** (Scheme 108).[126]



Scheme 108

With the pyrrolidine **423**, Boc deprotection occurred very smoothly to yield the HCl salt of the secondary amine **425** in 95% yield without any purification being required. In contrast, removal of the Boc group in the piperidine **424** did generate the hydrochloride salt **426** however ^{1}H-NMR analysis revealed this compound to be impure. The salt was therefore treated with base (NaOH) and the resulting free amine purified using flash column chromatography, to give the amine **427** in a slightly disappointing 48% yield.

Finally, attempts were then made to remove the tosyl group in both **425** and **427** using the Birch reduction conditions described previously (Na/ liq. NH_3) (Scheme 109).



Scheme 109

Disappointingly, isolation of the fully deprotected compounds proved to be very difficult. Although we suspected tosyl deprotection had occurred (analysis by mass spectrometry), problems were encountered when purification of the resulting diamines was attempted. Material suspected to be the fully deprotected compounds **402** and **403** was

isolated (in 39% and 64% respectively) although ^{1}H-NMR indicated this material to be impure (containing ~10% of unidentified impurity) (Scheme 109). We therefore suspected decomposition of the diamines may have occurred and in view of this and the small amounts of material recovered, no further attempts were made to resynthesise or purify these compounds.

3.4.1.4 Determination of stereochemistry

Unfortunately, none of the *N*-linked diol derivatives that had been synthesised were crystalline, so again NMR studies had to be undertaken in an attempt to determine the stereochemical outcome of the dihydroxylation step. We again suspected dihydroxylation had occurred *anti* to the C-2 substituent; however caution was taken in this assumption as there are reports in the literature where amide substituents have been used to direct dihydroxylation to the same face as the substituent.[127]

With the pyrrolidine azadisaccharide **425**, the resonances in the ^{1}H-NMR were adequately resolved and they were accurately assigned using COSY. A NOESY spectrum was subsequently run and analysis of this led to preliminary stereochemical assignments (Figure 28). Unsurprisingly a strong cross peak between the H_3 and H_4 resonances was observed, confirming dihydroxylation had gone *cis*.

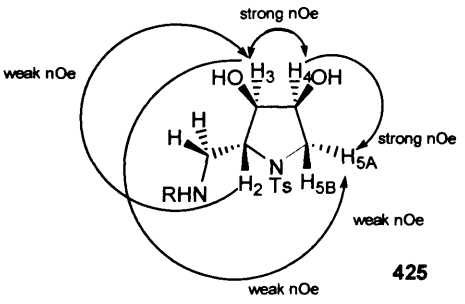


Figure 28

In contrast a small nOe cross peak was seen between the H_3 resonance and the H_2 and H_{5A} resonances. However in comparison no nOe cross peak was observed between the H_2 and H_4 resonances, suggesting these were on opposite faces of the pyrrolidine ring. Whilst not conclusive these results do suggest the *N*-linked azadisaccharide is the (2*R*, 3*R*, 4*S*) isomer as shown (Figure 28)

Unfortunately in the piperidine *N*-linked azadisaccharide **427**, many key resonances in the ^{1}H-NMR (D_2O) overlapped (*e.g.* H_3 and H_4 and both H_{6A} and H_{6B} resonated at very similar chemical shift) (Figure 29). Efforts to resolve these signals by running the NMR in CD_3OD (material insoluble in all other NMR solvents) unfortunately proved unsuccessful. Nevertheless, a NOESY spectrum was run however, as expected, due to the overlapping

signals, only limited information was obtained. The only evidence which did indicate dihydroxylation had gone *anti* to the C-2 substituent (as for all previous dihydroxylations), was a strong nOe cross peak between both CH_2N resonances and the H_3 and H_4 resonances (overlapping). If these protons were on the opposite face, a small cross peak may have been expected between H_3 and CH_2O. Thus the presence of a substantial cross-peak, does suggest that the H_3 hydrogen and the methylene substituent are on the same face (*i.e.* dihydroxylation has gone *anti*), although this is by no means conclusive and further experiments would need to be undertaken to fully establish this stereochemistry. These cross peaks also possibly suggest that **427** takes up a similar conformation as that proposed for the *O*-linked analogue **399** (see figure 26).

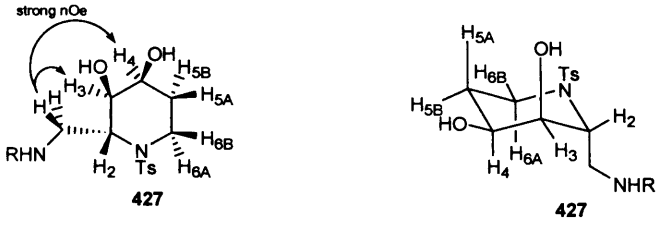


Figure 29

3.4.1.5 Summary

To summarise, the synthesis of two novel (1→6) linked homoaza-*N*-disaccharides **402** and **403** has been accomplished. Initial attempts to synthesise these compounds using a reductive amination approach proved to be problematic, however by undertaking a slightly modified version of the synthesis established for the *O*-linked azadisaccharides **399** and **400**, the amine functionality was conveniently introduced. Based on preliminary NOESY data, dihydroxylation is suspected to have been stereoselective occurring *anti* to the C-2 substituent and subsequently deprotection of the benzyl groups and the Boc group has given access to the *N*-tosyl protected derivatives **425** and **427**. Unfortunately, due to the instability of the final diamine compounds, their isolation after tosyl group removal proved to be very difficult and consequently full analysis of these azadisaccharides was not achieved. Nonetheless, the slightly impure deprotected compounds **402** and **403** have still been screened against a range of glycosidases and the results of these investigations are discussed in chapter 5.

3.5 Conclusion

A RCM-dihydroxylation strategy has been effectively used in the synthesis of four novel fully deprotected (1→6) linked homoaza-*O*- and *N*-disaccharides (**399**, **400**, **402** and **403**, Figure 30). The synthesis of the azasugar component in both the *O*- and *N*- linked systems was efficiently achieved from L-methionine **347** and after extensive investigations, displacement of the sugar derived triflate **377** proved to be the key in accessing the

disaccharide adducts. Initial NMR investigations have suggested dihydroxylation has been completely stereoselective in all cases and thus, each disaccharide was isolated as a single diastereoisomer.

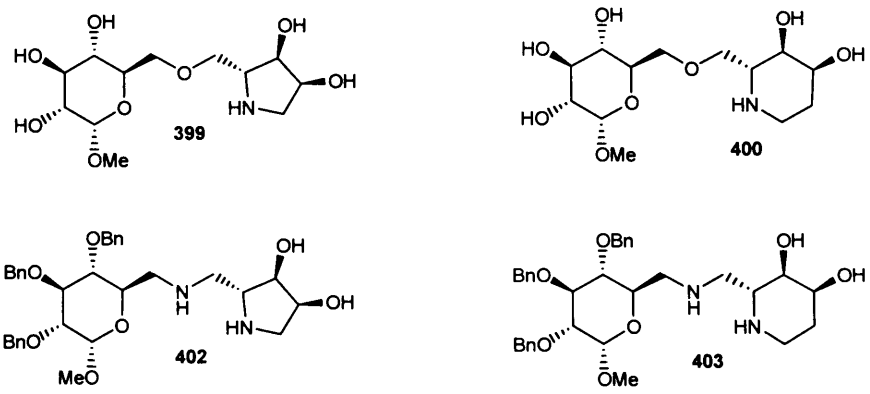


Figure 30

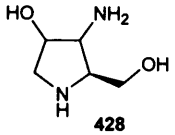
Although outside the scope of this thesis, there is potential to vary many structural aspects of the disaccharide compounds (*e.g.* azasugar ring size, the aglycon, sites of attachment, increased hydroxylation, length of tether between azasugar and aglycon *etc*) to investigate how these changes would affect biological activity. We believe the synthetic route we have established to the novel compounds discussed above, would easily permit such structural variations, hence allowing a small library of compounds to be synthesised and any potential structure activity relationships to be determined.

Chapter 4

A Stereoselective Approach to All Stereoisomers of 2-Hydroxy-3-amino-4-hydroxypyrrolidine

4.0 A Stereoselective Approach to All Stereoisomers of 2-Hydroxymethyl-3-amino-4-hydroxypyrrolidine

This chapter discusses new synthetic routes to 2-hydroxy-3-amino-4-hydroxypyrrolidines of the general structure **428** (Figure 31).



428

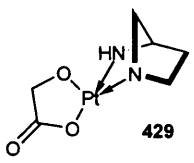
Figure 31

To review this area, and explain the synthetic significance of these compounds the syntheses and availability of compounds containing the key 3-aminopyrrolidine skeleton, together with further functionalised related structures, will be discussed first.

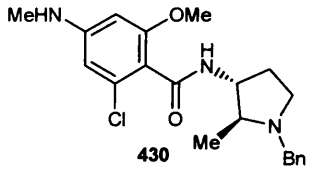
4.1 3-Aminopyrrolidines - general background

4.1.1 3-Aminopyrrolidines

The 3-aminopyrrolidine substructure is a ubiquitous moiety found in a range of compounds displaying extensive arrays of biologically activity.[128] For example, this structure is present in cyclopeptide alkaloids, neurotoxic fungal metabolites, anti-filarial agents, quinolone anti-bacterials and anti-tumour compounds.[129] Examples of such compounds include the anti-tumour complex **429** and the psychotropic drug **430** (Figure 32).[130]



429



430

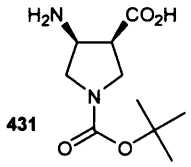
Figure 32

3-Aminopyrrolidine derivatives have therefore become attractive as intermediates in the synthesis of bioactive compounds. Optically active derivatives have also found applications in chromatography and as chiral auxiliaries in synthesis.[130,131] Consequently, the syntheses of simple aminopyrrolidines are widespread in the literature and many different strategies have been employed to access these functional compounds.[130-132]

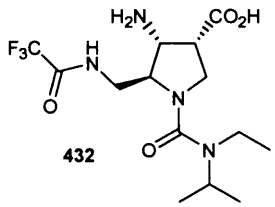
4.1.2 Further functionalised aminopyrrolidines

The syntheses of further functionalised aminopyrrolidines are less prevalent within the chemical literature. This is in spite of the fact that the introduction of additional functional groups onto the pyrrolidine skeleton often opens new possibilities with regard to bioactivity.

One example of a functionalised 3-aminopyrrolidine is A-87380 **431** (Figure 33). This compound was discovered as an initial lead by Wang and co-workers[133] as a moderately active neuramidase inhibitor (neuramidase is an attractive target in the treatment of influenza). By employing a combination of structure based drug design and combinatorial chemistry the related molecule **432** was synthesised, displaying a 250 fold improvement of activity (IC_{50} = 0.2μM) over the initial lead **431** (Figure 33).



431

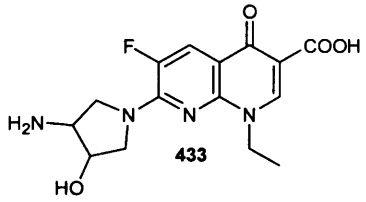


432

Figure 33

4.1.3 Hydroxy-3-aminopyrrolidines as components of antibacterials

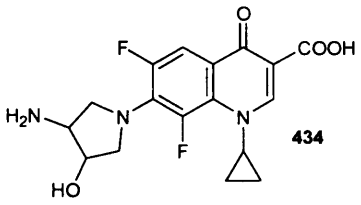
The introduction of a hydroxyl group onto the 3-aminopyrrolidine motif can significantly enhance biological interactions and the hydroxyaminopyrrolidine skeleton is found in many compounds displaying bioactivity. For example Matsumoto and co-workers[134] synthesised the compound **433** (Figure 34) incorporating a hydroxyaminopyrrolidine substituent (stereochemistry undefined) and found it to have substantial antibacterial activity (active against *S. aureus*, *E. coli*, *P. aeruginosa*).



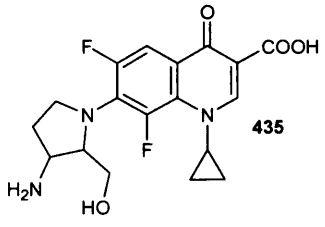
433

Figure 34

Okada and co-workers[135] have also synthesised a large number of related antibacterials incorporating this aminopyrrolidine substructure. For example, the quinoline derivatives **434** and **435** (stereochemistry undefined) (Figure 35) were synthesised as part of a quantitative structure-activity relationship study of various antibacterial agents. Compounds **434** and **435** were found to be moderate growth inhibitors of several strains of bacteria (*e.g. E. coli, K. pnemonia, P. morabilis, E. clocae, S. aureus*).



434



435

Figure 35

4.1.4 Hydroxy-aminopyrrolidines as glycosidase inhibitors

As discussed in chapter 1, carbohydrate furanoses in which the ring oxygen has been replaced by nitrogen are inert to metabolism. It has been proposed[136] that these compounds mimic the oxocarbenium ion-like transition state of glycosidic hydrolysis and therefore inhibit glycosidases and other carbohydrate processing proteins. Thus, these compounds have become important as potential anti-viral, anti-tumour and anti-diabetic agents. In replacing a hydroxyl group by an amino group on the azasugar ring (*i.e.* an aminopyrrolidine) there is potential for the discovery of new therapeutics and to also gain a greater understanding of mechanistic and structural factors important in glycosidase inhibition.

To investigate the effect of substituting a hydroxyl group for an amino group on glycosidase inhibition and to demonstrate their novel synthetic methodology, Lundt and co-workers[136] set out to synthesise 4-amino-3-hydroxypyrrolidines **436**, **437** and their corresponding enantiomers ent-**436** and ent-**437** (Figure 36) from readily accessible starting materials.

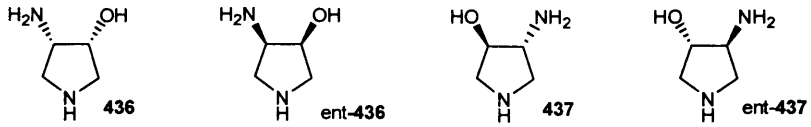
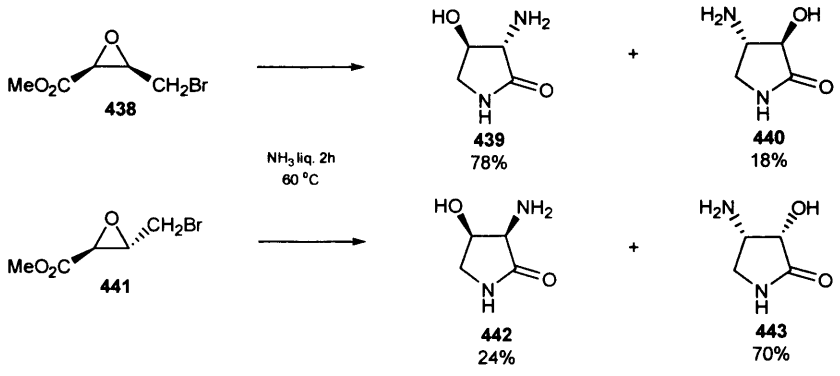


Figure 36

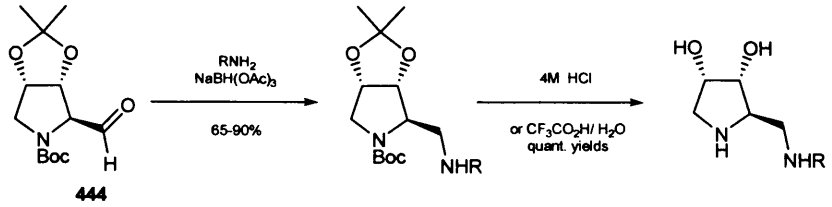
The synthetic approach involved the nucleophilic-mediated ring opening of 2,3-epoxides synthesised from D-erythronate derivatives (Scheme 110). Thus, opening of the 2,3-*cis*-epoxide **438** by heating in liquid ammonia yielded **439** and **440** (78%:18%). Likewise, reaction of the *trans*-2,3-epoxide **441** afforded **442** and **443** (24%:70%). The enantiomers of each lactam were also synthesised *via* the same route starting from an L-erythronate derivative. The lactams **439**, **442** and their corresponding enantiomers were reduced to the corresponding hydroxy-aminopyrrolidines **436**, **437**, ent-**436** and ent-**437** (Figure 36) using borane-dimethylsulfide.



Scheme 110

The pyrrolidines **436** and **437** were tested as glycosidase inhibitors but showed no activity against α-glucosidase from bakers yeast and β-glucosidase from *E. coli.* However, **437** showed inhibition of α-mannosidase from Jack beans exhibiting a K_i of 40μM, which is comparable with inhibition by deoxymannonojirimycin (K_i = 68μM)[137] for the same enzyme.

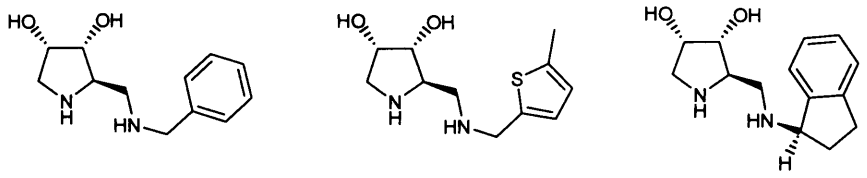
Vogel and co-workers[138,139] have also synthesised related compounds to investigate glycosidase inhibition. A collection of (2*R*, 3*R*, 4*S*)-3,4-dihydroxypyrrolidin-2-yl derivatives were synthesised in an attempt to generate selective mannosidase inhibitors as potential anti-cancer agents. These compounds were successfully synthesised from the key aldehyde **444** (derived from D-gluconolactone) *via* reductive amination with a variety of amines followed by full deprotection (Scheme 111).



444

Scheme 111

The final products were tested for inhibitory activity towards 25 glycosidases and the most potent inhibitors towards α-mannosidase from Jack bean **445**→**447** are shown below (Figure 37).



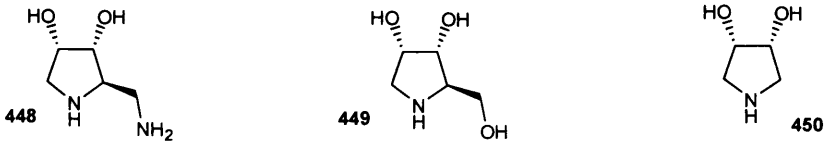
445
IC_{50} = 5μM
K_i = 2.3μM

446
IC_{50} = 10μM
K_i = 3.5μM

447
IC_{50} = 17μM
K_i = 2.3μM

Figure 37

Interestingly the results of this investigation also revealed the simple diamine **448** to be a better inhibitor of α-mannosidases (and many other glycosidases) than its hydroxylated counterpart **449** and the diol **450** (Figure 38).



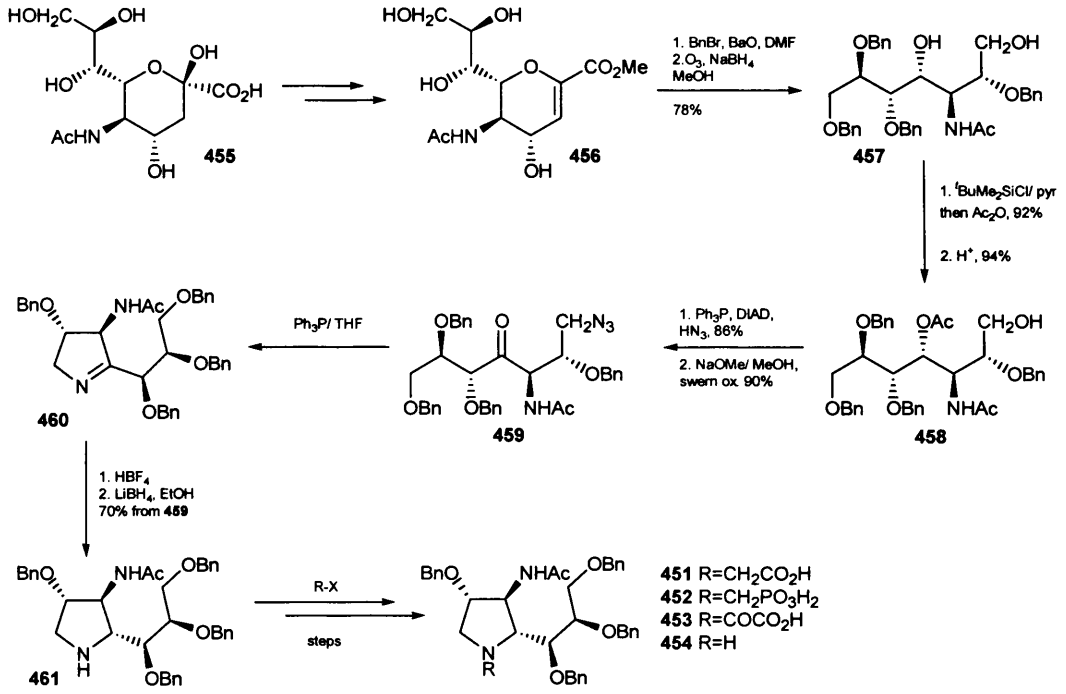
448 **449** **450**

Figure 38

4.1.5 2-Hydroxymethyl-3-amino-4-hydroxy-pyrrolidines (3-aminodeoxyazasugars)

There are relatively few literature reports describing the synthesis of our target compounds, 3-amino-4-hydroxypyrrolidines having a C-2 hydroxymethyl substituent (or the equivalent).

One report by Vasella and co-workers[140] describes the synthesis of the pyrrolidines **451**→**454** (Scheme 112). These compounds were synthesised from *N*-acetylneuramic acid (Neu5Ac) **455** as potential inhibitors of the enzyme *N*-acetylneuramidase.

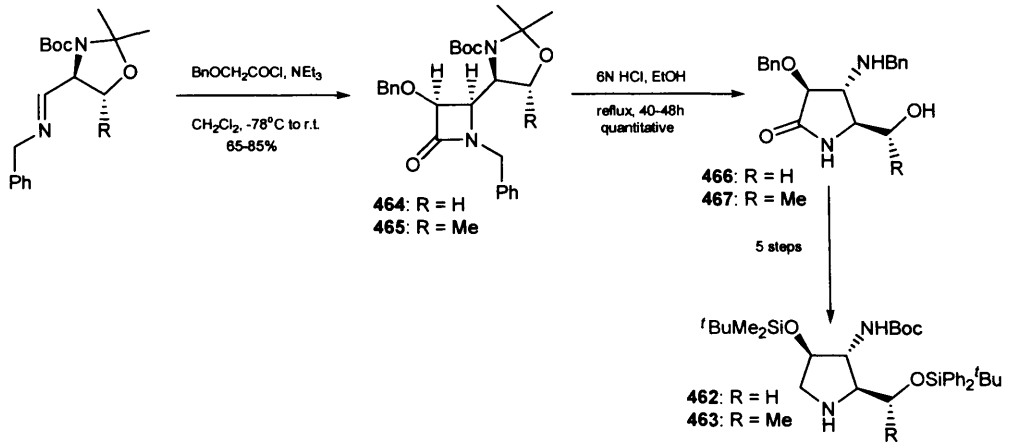


Scheme 112

The initial conversion of Neu5Ac **455** to the methyl ester of Neu2en5Ac **456** (according to known procedures) was followed by benzylation, ozonolysis and reduction (of the crude ozonides) to give the diol **457**. Silylation of the primary alcohol, acetylation of the remaining alcohol and subsequent desilylation (under acidic conditions) then afforded the monoacetate **458**. The azido group was next introduced using a Mitsonobu reaction after attempts to mesylate and displace with azide failed. Deacetylation, followed by oxidation furnished the ketone **459**. The azide moiety was reduced (Ph_3P, THF) to the amine, which underwent subsequent cyclisation to afford the imine **460**. Protonation of the imine (HBF_4) followed by a diastereoselective reduction ($LiBH_4$) yielded the key amine **461**. Alkylation of the amine with various alkyl halides subsequently afforded the target compounds **451**→**454** of which **451**→**453** were found to be competitive inhibitors of *Vibrio cholerae* sialidase with K_i values of $4x10^{-3}$M, $5.3x10^{-5}$M, and $4x10^{-2}$M respectively.

An additional report describes the synthesis of the protected 3-amino-4-hydroxypyrrolidines **462** and **463** (Scheme 113). Palomo and co-workers[141] synthesised these derivatives as an extension to their studies in the area of β-lactam synthesis. The authors

employed an asymmetric [2+2] cycloaddition reaction (the Staudinger reaction) to synthesise the β-lactams **464** and **465**. Intramolecular lactonisation of these intermediates under acidic conditions gave the 3-amino-hydroxyproline derivatives **466** and **467** in one step. Finally, selective protection yielded the differentially protected pyrrolidines **462** and **463**.



Scheme 113

Herdeis and co-workers[142] have also produced hydroxy-aminopyrrolidine derivatives as part of investigations towards the syntheses of proline analogues. The basis behind these studies was the recent finding that non-proteinogenic highly substituted proline derivatives (*e.g.* **468**→**470**) (Figure 39) in novel cyclic peptides, exhibit considerable bioactivity.

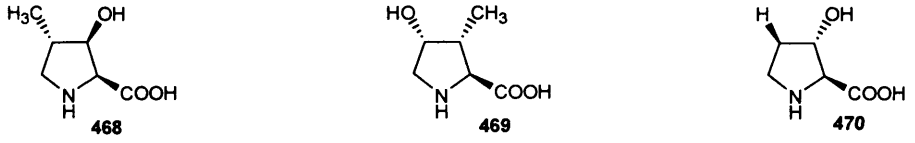
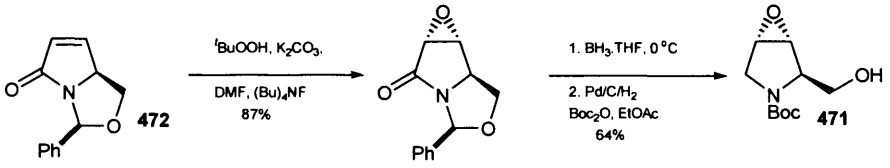


Figure 39

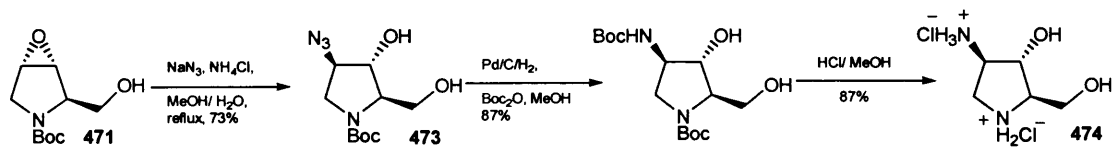
Herdeis and co-workers envisaged the epoxide **471** (Scheme 114) as a key intermediate in the synthesis of such compounds. This epoxide **471** was synthesised from the stereoselective epoxidation of compound **472** (itself derived from a saturated pyroglutaminol derivative). Subsequent reduction of the lactam, benzyl deprotection and re-protection with Boc yielded the epoxy-pyrrolidine **471**.



Scheme 114

To generate additional compounds of interest other than the target compounds above (Figure 39), the epoxide **471** was regioselectively opened at C-4 with sodium azide/ammonium chloride to give **473** (Scheme 115). Reduction of the azide moiety, Boc

protection and deprotection under acidic conditions, afforded the 2-hydroxymethyl-3-hydroxy-4-aminopyrrolidine **474**. The report does not state whether any biological testing has been undertaken on these compounds.



Scheme 115

In a related report by Schofield and co-workers[143] the synthesis of analogous epoxides **475** and **476** is described (Figure 40). The aim of this work was to prepare samples of **475** and **476** to compare them to the products from the enzymatic epoxidation by proline-4-hydroxylase on the unnatural substrate 3,4-dehydro-L-proline **477** (Figure 40). Whilst synthesising these key intermediates some interesting hydroxy-aminopyrrolidines were also produced.

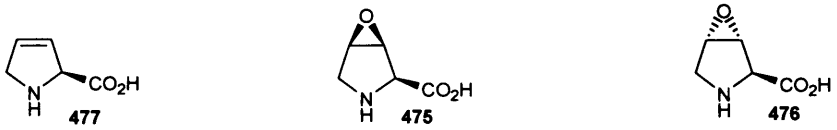
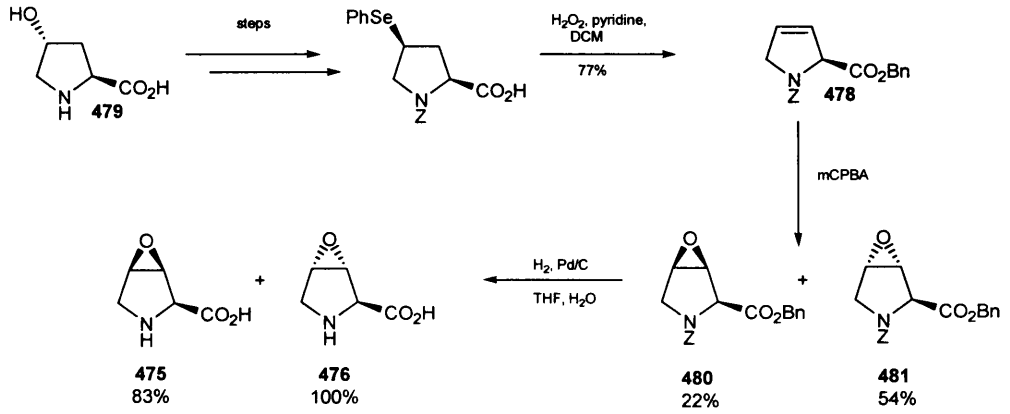


Figure 40

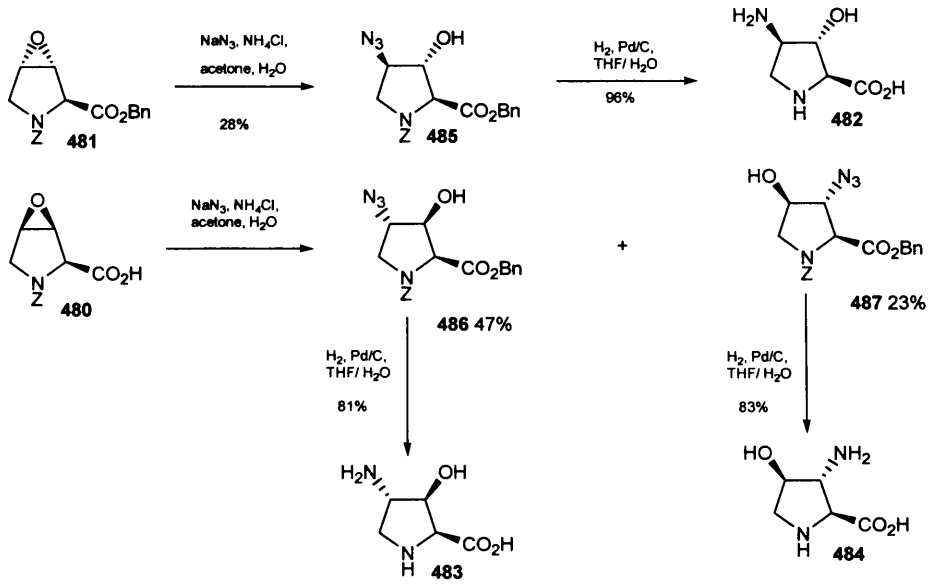
The requisite 3,4-dehydro-L-proline derivative **478** for this synthesis was made from *trans*-hydroxy-L-proline **479** using a modified version of a literature method (Scheme 116).[144] This involved a 6-step synthesis incorporating a selenoxide elimination step to introduce the alkene functionality (Scheme 116).

Epoxidation of **478** was carried out using m-CPBA to yield both the *syn*- and *anti*-epoxides **480** and **481** (1:2.5 ratio respectively, separable by column chromatography) which were subsequently converted to the target carboxylic acids **475** and **476**. The synthetic potential of the epoxyproline derivatives **480** and **481** was illustrated by the synthesis of three hydroxyaminoprolines **482, 483** and **484** (Scheme 117).



Scheme 116

Epoxide opening of the *anti*-epoxide **481** with sodium azide/ammonium chloride was again found to be regioselective (in agreement with the findings of Herdeis[142]) affording **485** only. In contrast opening of the *syn*-epoxide **480** under the same conditions yielded two products **486** and **487** (in a 2:1 ratio respectively). All three azidohydroxyprolines underwent catalytic hydrogenation to give the corresponding hydroxyaminoprolines **482** (also prepared by Herdeis[14]), **483** and **484**. Again, no biological testing was carried out on these compounds.



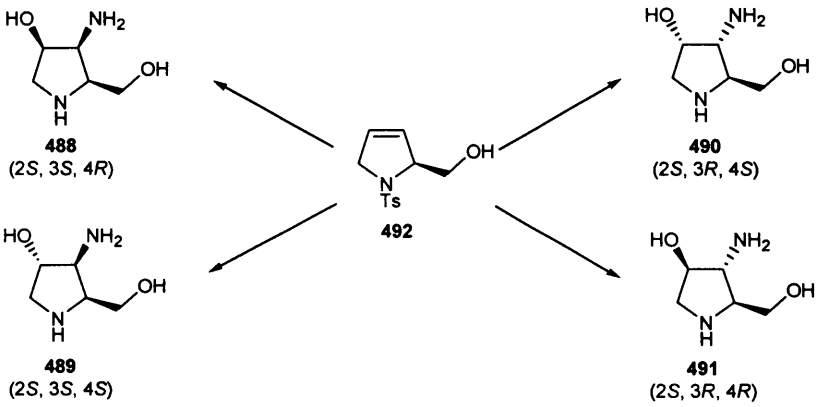
Scheme 117

4.1.6 Summary

To summarise, it can be concluded that the aminopyrrolidine framework is a valuable asset within synthetic chemistry. Simple 3-aminopyrrolidines and hydroxy-3-aminopyrrolidines are found in a number of compounds displaying biological activity and consequently there is a demand for the syntheses of these systems. However, the synthesis and reported biological activity of 3-amino-4-hydroxypyrrolidines (with a substituent at C-2) is limited. For example Vasella and co-workers[140] have used a natural carbohydrate starting material as a framework to construct the 3-amino-4-hydroxy pyrrolidines **451→453** and found them to have glycosidase inhibitory activity. Palomo[141] also synthesised this framework using a β-lactam expansion whilst Herdeis[142] and Schofield[143] synthesised 4-amino derivatives from the nucleophilic ring opening of epoxyprolines (Schofield did report the formation of a 3-amino derivative **484** although only as a minor product). This lack of previous reports of the synthesis of this class of compound together with their potential biological activity makes them attractive targets.

4.2 Research aims - regio- and stereoselective synthesis of 2-hydroxymethyl-3-amino-4-hydroxypryrrolidines

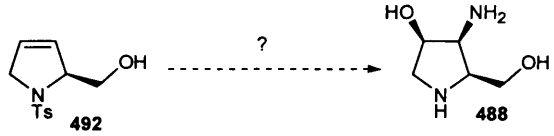
The aim of this section of research is to develop new regio- and stereoselective synthetic routes to the four diastereoisomers of 2-hydroxymethyl-3-amino-4-hydroxypyrrolidines **488**→ **491** from one common starting material **492** (Scheme 118).



Scheme 118

4.3 Studies into the synthesis of (2*S*, 3*S*, 4*R*)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 488

4.3.1 Synthetic Approach



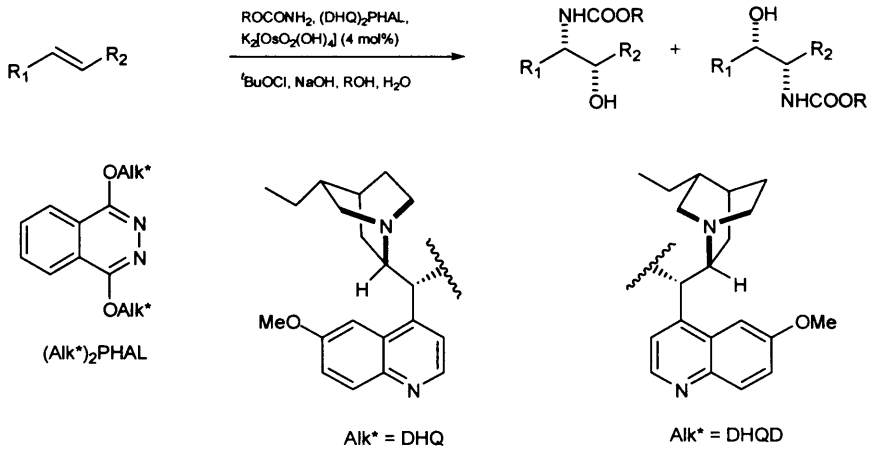
Scheme 119

In synthesising the (2*S*, 3*S*, 4*R*) isomer **488**, the C-3 amine and C-4 hydroxyl groups needed to be introduced *cis* to each other and *syn* to the C-2 hydroxymethyl substituent (Scheme 119). The Sharpless aminohydroxylation has been extensively employed to access *cis*-amino alcohols, and we saw this as a possible route to access this target diastereoisomer. We suspected that this approach would confer addition *anti* to the C-2 substituent (as in previous dihydroxylations of analogous systems see chapter 3, section 3.2.4). However, we had become aware of a tethered aminohydroxylation developed by Donohoe and co-workers[91] and saw this as a viable route to access the all *syn* isomer **488**.

4.3.2 The tethered aminohydroxylation

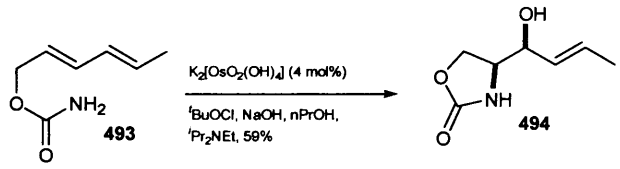
The tethered aminohydroxylation developed by Donohoe is based on, and is an advancement of, the Sharpless asymmetric aminohydroxylation (AA) reaction.[145] The AA

reaction was developed in the mid-nineties by Sharpless and co-workers[146] and has now become a powerful tool in the synthesis of chiral amino alcohols from alkenes. The reaction, typified by the conversion shown in Scheme 120 employs a catalyst consisting of Cinchona alkaloid derived ligands (*e.g.* $(DHQ)_2PHAL$ and $(DHQD)_2PHAL$), an osmium species (commonly $K_2[OsO_2(OH)_4]$) and a nitrogen source (*e.g.* a carbamate) together with an oxidant ^{t}BuOCl. This reaction has many similarities to the more widely known asymmetric dihydroxylation in that it is stereospecific; however there is an added complication in that control of regioselectivity is required when unsymmetrical olefins are used.



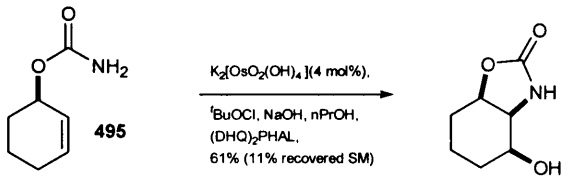
Scheme 120

The tethered aminohydroxylation overcomes the problem of regioselectivity by attaching the source of nitrogen (carbamate) to an achiral alcohol *e.g.* **493** (Scheme 121).



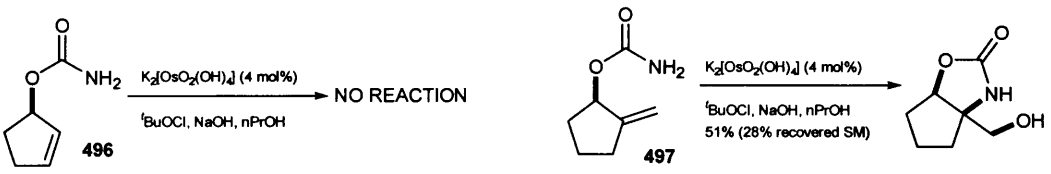
Scheme 121

A range of acyclic achiral allylic alcohols can be converted to their carbamates and under standard Sharpless AA conditions (without the chiral ligand), hydroxyoxazolidinones (*e.g.* **494**) can be isolated as single regioisomers displaying total *syn* selectivity (Scheme 121). This methodology has also been applied to chiral cyclic systems where both regio- and stereoselectivity can be controlled (Scheme 122).



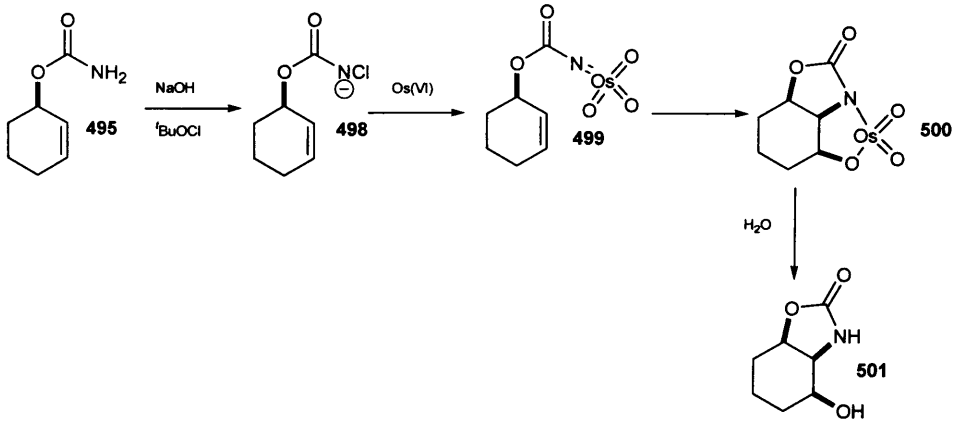
Scheme 122

Whilst working well for six membered endocyclic alkenes *e.g.* **495** (Scheme 122) this reaction has proved to be incompatible with the five membered equivalent **496**, with only exocyclic alkenes *e.g.* **497** reacting successfully (Scheme 123).



Scheme 123

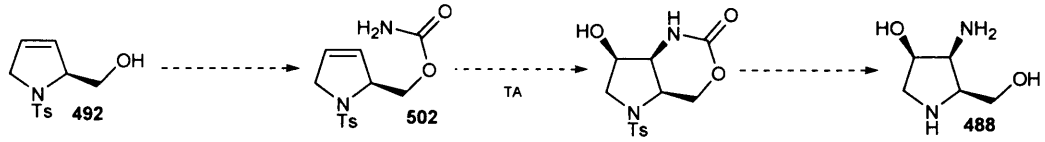
The mechanism proposed by Donohoe[91] for this tethered reaction is analogous to the proposal by Sharpless[146] for the asymmetric aminohydroxylation (Scheme 124). Thus, after an *in situ* chlorination and deprotonation of the carbamate **495** the nitrene equivalent **498** is formed. Intermediate **498** then combines with potassium osmate to form the active osmium species **499**, which subsequently is delivered to the alkene to form the all *syn* azaglycolate **500**. The azaglycolate **500** is then oxidised (by the addition of a second nitrene equivalent) and hydrolysed *in situ* to give the product **501** (Scheme 124). It is therefore suspected that endocyclic five membered alkenes are not compatible with this reaction due to the excessive strain, which would be present in the corresponding azaglycolate osmate ester.



Scheme 124

4.3.3 A tethered aminohydroxylation approach to the (2*S*, 3*S*, 4*R*)-2-hydroxymethyl-3-amino-4-hydroxyaminopyrrolidine 488

Due to the excellent control of regioselectivity and stereospecificity in the tethered aminohydroxylation reaction, we envisaged it could be employed with our key intermediate **492** to yield the target compound **488** as shown (Scheme 125).

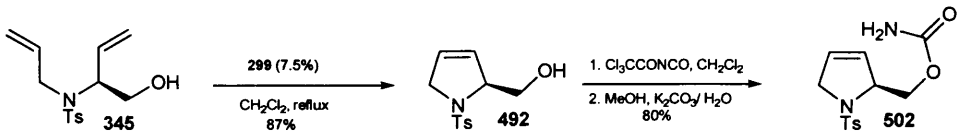


Scheme 125

With reference to this reaction, we had three major concerns; firstly at the time of our initial studies, all examples of this reaction had been undertaken using allylic carbamates. The key intermediate **492** that we wished to employ would involve a homoallylic carbamate **502** so we were slightly apprehensive about the effect of this on the reaction. Secondly, **492** contains a five membered endocyclic alkene, which was shown to be incompatible with this reaction in allylic systems. However, due to the extra carbon in the homoallylic system we were hopeful that this would relieve some of the strain in the azaglycolate species (5:6:6 *vs.* 5:5:5 tricyclic intermediate) allowing the reaction to be successful. Finally, our third concern was the effect of the electron withdrawing *N*-toluenesulfonamide substituent on the reactivity of the alkene. Again, previous examples of the reaction had involved carbocyclic alkenes so therefore we did not know how the relatively electron poor alkene **502** would react.

4.3.4 Initial studies

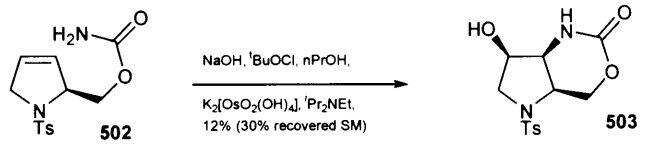
Initially to test this reaction the homoallylic alcohol **492** was synthesised using a RCM reaction[147] on the diene **345** (Scheme 126) (**345** had previously been synthesised from L-methionine as part of the azadisaccharide work described in chapter 3, section 3.2.1). The RCM reaction proceeded efficiently and in good yield (87%) and the resulting alcohol **492** was converted to the carbamate **502** using the procedure reported by Donohoe.[91] This involved an initial reaction of the alcohol with trichloroacetyl isocyanate ($Cl_3CCONCO$) and an *in situ* hydrolysis with K_2CO_3 (aq.) in methanol to form the carbamate **502** in excellent (80%) yield (Scheme 126).



Scheme 126

With carbamate **502** in hand the tethered aminohydroxylation was undertaken using the reaction procedure reported by Donohoe (Scheme 127). In this procedure it is stated that a black colour is indicative of the end of turnover and the reaction being complete. In our initial attempt the reaction was left a total of 18 hours and although a darkening of colour (light yellow to brown) was observed, the reaction mixture did not turn black suggesting the catalyst had not completed turnover. Nevertheless, after work-up and purification by column chromatography a small amount (12%) of a new compound was isolated together with recovered starting material (30%). Analysis by ^{1}H- and ^{13}C-NMR spectroscopy and mass spectrometry agreed with the expected structure **503** although the stereochemistry could not be accurately assigned (Scheme 127). Although the yield of this initial attempt was relatively

poor we suspected the target compound **503** had been isolated and so we set about optimising this reaction.



Scheme 127

To optimise this reaction, many conditions were varied *e.g.* catalyst loading, addition of catalyst at intervals, concentration of the sodium hydroxide solution, different temperatures, amount of ^{t}BuOCl, ligand *etc*. However, it was found that on a small scale (<0.1mmol), this reaction was very unpredictable and although an improved yield was achieved (optimum yield of **503** 40%, 60% based on recovered starting material **502**) this result was not repeatable and yields of **503** were found to vary between 4% and 40%. Furthermore, as the reaction was repeated a number of times, an unknown side product (which appeared to be an isomer of **503**) was isolated in varying amounts. Fortunately both **503** and the side-product **504** were crystalline and x-ray crystal structures were obtained for both (Figure 41 and see appendix).

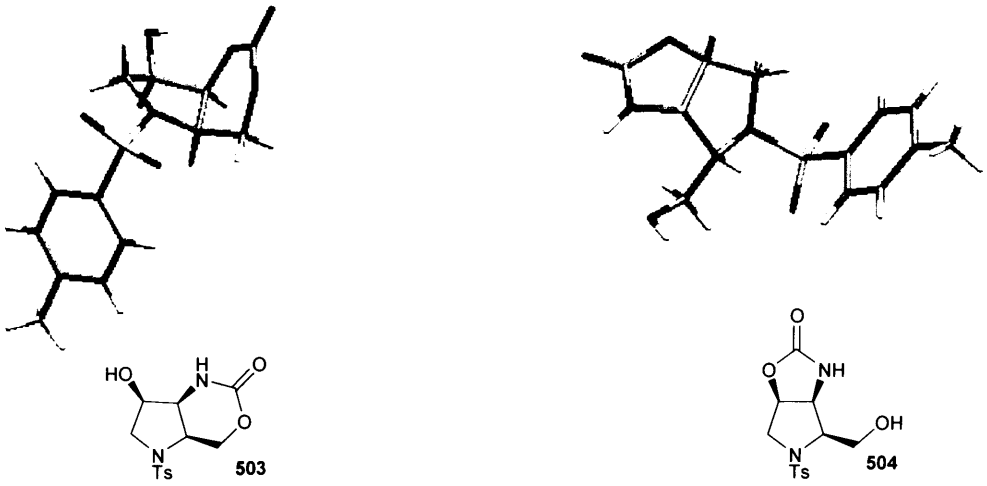
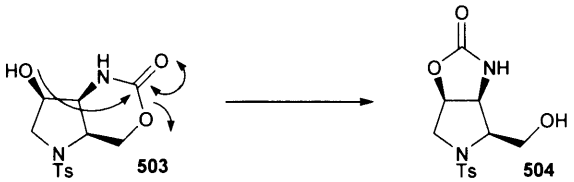


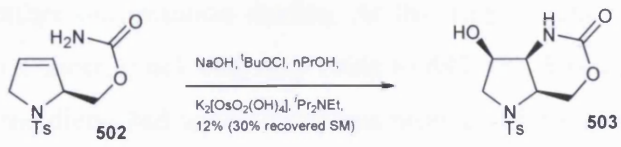
Figure 41

As can be seen, these x-ray structures confirmed that the aminohydroxylation had been successful yielding the desired (2*S*, 3*S*, 4*R*) cyclic carbamate **503** and confirmed the identity of the unknown to be the isomeric carbamate **504** resulting from migration of the carbonyl from the primary to secondary hydroxyl group (Scheme 127). This isomeric carbamate **504** seems to be energetically preferred as on standing in solution for long periods (*e.g.* an NMR sample in MeOD) **503** slowly converted to substantial amounts of **504**.



Scheme 127

poor we suspected the target compound **503** had been isolated and so we set about optimising this reaction.



Scheme 127

To optimise this reaction, many conditions were varied *e.g.* catalyst loading, addition of catalyst at intervals, concentration of the sodium hydroxide solution, different temperatures, amount of ^{t}BuOCl, ligand *etc*. However, it was found that on a small scale (<0.1mmol), this reaction was very unpredictable and although an improved yield was achieved (optimum yield of **503** 40%, 60% based on recovered starting material **502**) this result was not repeatable and yields of **503** were found to vary between 4% and 40%. Furthermore, as the reaction was repeated a number of times, an unknown side product (which appeared to be an isomer of **503**) was isolated in varying amounts. Fortunately both **503** and the side-product **504** were crystalline and x-ray crystal structures were obtained for both (Figure 41 and see appendix).

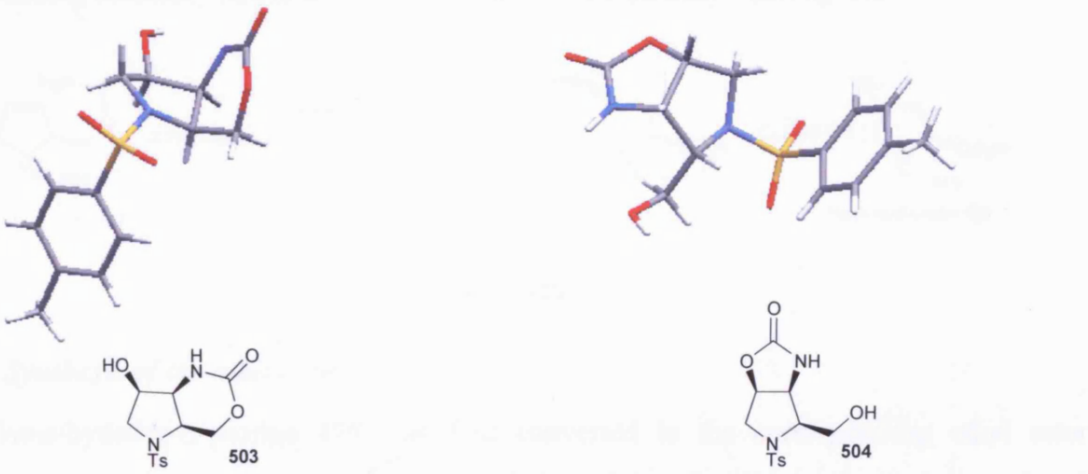


Figure 41

As can be seen, these x-ray structures confirmed that the aminohydroxylation had been successful yielding the desired (2*S*, 3*S*, 4*R*) cyclic carbamate **503** and confirmed the identity of the unknown to be the isomeric carbamate **504** resulting from migration of the carbonyl from the primary to secondary hydroxyl group (Scheme 127). This isomeric carbamate **504** seems to be energetically preferred as on standing in solution for long periods (*e.g.* an NMR sample in MeOD) **503** slowly converted to substantial amounts of **504**.

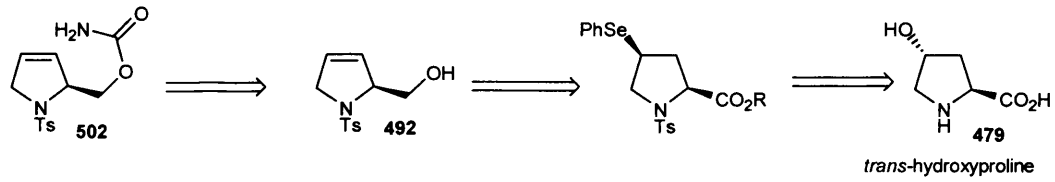
Scheme 127

In view of these promising results and due to the capricious nature of the reaction we decided to explore further optimisation studies. At this time stocks of the key alcohol **492** were depleting and so a short, quick and easy route to **492** was developed. Our original route to **492** had employed the diene **345** which itself was produced from L-methionine **347**. Whilst this route was perfectly suited for the synthesis of azadisaccharides (chapter 3, section 3.2.1) as it allows for the heterocyclic ring size to be readily varied, it was somewhat long-winded for the synthetic studies to hand. Therefore an alternative route was developed from *trans*-hydroxy-L-proline **479** as discussed below.

4.3.5 An 'improved' synthesis of 492 from *trans*-hydroxy-L-proline 479

4.3.5.1 Retrosynthetic analysis

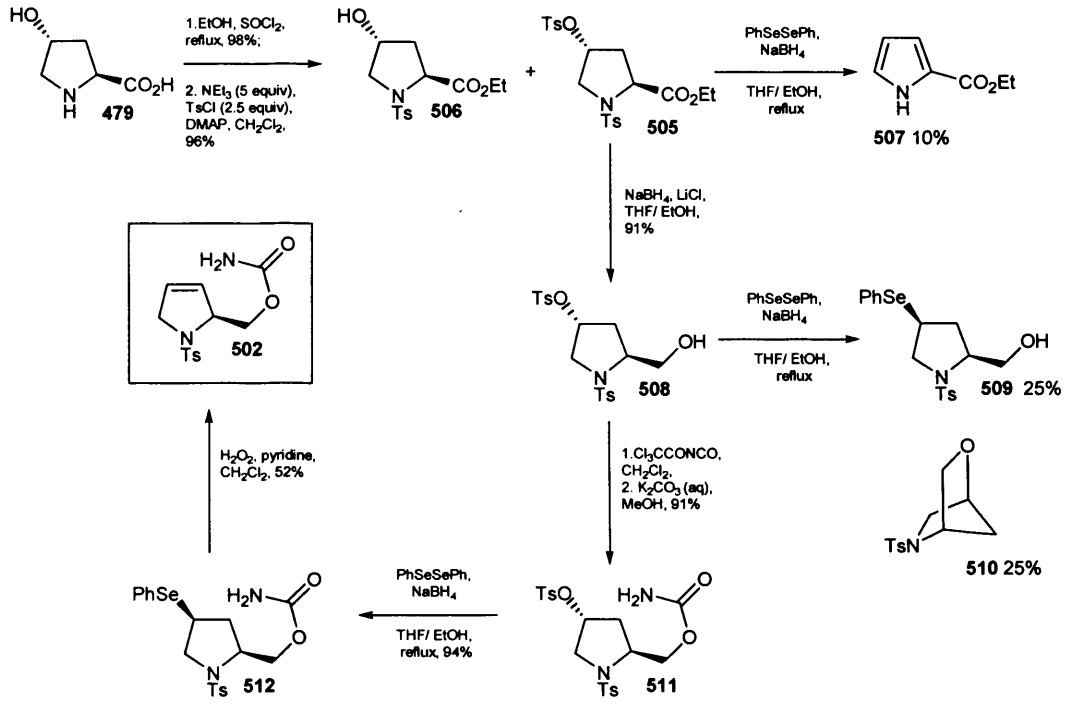
A modified version of the synthesis from *trans*-hydroxy-L-proline **479** reported by Schofield[143] was envisaged as a route to the carbamate **502**. This followed the retrosynthetic plan described in Scheme 128 where the elimination of a selenoxide (formed from the corresponding selenide) would introduce the alkene functionality yielding **492**.



Scheme 128

4.3.5.2 Synthesis of the alkene 502

Trans-hydroxy-L-proline **479** was first converted to the corresponding ethyl ester (Scheme 129). A di-tosylation of the amine and alcohol functional groups was then achieved following a procedure reported by Walker and co-workers.[148] Using a long reaction time (48 hours) and an excess of *p*-toluenesulfonyl chloride and triethylamine (2.5 and 5 equivalents respectively) the *bis*-toluenesulfonate **505** was accessed in an excellent 96% yield after chromatography (accompanied by 3% of the monotosylated product **506**). Nucleophilic displacement of the tosylate using the phenylselenide anion (generated *in situ* from PhSeSePh and $NaBH_4$) in THF was subsequently attempted. However, none of the desired product was formed and only the pyrrole **507** could be isolated from the reaction. The pyrrole **507** presumably arises by the base-mediated elimination of both sulfonates and this maybe facilitated by the acidic C-2 proton in **505**.



Scheme 129

In an effort to decrease the acidity of this proton the carboxyester functionality was reduced to the corresponding alcohol **508**. Thus, reduction of **505** was achieved using $NaBH_4$ and LiCl in methanol (to form $LiBH_4$ *in situ*) following a procedure reported by Ibuka and co-workers[149] to give **508** in excellent yield.

Displacement of the tosylate in **508** with phenylselenide was subsequently undertaken and was successful with the product **509** being isolated in 25% yield. However, **509** was accompanied by significant amounts of the bicyclic ether **510** (25%) formed *via* an intramolecular nucleophilic displacement of the tosylate by the primary alcohol. In view of this result we decided to protect the hydroxyl group before the selenide displacement and chose the carbamate group, which would be utilised in the tethered aminohydroxylation reaction. Thus the alcohol **508** was converted to the carbamate **511** in excellent yield (91%) using the conditions previously discussed. Pleasingly, the nucleophilic displacement step now proceeded smoothly to give selenide **512** in excellent yield (94%). Finally oxidation of the selenide and subsequent *syn*-elimination of the selenoxide thus produced, gave the homoallylic carbamate **502** in moderate yield (52%).

Thus, in conclusion we have developed an efficient synthesis of our key intermediate **502** in six steps from *trans*-hydroxy-L-proline **479** in 38% overall yield and requiring only minimal purification steps.

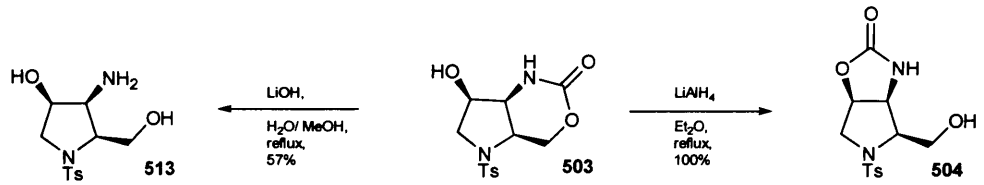
4.3.6 Further investigations into the tethered aminohydroxylation reaction

With a substantial amount of the carbamate **502** in hand further investigations regarding the tethered aminohydroxylation were undertaken. After exploring many conditions on a small scale (0.1mmol) no real trends became apparent and again the reaction proved to be extremely variable. However, repeating the reaction on a larger scale (1.5mmol), proved more reliable and repeatable, and the yield of products (**503** and **504**) was increased to 52% (80% based on recovered starting material) (Scheme 127). The reaction mixture was also turning the black colour reported by Donohoe indicating the catalyst had completed turnover.

Recently Donohoe and co-workers published a report[150] concerning the tethered aminohydroxylation of homoallylic systems. Yields for these systems were reported to be in the range of 30-65% (depending on the substitution pattern on the olefin). These yields are in agreement with the results we have obtained and so we are very pleased with the success of this reaction.

4.3.7 Deprotection of the key intermediates 503 and 504 - synthesis of 488

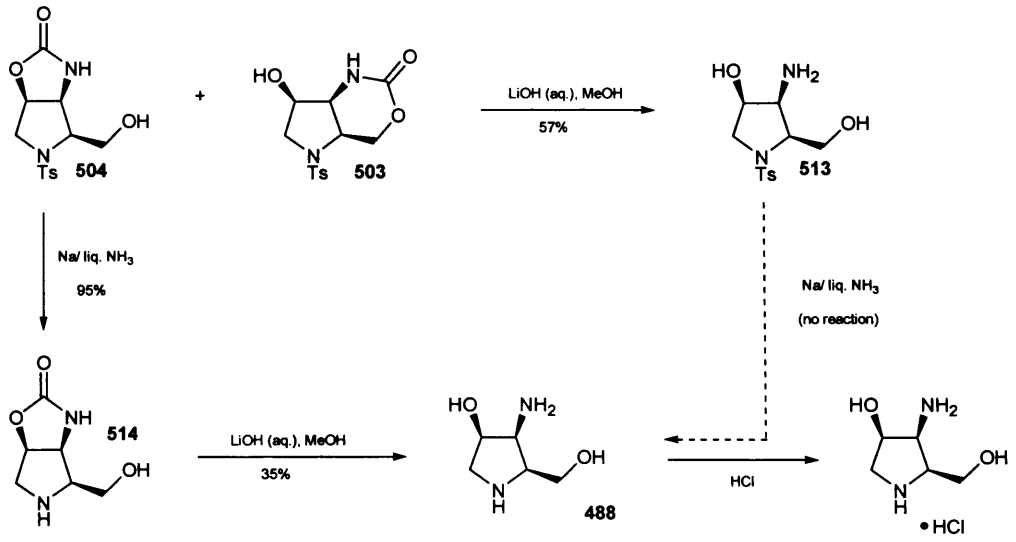
Initially deprotection of the carbamate group in **503** was attempted using $LiAlH_4$. However, after heating **503** in diethyl ether no deprotection was observed and only the 5,5-bicyclic carbamate **504** (Scheme 130) was isolated. Deprotection was next attempted with LiOH (aq.) in refluxing methanol following a procedure reported by Gotor and co-workers.[151] Pleasingly, this reaction was successful and after chromatography the tosyl-protected pyrrolidine **513** was accessed in reasonable yield (57%) (Scheme 130).



Scheme 130

Subsequent studies found that this hydrolysis could be carried out successfully on an isomeric mixture of **503** and **504** giving **513** (68%) and this meant that the two isomers **503** and **504** did not have to be separated thus, making the whole process considerably simpler. From this intermediate **513**, efforts were then undertaken to remove the tosyl group. Formation and isolation of the target compound **488** using standard tosyl deprotection conditions[80] ($Na/liq.NH_3$) was found to be very difficult and it was suspected that either **513** and/or the product **488** were unstable to such conditions. Therefore an alternative approach to the fully deprotected pyrrolidine was investigated which involved removal of the tosyl group prior to hydrolysis of the carbamate. Thus, **503** was subjected to tosyl deprotection yielding **514** in

good yield (95%) with no evidence of carbamate reduction (Scheme 131). Hydrolysis of the carbamate **514** (LiOH (aq.)/methanol) was then achieved providing the target pyrrolidine **488** in reasonable yield (35%) after column chromatography.



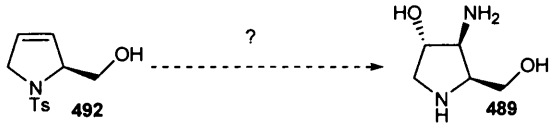
Scheme 131

The amine **488** was found to be particularly unstable on storage and consequently, to try and increase its stability the corresponding HCl salt was formed and characterised. However, even the HCl salt of **488** seemed to readily undergo decomposition and therefore only minimal data for this compound was obtained.

To summarise, the tethered aminohydroxylation reaction has successfully been used to regio- and stereoselectively form a novel 2-hydroxymethyl-3-amino-4-hydroxypyrrolidine. Deprotection studies have shown that the protecting groups can be manipulated to give intermediates having a variety of the hydroxyl groups and amino groups selectively protected (*i.e.* **503**, **504**, **513**, **514** and **488**), thus, demonstrating potential as synthetic intermediates. Compounds **503**, **504**, **513**, **514** and **488** were tested (in collaboration) against a range of glycosidase enzymes and the results are discussed in chapter 5.

4.4 Studies into the synthesis of the (2*S*, 3*S*, 4*S*)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 489

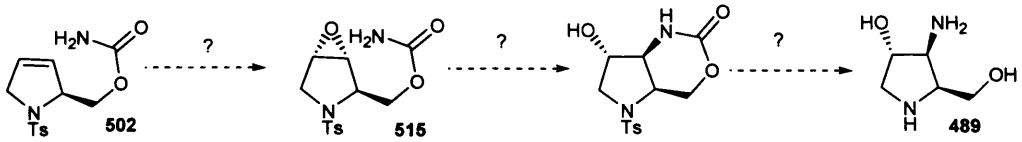
4.4.1 Approach



Scheme 132

Having successfully accessed the all *syn*-pyrrolidine **488**, we next turned our attention to the diastereoisomer **489**, epimeric at the C-4 position (Scheme 132). The (2*S*, 3*S*, 4*S*)

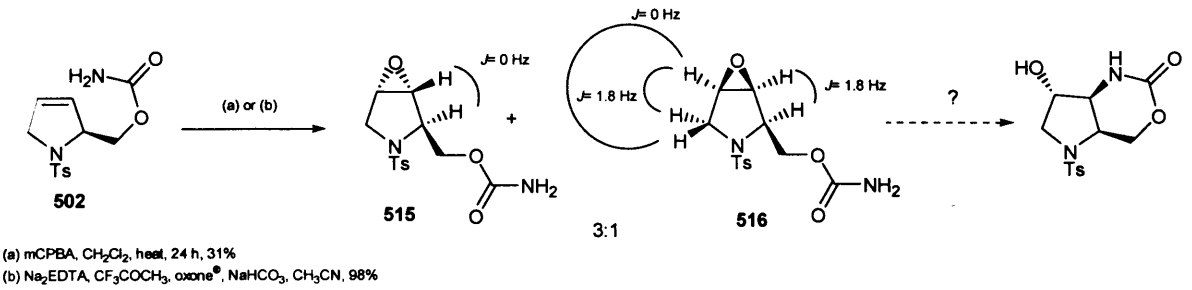
isomer **489** has the C-3 amine group and C-4 hydroxyl group in a *trans* arrangement and so we envisaged an epoxide opening could be used to access this stereoisomer. Whilst there would potentially be few problems in selectively forming the requisite *anti*-epoxide **515** (Scheme 133), we were concerned about the regioselectivity of epoxide opening especially in light of results reported by Heredis[142] and Schofield[143] which showed selective nucleophilic attack at C-4 in analogous *anti*-epoxide intermediates (see section 4.1.5). We therefore entertained the idea of tethering the nucleophile to the C-2 position (analogous to the tethered aminohydroxylation) allowing the epoxide ring opening to take place in an intramolecular fashion in a regio- and stereoselective manner. We saw the carbamate **502** as a viable source to achieve this synthetic target (Scheme 133).



Scheme 133

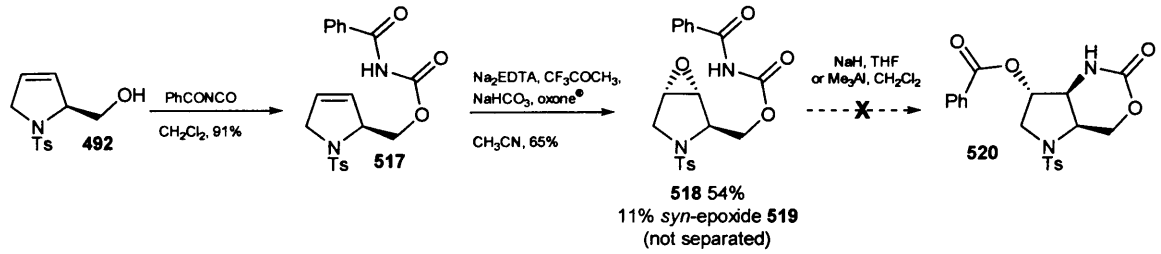
4.4.2 Epoxidation and intramolecular ring opening

Epoxidation of the homoallylic carbamate **502** was initially attempted using mCPBA and following the procedure reported by Schofield.[143] However, whilst the epoxide **515** was formed in poor yield (31% of a 3:1 mixture of *anti*:*syn* isomers respectively) the reaction was not very efficient and even after prolonged reaction times (heating for 24 hours) significant quantities (48%) of starting material remained. These observations are in agreement with results reported by Schofield with the analogous *N*-benzyloxycarbonyl-3,4-dehydroproline benzyl ester **478** (section 4.1.5, Scheme 116) where prolonged heating was also required to obtain a 1:2.5 epoxide mixture although in a 76% yield. Pleasingly, an alternative procedure involving the formation of a dioxirane[152] *in situ* (formed from CF_3COCH_3 and oxone®) was more successful and gave a mixture of the epoxides **515** and **516** (again as a 3:1 mixture by ^{1}H-NMR analysis) in an excellent 98% yield (Scheme 134). Although, these epoxide isomers proved to be frustratingly inseparable by column chromatography. Although inseparable, examination of ^{1}H-NMR *J* values between H-2 and H-3 (0 Hz for the major isomer **515** and 1.8 Hz for the minor isomer **516,** see Scheme 134) suggested that the major isomer **515** had the anticipated stereochemistry with the epoxide *anti* to the C-2 substituent. This assignment was later confirmed by the unambiguous synthesis of both isomers (see sections 4.4.3 and 4.5.3). In view of this, investigations into the intramolecular epoxide opening were then carried out on the isomeric mixture.



Scheme 134

Having researched the literature in this area it was discovered that no intramolecular epoxide opening had been reported using an unsubstituted carbamate, although there were reports of similar reactions employing *N*-substituted carbamates (*e.g.* methyl,[153] benzyl,[154] benzoyl),[155] where bases (*e.g.* NaH) and Lewis acids (*e.g.* Me_3Al) [156] had been employed to achieve epoxide opening. Based on these reports, cyclisation was initially attempted under basic conditions. Treating the mixture of epoxides **515** and **516** with either ^{t}BuOK or NaH in refluxing THF however, gave only recovered starting materials. In order to increase the acidity of the N-H proton the alcohol **492** was next derivatised to the benzoyl carbamate **517** (using benzoyl isocyanate[155]) (Scheme 135). Epoxidation (CF_3COCH_3 and oxone®) of **517** gave the epoxides **518** and **519** (1:4.5 ratio by ^{1}H-NMR analysis, the major isomer was assumed to be the *anti*-epoxide **518** as shown, Scheme 135). Epoxidation was found to be much slower and not as 'clean' as with the corresponding carbamate **502** and again the epoxide isomers **518** and **519** were inseparable by column chromatography.

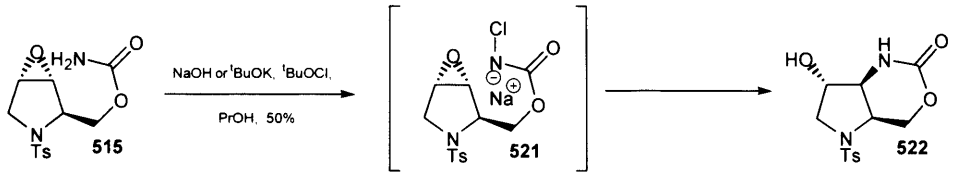


Scheme 135

This mixture was subsequently treated with NaH following the procedure by Knapp and co-workers[155] however, after heating at reflux for 5 hours no reaction was apparent and only starting material was recovered. Attempting the reaction under Lewis acid conditions (Me_3Al in CH_2Cl_2)[156] also gave none of the desired product **520** and once again only starting material was recovered.

In view of this failure we returned to examining the reactions of the unsubstituted carbamate **502**. This intermediate had previously been shown to successfully undergo the tethered aminohydroxylation reaction (see section 4.3.2) the first step of which involves deprotonation to form the corresponding carbamate anion, which then reacts with ^{t}BuOCl to

form a nitrene (section 4.3.2, Scheme 124). Thus, we reasoned that treatment of **515** with the same base used in the aminohydroxylation (sodium hydroxide in propanol) would guarantee formation of the anion and thus potentially give the intramolecular epoxide opening. However, prolonged heating of **515** with aqueous sodium hydroxide in propanol gave only recovered starting material **515**. Despite this failure we repeated the reaction but this time included ^{t}BuOCl in the basic mixture in the hope that isolation of the *N*-chloro-carbamate salt **521** would at least give evidence for the formation of the carbamate anion from **515**. Intriguingly no *N*-chloro products were isolated from the reaction but to our surprise the target ring opened product **522** was isolated in moderate yield (22%) (Scheme 136).



Scheme 136

Subsequent optimisation of the reaction increased this yield to 50%. Fortunately, the product **522** was crystalline and an x-ray structure could be obtained (Figure 42 and see appendix). This confirmed the product to have the stereochemistry shown and confirmed that opening of the *anti*-epoxide **515** had occurred.

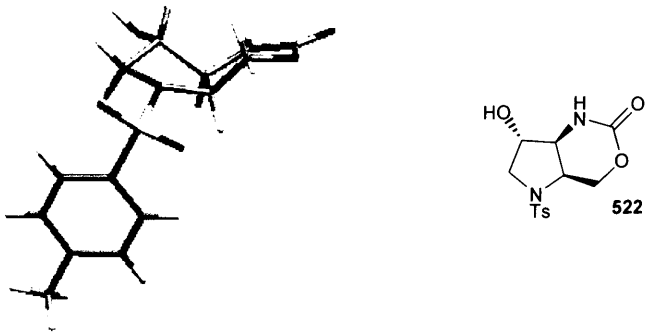
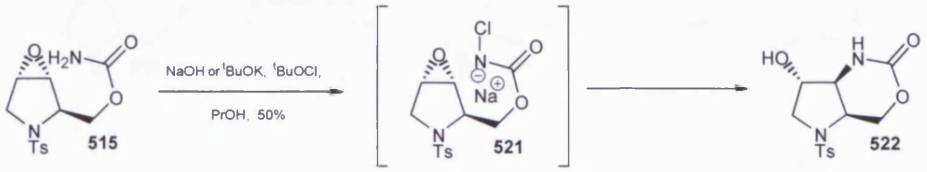


Figure 42

Regrettably, none of the *syn*-epoxide **516** or the product resulting from its epoxide opening was ever isolated from these reactions and it was suspected that other side reactions were occurring. To try and minimise these, the reaction was also undertaken using ^{t}BuOK in place of NaOH, however, the outcome was the same and comparable yields were obtained (Scheme 136).

form a nitrene (section 4.3.2, Scheme 124). Thus, we reasoned that treatment of **515** with the same base used in the aminohydroxylation (sodium hydroxide in propanol) would guarantee formation of the anion and thus potentially give the intramolecular epoxide opening. However, prolonged heating of **515** with aqueous sodium hydroxide in propanol gave only recovered starting material **515**. Despite this failure we repeated the reaction but this time included ^{t}BuOCl in the basic mixture in the hope that isolation of the *N*-chloro-carbamate salt **521** would at least give evidence for the formation of the carbamate anion from **515**. Intriguingly no *N*-chloro products were isolated from the reaction but to our surprise the target ring opened product **522** was isolated in moderate yield (22%) (Scheme 136).



Scheme 136

Subsequent optimisation of the reaction increased this yield to 50%. Fortunately, the product **522** was crystalline and an x-ray structure could be obtained (Figure 42 and see appendix). This confirmed the product to have the stereochemistry shown and confirmed that opening of the *anti*-epoxide **515** had occurred.

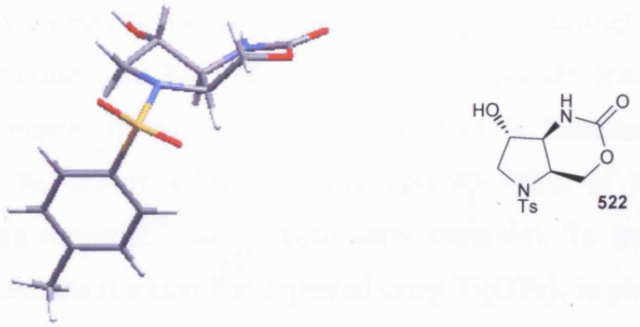
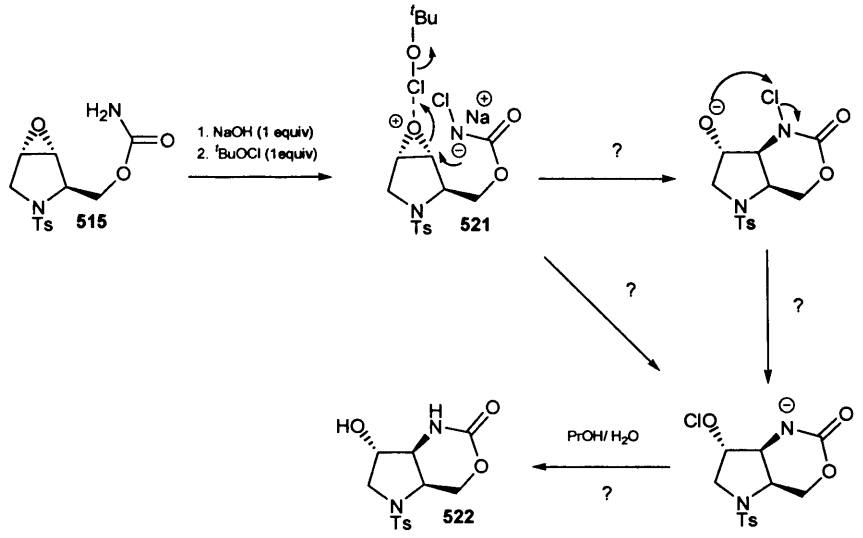


Figure 42

Regrettably, none of the *syn*-epoxide **516** or the product resulting from its epoxide opening was ever isolated from these reactions and it was suspected that other side reactions were occurring. To try and minimise these, the reaction was also undertaken using ^{t}BuOK in place of NaOH, however, the outcome was the same and comparable yields were obtained (Scheme 136).

Whilst we were pleased with this successful intramolecular epoxide opening, we were unsure of the mechanism of the reaction, especially as to the vital role being played by the ^{t}BuOCl. We supposed that the mechanism may involve initial *N*-chlorination of **515** (as in the aminohydroxylation) followed by formation of the anion **521**, which subsequently carried out the nucleophilic attack at C-3 (Scheme 137). Alternatively, or perhaps in combination, the ^{t}BuOCl may also facilitate epoxide ring opening through coordination as shown (Scheme 137).



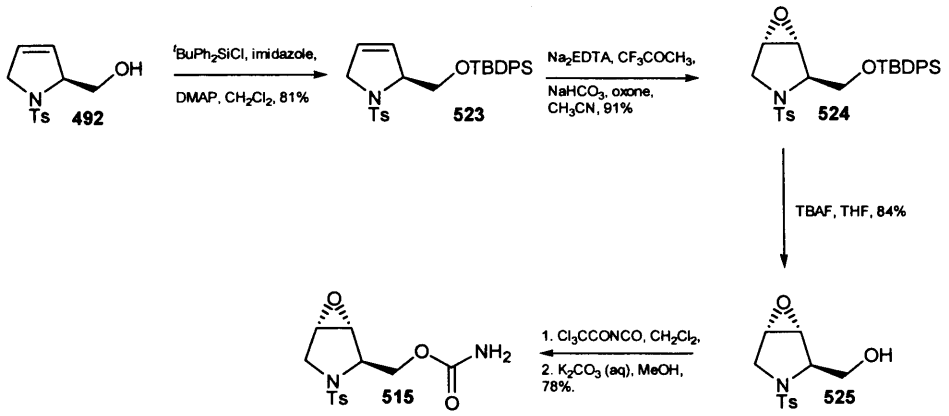
Scheme 137

In an effort to investigate these mechanistic proposals, attempts were made to try and isolate the *N*-chlorosodium salt **521** and to carry out the epoxide opening. However attempts to form **521** using either NaOCl following a procedure by Bachand and co-workers[157] or ^{t}BuOCl proved to be unsuccessful and only decomposition of **515** was apparent (*N*-chlorocarbamates are reported[157] to be particularly unstable). To investigate if Lewis acid catalysis was involved, the reaction was repeated using Ti(O^{i}Pr)$_4$ in place of ^{t}BuOCl, however no reaction was evident and only starting material was recovered. Therefore whilst the mechanism of the epoxide opening was not fully elucidated the synthesis of the target intermediate **522** had been achieved.

4.4.3 Stereoselective synthesis of the *trans*-epoxide 515

In order to prove that the major isomer in the epoxidation reaction of **502** (Scheme 134) was the *anti*-epoxide **515**; a route was devised to synthesise this isomer stereoselectively (Scheme 138). The alcohol **492** was firstly converted to the sterically demanding TBDPS ether[158] **523** to favour epoxide formation *trans* to the substituent. Subsequent epoxidation of **523** did indeed yield one major epoxide (>98% stereoselective) suspected to be the *trans*-epoxide **524**. Subsequent silyl deprotection[159] yielding **525** and carbamate formation gave the

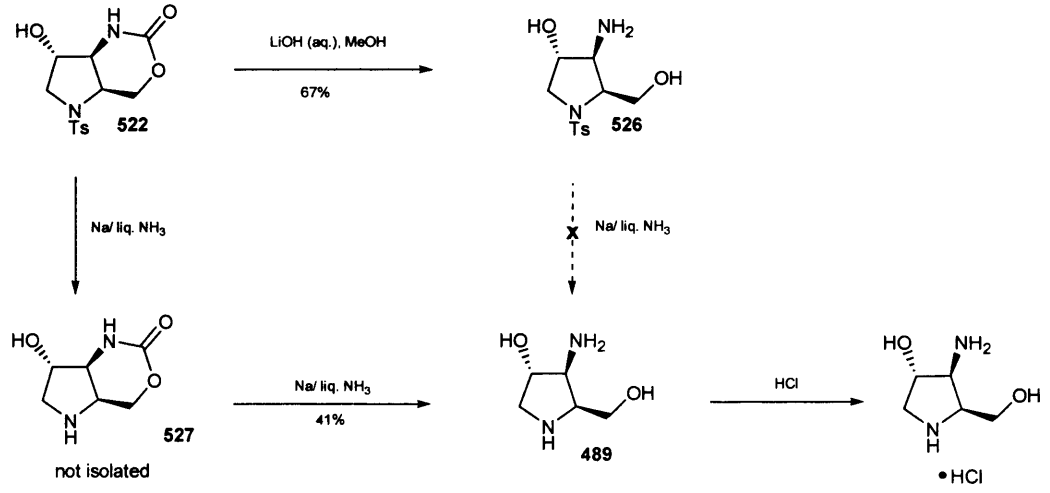
epoxide **515** and comparison of the NMR data (^{1}H, ^{13}C) with that of the major isomer formed previously (Scheme 134) indicated both to be identical. Fortunately **515** was crystalline and an x-ray structure was obtained (see appendix) confirming the already predicted stereochemistry.



Scheme 138

4.4.4 Deprotection of the key intermediate 522

As with the (2*S*, 3*S*, 4*R*) isomer selective deprotections were carried out on **522** (Scheme 139). Thus, heating **522** with LiOH (aq.) in methanol at reflux gave the tosyl-protected aminopyrrolidine **526** in good (68%) yield. Again attempts to remove the tosyl group from this intermediate (using Na/ liq. NH_3) proved unsuccessful and it was suspected that degradation of either the starting material **526** or the resulting product **489** was occurring under the reaction conditions. Additionally, attempts at tosyl deprotection on the carbamate **522**, proved difficult to control and resulted in varying amounts of tosyl (forming **527**) and/or carbamate deprotection.



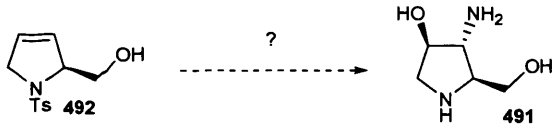
Scheme 139

However, these results suggested that it may prove feasible to carry out two steps in one pot and pleasingly treatment of **522** with an excess of sodium in liquid ammonia resulted in clean removal of both tosyl and carbamate functionalities. Again as with the (2*S*, 3*S*, 4*R*) isomer **488**, this pyrrolidine **489** was characterised as the corresponding hydrochloride salt.

To summarise, the target (2*S*, 3*S*, 4*S*) 2-hydroxymethyl-3-amino-4-hydroxypyrrolidine **489** has been accessed using an intramolecular regio- and stereoselective nucleophilic epoxide opening. Deprotection studies have again shown that the protecting groups can be manipulated to give intermediates having a variety of the hydroxyl groups and amino groups selectively protected (*i.e.* **522**, **526**, and **489**). Compounds **522**, **526**, and **489** were tested (in collaboration) against a range of glycosidase enzymes and the results are discussed in chapter 5.

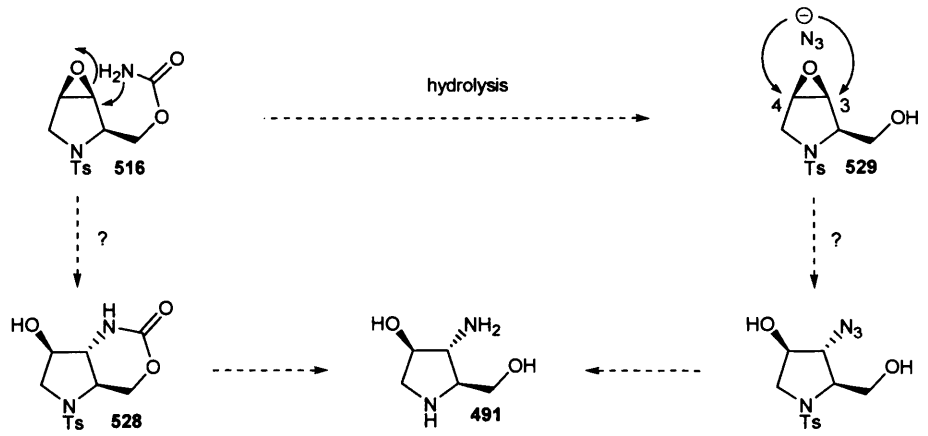
4.5 Studies into the synthesis of the (2*S*, 3*R*, 4*R*)-2-hydroxymethyl-3-amino-4-hydroxy-pyrrolidine 491

4.5.1 Synthetic Approach



Scheme 140

The (2*S*, 3*R*, 4*R*) diastereoisomer **491**, also contained a *trans* relationship between the 3-amino and 4-hydroxyl substituents (Scheme 140). We envisaged two possible routes to this stereoisomer both starting from the *syn*-epoxide **516** as shown (Scheme 141). In the first case intramolecular epoxide opening would lead to the bicyclic carbamate **528** having a *trans* ring junction. Whilst this is chemically possible we were slightly concerned that there had been no indication of **528** being formed in the epoxide opening reactions carried out with the 3:1 mixture of **515** and **516**. The alternative strategy involved intermolecular opening of the epoxide **529** using a suitable nitrogen nucleophile (*e.g.* NaN_3). However, the disadvantage of this approach was the possible lack of regiocontrol and indeed the previous studies by Schofield[143] with similar epoxides (see section 4.1.5) had suggested that intermolecular nucleophilic attack would be favoured at the C-4 rather than the required C-3 carbon (a 2:1 ratio of C-4 : C-3 attack was found in these studies). To attempt either of these approaches a stereoselective synthesis of *syn*-epoxide **516** was needed.

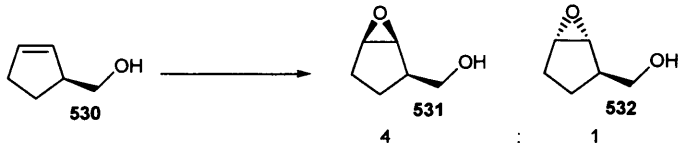


Scheme 141

4.5.2 Stereoselective synthesis of the *syn*-epoxide 516

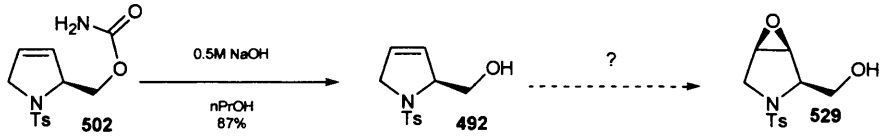
4.5.2.1 Alcohol directed epoxidation

The stereoselectivity of epoxide formation in allylic alcohols can be controlled using alcohol directed epoxidation.[160] This approach employs an epoxidising reagent that will hydrogen bond or chelate to an adjacent hydroxyl group (usually an allylic alcohol), causing the epoxide to form on the same face of the olefin. Indeed, Morizawa and co-workers[161] have shown that the cyclopentane **530** undergoes reaction with mCPBA to give a 4:1 mixture of the *syn* : *anti* (**531** : **532**) epoxides as shown (Scheme 142).



Scheme 142

It was therefore considered that this approach could also be applied to our heterocyclic system *i.e.* the alcohol **492** and this was generated from the carbamate **502** by alkaline hydrolysis as shown (Scheme 143).



Scheme 143

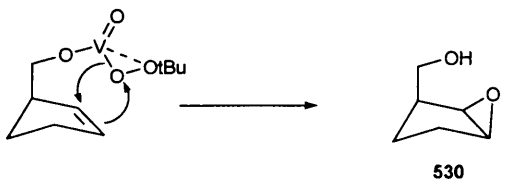
As discussed previously (section 4.4.2) epoxidation of the carbamate **502** with mCPBA or a dioxirane (formed *in situ* using oxone® and trifluroacetone) generated an inseparable 3:1 (*anti* : *syn*) mixture of epoxides **515** and **516** (Table 1, entries 1 and 2).

Entry	Substrate	Epoxidation method	*anti* : *syn*	Yield
1	**502** (carbamate)	mCPBA	3:1	31% (62% based on recovered **502**)
2	**502** (carbamate)	Oxone, CF_3COCH_3	3:1	98%
3	**492** (alcohol)	mCPBA	3:2	90%
4	**492** (alcohol)	Oxone, CF_3COCH_3	4:1	96%
5	**492** (alcohol)	$Mo(CO)_6$, ^tBuOOH	5:2	16%
6	**492** (alcohol)	$VO(acac)_2$, ^tBuOOH	-	-

Table 1

Initially epoxidation was attempted on the free alcohol **492** using mCPBA and as with the carbamate **502**, the reaction was found to be sluggish and required heating and long reaction times (24 hours). Nevertheless, the reaction yielded epoxides **525** and **529** in a good yield and as a 3:2 (*anti* : *syn*) mixture (Table 1, entry 3), although unfortunately the two isomers proved to be inseparable by flash column chromatography. This slight increase in the amount of the *syn*-epoxide (in comparison to the reaction with carbamate **502**) may indicate a slight hydrogen bonding effect between the peroxy-acid and the hydroxyl group, although, this does not compare to the selectivity achieved with the carbocycle **530** mentioned above (Scheme 142). Interestingly, reaction of the alcohol **492** with the dioxirane (generated *in situ* using oxone® and trifluoroacetone) actually resulted in an increased selectivity for the *anti*-epoxide **525** in comparison to the carbamate **502** (Table 1, entry 4).

With the failure of either of these methods to give increased levels of the *syn*-epoxide we turned our attentions to metal-mediated reactions. The use of organic peroxides combined with group 5B or 6B transition metal (*e.g.* Mo, W, V) catalyst systems have been reported[162] as useful reagents for stereoselective epoxidations of allylic alcohols. It is proposed that the metal chelates both the reagent and the alcohol and delivers the reactive oxygen atom to the same face hydroxyl substituent generating the *syn*-epoxide (Scheme 144). The most commonly used reagents are ^tBuOOH in combination with $Mo(CO)_6$ or $VO(acac)_2$ and these reagents have previously been used to selectively access the *syn*-epoxide **531** above (90% stereoselective) from the corresponding alkene **530** (Scheme 144).[161]



530

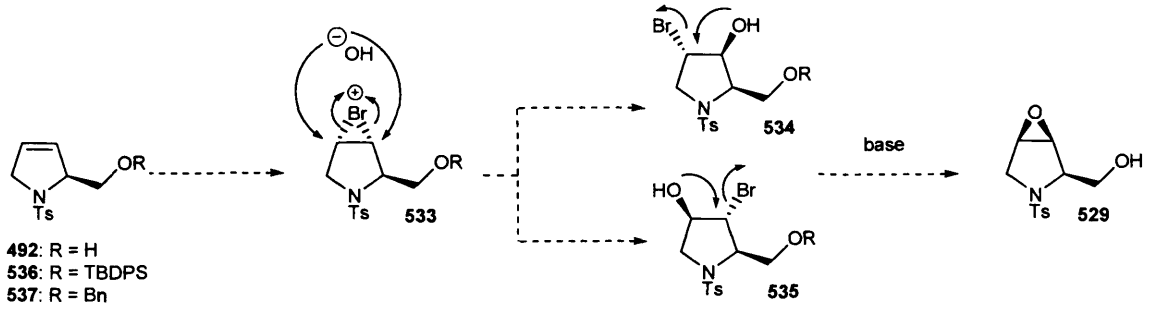
Scheme 144

However reaction of these reagents with the homoallylic alcohol **492** was not so successful. Thus, $Mo(CO)_6$ and ^tBuOOH in refluxing toluene (Table 1, entry 5) still gave an *anti*-selective reaction and in poor yield. Reaction with the vanadyl complex $VO(acac)_2$ gave

neither epoxide **525** or **529** and only a complex mixture of unidentifiable products (by ^{1}H-NMR analysis) was obtained (Table 1, entry 6). To conclude this investigation, alcohol directed epoxidation with mCPBA did slightly increase the ratio of the *syn*-epoxide **529** but not to an extent, which would be synthetically useful. Therefore alternative approaches to the *syn*-epoxide were explored.

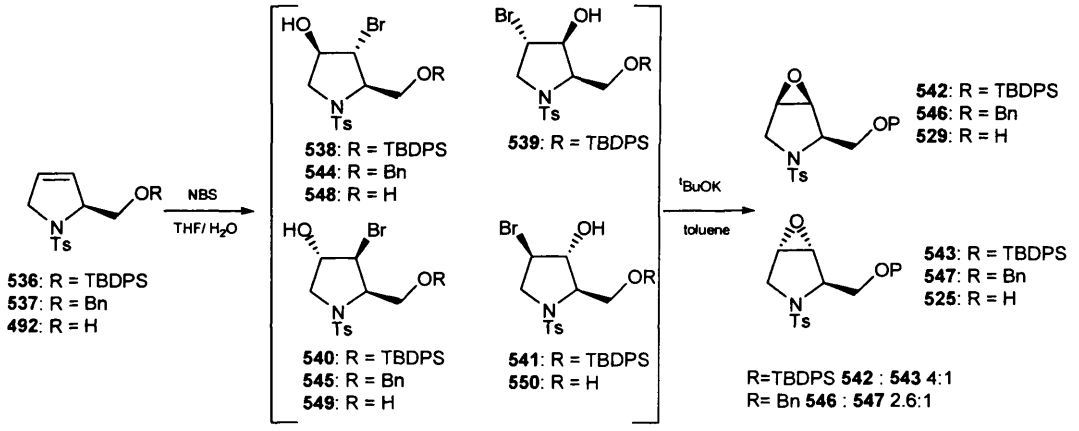
4.5.2.2 A bromohydrin based approach to the syn-epoxide 529

Our studies so far had indicated a strong preference for the alkene in a 2-substituted pyrrolidine such as **492** to undergo attack in an *anti*-selective manner. We postulated that it may prove possible to use this inherent diastereoselectivity to access the target *syn*-epoxide **529** using the bromohydrin based approach outlined in Scheme 145.



Scheme 145

Thus bromonium ion formation would be predicted to be face selective giving intermediate **533** and subsequently nucleophilic attack by water would generate **534** and/or **535**. Treatment of either of these bromohydrins with base and deprotection (as necessary) would then generate the target *syn*-epoxide **529**. Studies were carried out on the *O*-protected silyl and benzyl ethers **536** and **537** respectively as well as the free alcohol **492** as summarised in Scheme 146.



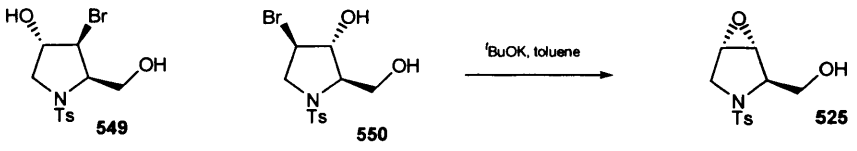
Scheme 146

Reaction of the silyl ether **536** with NBS in THF-water following a literature procedure[163] gave material which, by ^{1}H-NMR analysis contained a mixture of the bromohydrins **538**→**541** resulting from non-selective attack on both the alkene and

bromonium ion (Scheme 146). Additionally silyl cleavage was also observed complicating the analysis. Treatment of this mixture of products with base (^{t}BuOK in toluene) gave a 4:1 mixture of epoxides initially assigned as the *syn* **542** and *anti* **543** isomers respectively. This assignment was confirmed by silyl deprotection (TBAF in THF) generating a 4:1 mixture of **529** : **525** from which the *syn* stereochemistry was evident by comparison of ^{1}H-NMR data to previous studies (section 4.5.2). Whilst the *syn*-epoxide was now the major isomer, the overall yield for the process was extremely poor (2.6% of **529** and **525** from **536**) due in main to the instability of the silyl protecting group under the reaction conditions.

In view of the unwanted silyl deprotection with **536**, we turned our attentions to the benzyl ether **537** which was formed from **492** by reaction with sodium hydride and benzyl bromide. Treatment of **537** with NBS gave a mixture of two inseparable bromohydrins (presumed to **544** and **545**) in reasonable (56%) yield. This mixture was treated with ^{t}BuOK in toluene giving a 2.6:1 mixture of two inseparable epoxides in 64% overall yield, assumed to be the stereoisomers **546** and **547** (Scheme 146). Due to this poor selectivity and the inability to separate the isomers this route was taken no further.

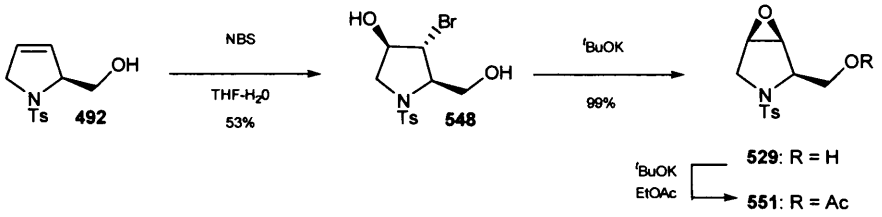
Treatment of alcohol **492** with NBS produced a complex mixture of products, which was not purified but was directly treated with base, to give, after purification by flash column chromatography, two main fractions. Whilst one fraction contained an unidentifiable mixture of compounds the other was shown to be relatively pure by ^{1}H-NMR spectroscopy. Fortunately this pure compound was crystalline and an x-ray structure was obtained (see appendix) revealing the compound to actually be the unreacted bromohydrin **548** (Scheme 146). Repetition of the reaction of **492** with NBS clearly showed that the bromohydrin **548** was the pure product and it could be isolated in moderate yield (53%) (Scheme 146). The reaction also produced a 1:1 mixture of two isomeric bromohydrins which were suspected to be regioisomers **549** and **550** (Scheme 146) as treatment of this mixture with ^{t}BuOK in toluene gave solely the *trans*-epoxide **525** (Scheme 147).



Scheme 147

With the pure bromohydrin **548** in hand, treatment with base yielded the required *syn*-epoxide **529** in good yield (99%) (Scheme 148). It was noted that after column chromatography (using ethyl acetate: petroleum ether) we sometimes saw the formation of the corresponding acetate **551** (Scheme 148). This was tested by reaction of the alcohol **529** with ethyl acetate under basic conditions to give an excellent (80%) yield of **551**. Fortunately the

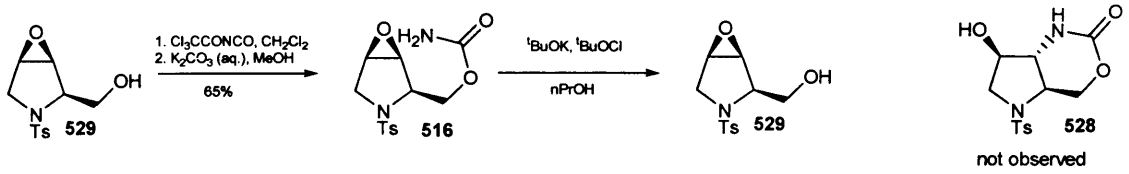
acetate **551** was crystalline and x-ray diffraction analysis confirmed the *syn* stereochemistry shown (see appendix).



Scheme 148

4.5.3 Intramolecular ring opening of the *syn*-epoxide 516

With the pure *syn*-epoxide **529** in hand the intramolecular epoxide opening was investigated (Scheme 149). The alcohol **529** was hence converted to the carbamate **516** and subjected to the established cyclisation conditions (^{t}BuOK, ^{t}BuOCl, ^{n}PrOH). The reaction mixture was initially stirred at room temperature; however after 4 hours TLC analysis confirmed only starting material was present. After heating at reflux for a further 18 hours all starting material had been consumed however only the alcohol **529** could be detected (presumably by hydrolysis) in the crude reaction mixture and there was no evidence of the target cyclic carbamate **528**.

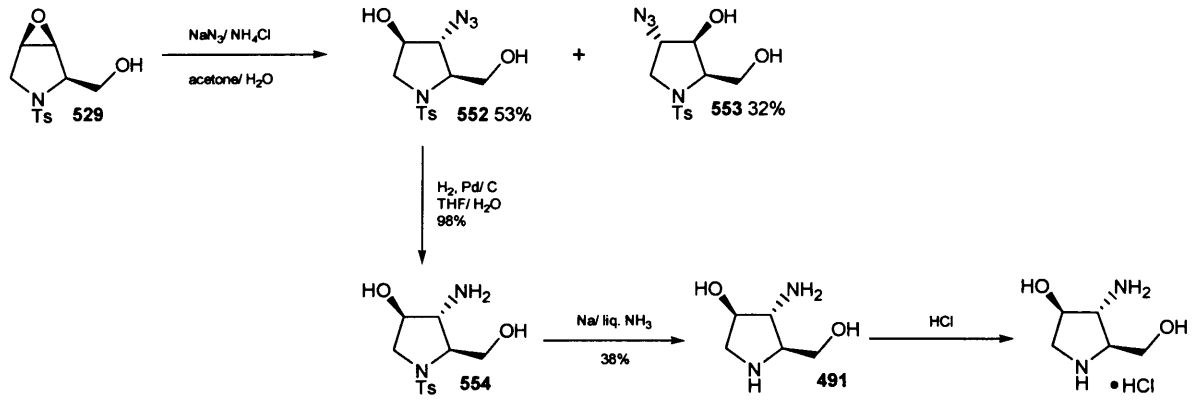


Scheme 149

Based on this result it was decided that either cyclisation was very slow and other side reactions predominated under the reaction conditions, or that the 5:6 bicycle **528** was just too strained and high in energy to be formed. Our attentions therefore turned to an intermolecular reaction.

4.5.4 Intermolecular opening of the *syn*-epoxide 529

Following the procedure reported by Schofield[143] ring opening of epoxide **529** was achieved with sodium azide and NH_4Cl in acetone/ water (Scheme 150). Analysis of the ^{1}H-NMR of the crude material from the reaction revealed that a 7:3 mixture of isomeric products had been formed. These two isomers were readily separated by chromatography to give 58% of the major isomer **552** and 32% of the minor isomer **553**. Fortunately the major isomer was crystalline and x-ray diffraction analysis revealed the compound to have arisen from nucleophilic attack at the C-3 carbon (see appendix) (Scheme 150).



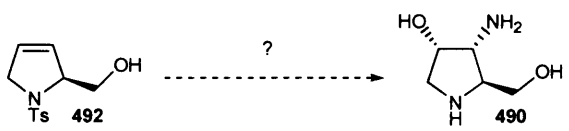
Scheme 150

This result was somewhat surprising as literature reports had suggested that nucleophilic attack at C-4 would be favoured (section 4.1.5). Thus, reports of reactions with *anti*-epoxides similar to **529**[142,143] are all highly regioselective for nucleophilic attack at C-4. The only report of the reaction of a *syn*-epoxide similar to **529** again resulted in attack at C-4 albeit with lower selectivity (C-4 : C-3 attack took place in 2:1 ratio).[143] The selectivity observed in the reaction of **529** is therefore unprecedented and is difficult to explain. Nonetheless this unexpected selectivity enabled the desired azidohydroxypyrrolidine derivative **552** to be obtained in good (53%) yield.

Reduction of the azide **552** by catalytic dehydrogenation (H_2, Pd/ C in THF and H_2O)[143] afforded the *N*-tosyl protected 3-amino-4-hydroxypyrrolidine **554** in 98% yield. Finally, removal of the tosyl group was accomplished using sodium in liquid ammonia and the resulting diamine **491** characterised as the corresponding HCl salt. Whilst derivatives of this 3-amino-4-hydroxyaminopyrrolidine regioisomer have been reported by Palmo[141] and Schofield,[143] this is the first example of the synthesis of the pyrrolidine **491**.

4.6 Studies into the synthesis of the (2*S*, 3*R*, 4*S*)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 490

4.6.1 Approach



Scheme 151

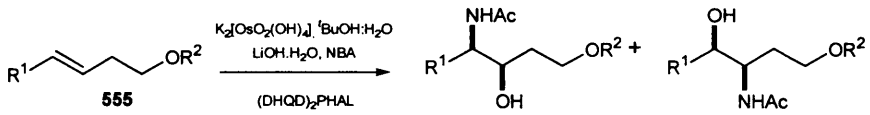
In this final isomer the C-3 amine and C-4 hydroxyl groups needed to be introduced *cis* to each other and *anti* to the C-2 substituent (Scheme 151). Based on our previous results indicating the *anti*-directing effects at the C-2 substituent we believed a Sharpless

aminohydroxylation could be employed to obtain this isomer (see the results of previous dihydroxylation reactions in chapter 3, section 3.2.4). However we anticipated possible problems in control of regioselectivity.

4.6.2 Investigations into non-tethered aminohydroxylation reactions

4.6.2.1 Background

As discussed in section 4.3.2, control of regioselectivity in aminohydroxylation reactions is very difficult to achieve when using unsymmetrical olefins. The problem of regioselectivity is very complex and consequently many investigations have been undertaken in an attempt to understand the important factors in this area.[146] One such investigation by Janda and co-workers[164] describes a substrate based approach. Substrates were synthesised in order to investigate how substrate-catalyst shape, steric and electronic factors influenced regioselectivity. Their results indicated that with *O*-protected homoallylic alcohols *e.g.* **555** (using *N*-bromacetamide as a nitrogen source) the nitrogen prefers to add to the less substituted and more electrophilic end of the alkene (Scheme 152, entry 1).



1. R^1= H R^2= TBDPS	20	:	1
2. R^1= H R^2=PMP	1.2	:	1
3. R^1= Me R^2=PMP	1	:	3.2
4. R^1= Et R^2= PMP	1	:	3.5

Scheme 152

However, the nature of the alcohol protecting group can reverse this selectivity, with aromatic protecting groups (*e.g.* PMP) favouring amination at the more substituted carbon (Scheme 152, entry 2). This is thought to be due to an aryl-aryl interaction occurring between the substrate and the cleft of the catalyst leading to the proposed binding mode depicted in Figure 43. This effect becomes even more prominent with equally substituted alkenes (Scheme 152, entries 3 and 4).

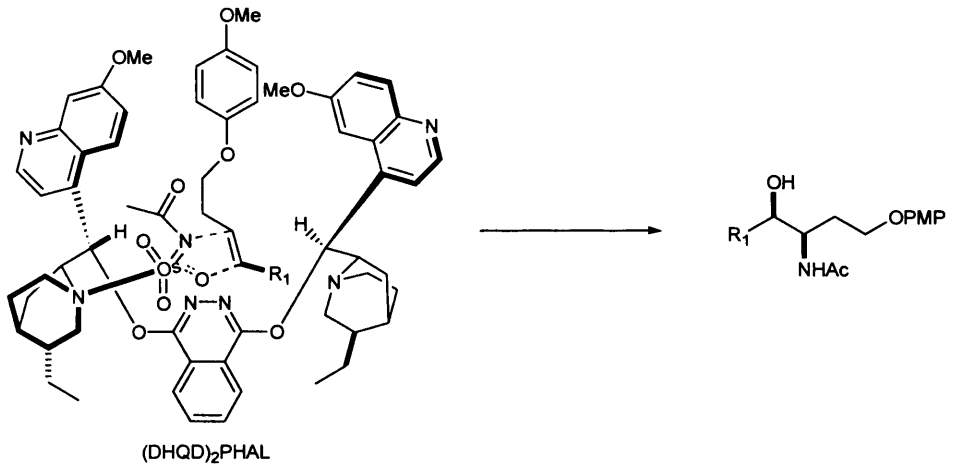
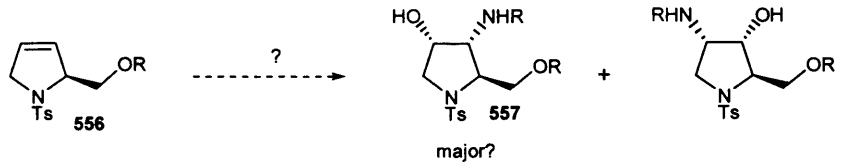


Figure 43

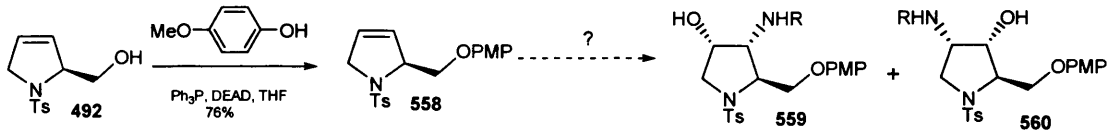
On the basis of this report we postulated that some control could be exerted over the regioselectivity of oxyamination with the alkene **556** to allow access to the required aminoalcohol **557** (Scheme 153).



Scheme 153

4.6.2.2 Synthesis and aminohydroxylation of the PMP ether 558

In view of the good control of regioselectivity obtained by Janda with aromatic protecting groups, we decided to synthesise the PMP derivative **558** (Scheme 154). This was achieved using a Mitsonobu reaction of the alcohol **492** with 4-methoxyphenol following a procedure by Corey and co-workers.[165] With the PMP ether **558** in hand the aminohydroxylation reaction (**558**→**559** and/ or **560**) was attempted.



Scheme 154

A detailed literature search indicated that there are many variables involved in the experimental procedure for aminohydroxylation, and no definitive conditions are reported. Therefore a range of conditions were tested for the reaction of **558** and the results are summarised in Table 2.

Firstly, the procedure reported by Janda[164] was employed (Table 2, entry 1). In this initial attempt no ligand was used as we wanted to see if there was any inherent control of diastereoselectivity (*i.e.* reaction *anti* to the C-2 substituent as in the dihydroxylation reactions discussed in chapter 3). Unfortunately, this reaction was unsuccessful and starting material

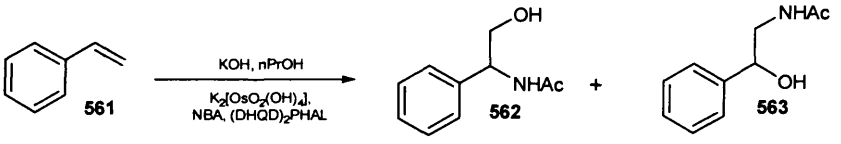
558 was recovered. It has been proposed[146] the Cinchona based ligands, as well as controlling enantioselectivity, also accelerate aminohydroxylation reactions. Therefore the NBA-mediated reaction was repeated (Table 2, entry 2) with the addition of $(DHQD)_2PHAL$ to increase reactivity but unfortunately only starting material was recovered again. At this point we were unclear whether the reaction was unsuccessful due to the nature of the substrate, the reaction conditions or the quality of the reagents.

Entry	Substrate	Nitrogen source	Base	Solvent	Ligand	Outcome of reaction
1	**558**	NBA	LiOH	^{t}BuOH	-	recovered **558**
2	**558**	NBA	LiOH	^{t}BuOH	$(DHQD)_2$ PHAL	recovered **558**
3	Styrene **561**	NBA	KOH	^{n}PrOH	$(DHQD)_2$ PHAL	1:1 ratio of aminoalcohols **562** and **563**
4	**558**	NBA	KOH	^{n}PrOH	$(DHQD)_2$ PHAL	recovered **558**
5	**558**	NBA	KOH	CH_3CN	$(DHQD)_2$ PHAL	recovered **558**
6	**558**	Chloramine T-trihydrate	-	^{t}BuOH	$(DHQD)_2$ PHAL	dihydroxylation (71%)

All reactions were undertaken using $K_2[OsO_2(OH)_4]$ as oxidising agent

Table 2

To try and eliminate two of these factors a control reaction using styrene **561** was undertaken. This was achieved following a procedure reported by Sharpless and co-workers[166] using KOH and ^{n}PrOH (Table 2, entry 3). Pleasingly, this reaction was successful and the literature result was reproduced giving a 1:1 ratio of regioisomeric aminoalcohols **562** and **563** in quantitative yield (Scheme 155).

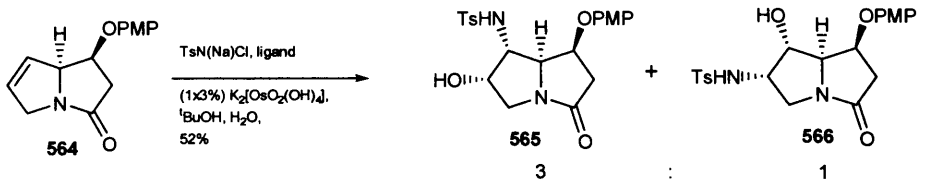


Scheme 155

In view of this success, this procedure was tested on the PMP ether **558**. Unfortunately the reaction was again unsuccessful with only starting material recovered (Table 2, entry 4). At this point it was thought that solubility maybe a factor influencing the reaction as the PMP derivative **558** was not particularly soluble in ^{n}PrOH or ^{t}BuOH. Therefore the reaction was undertaken in acetonitrile (Table 2, entry 5) but was again unsuccessful (**558** did not appear to be any more soluble).

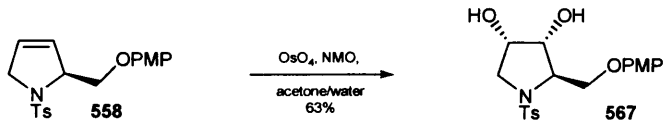
The above results demonstrated the low reactivity of the substrate **558** in the reaction and it was suspected that either the limited solubility of **558** or the low olefin reactivity (due to the electron withdrawing *N*-sulfonamide) was the cause. A subsequent literature search revealed a report by White and co-workers[167] in which a similar substrate **564** had undergone

aminohydroxylation to give the two regioisomers **565** and **566** in reasonable yields (18-52%) employing chloramine T-trihydrate as the nitrogen source (although dihydroxylation was found to be a competing reaction (29-48%)) (Scheme 156).



Scheme 156

Unfortunately, when employed with **558**, this procedure yielded only the product resulting from dihydroxylation (71%) and no aminohydroxylation was observed (Table 2, entry 6). To confirm dihydroxylation had occurred and to gauge the reactivity of the olefin (it was suspected the PMP group could be hindering or deactivating the alkene against attack) **558** was reacted with osmium tetroxide-NMO as described in chapter 3. This dihydroxylation was found to be quite sluggish (compared to the disaccharide precursors) with a reaction time of 72 hours being required to obtain 63% of the corresponding diol **567** (20% recovered starting material) (Scheme 157). This result does suggest the PMP derivative **558** is not exceptionally reactive towards dihydroxylation signifying the PMP group could be hindering attack at the olefin. With regards to the aminohydroxylation, it is suspected that this low reactivity and the poor solubility of the starting material are both contributing factors towards the poor conversions observed.



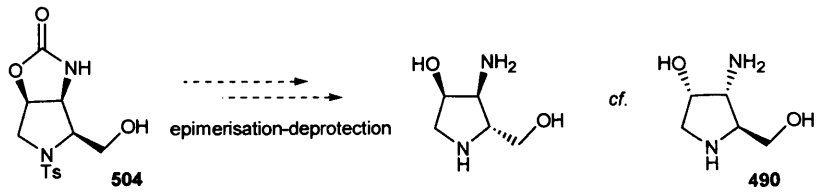
Scheme 157

4.6.3 Alternative approaches

With the limited success of the aminohydroxylation reaction other approaches were explored to access the target (2*S*, 3*R*, 4*S*) isomer **490** or its enantiomer.

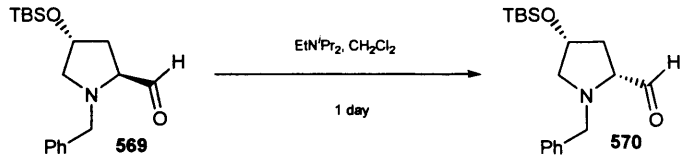
4.6.3.1 Epimerisation of the C-2 stereocentre

Epimerisation of the C-2 stereocentre of the all *syn*-carbamate **504** resulting from the tethered aminohydroxylation reaction (scheme 158) would produce the enantiomer of the target stereoisomer **490**. Conversion of the alcohol **504** to the corresponding aldehyde **568** should facilitate this process by increasing the acidity of the C-2 proton.



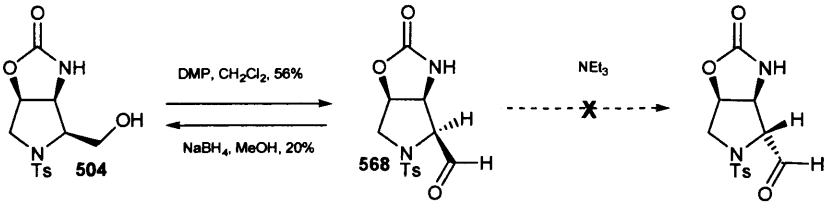
Scheme 158

This theory was also reinforced by a report by Stadau and Nubbemeyer[168] where the related aldehyde **569** underwent base catalysed (EtN^iPr_2, CH_2Cl_2) epimerisation to generate the *cis*-hydroxyproline derivative **570** (Scheme 159).



Scheme 159

Therefore the alcohol **504** was oxidised to the corresponding aldehyde **568** using Dess-Martin periodinane[169] (Scheme 160). After work-up and purification by column chromatography ^{1}H-NMR indicated the presence of a single diastereoisomer. This was presumed to be the aldehyde **568**, although to test if complete epimerisation had occurred during the oxidation a small portion of this aldehyde was reduced back to the alcohol ($NaBH_4$, MeOH).[170] Comparison of ^{1}H-NMR indicated no epimerisation had occurred as **504** was recovered from the reduction. To try and epimerise the C-2 centre, the aldehyde **568** was stirred in CH_2Cl_2 with 1.1 equivalents of NEt_3. After work up, ^{1}H-NMR analysis of the crude material indicated a complex mixture of products had been produced suggesting numerous side reactions had occurred.



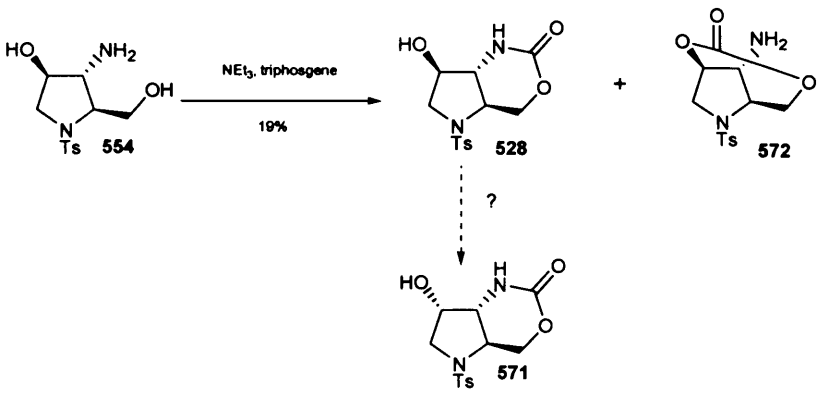
Scheme 160

To conclude, although the epimerisation of **504** was unsuccessful we have demonstrated the configurational stability of the C-2 stereocentre in the novel derivative **568**. This maybe particularly useful if this compound is to be used as a chiral building block in the synthesis of further elaborated compounds.

4.6.3.2 Inversion of the C-4 stereocentre

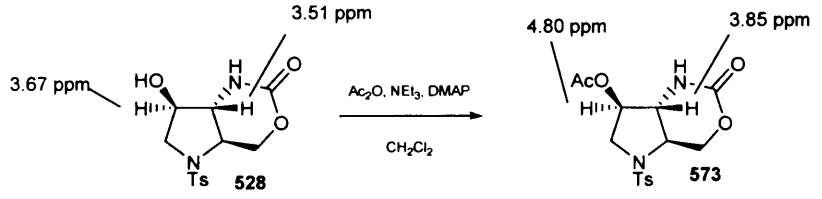
The synthesis of the target compound **490** could also be achieved by manipulating the (2*S*, 3*R*, 4*R*) aminopyrrolidine **554**. It was anticipated that if this isomer **554** could be converted to the carbamate **528** this would allow the remaining C-4 centre to be inverted (by a Mitsonobu reaction or a nucleophilic displacement) yielding the desired stereoisomer in the form of the derivative **571** (Scheme 161).

. Reaction of the pyrrolidine **554** with trisphosgene[90] at room temperature for 48 hours followed by work-up and purification yielded a compound containing a carbonyl group (indicated by mass spectrometry and ^{13}C-NMR). However, we were unable to determine whether this compound was the required carbamate **528** or the seven membered carbonate **572** (Scheme 161).



Scheme 161

Whilst we would normally predict the preferential formation of the six membered **528** over **572**, the failure of the intramolecular epoxide opening reactions with the *syn*-epoxide (*i.e.* **516→528**; section 5.3.2, Scheme 149) had led us to question the stability of the potentially strained 5:6 bicycle. Additionally a detailed analysis of the chemical shifts in the ^{1}H-NMR of the product (made in comparison with the previously synthesised carbamates **503**, **504** and **522**) failed to shed further light on the identity of the isolated material. Acylation of this unknown (Ac_2O, NEt_3, DMAP (cat), CH_2Cl_2) gave the corresponding acetylated product and ^{1}H-NMR analysis of this material indicated significant changes in the chemical shifts for the C-3 and C-4 hydrogen atoms. Thus, the C-3 hydrogen was shifted by 0.34 ppm (3.51→3.85 ppm) on acetylation where as the C-4 hydrogen was shifted by 1.13 ppm (3.67→4.80 ppm) (Scheme 162).



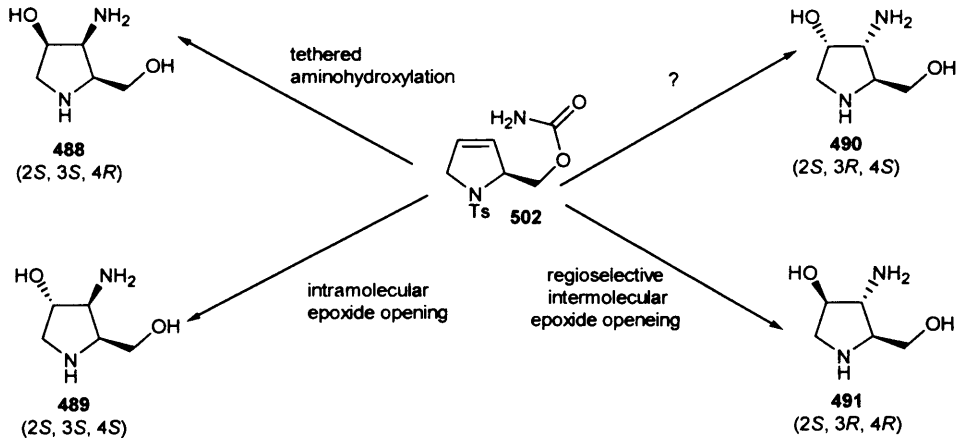
Scheme 162

These initial results are in agreement with formation of the target 5:6 carbamate **528** and subsequent acetylation of the C-4 hydroxyl group yielding **573**. Unfortunately these studies were undertaken on a very small scale and so adequate material was not available to investigate the subsequent inversion of the C-4 stereocentre of **528**.

To summarise, preliminary experiments have shown that selective protection of the 1,3-amino alcohol to give the 5:6 bicycle **528** incorporating a *trans* ring junction can be achieved. To put this in context with previous attempts to cyclise the carbamate in the *syn*-epoxide **516**, (section 5.3.2) we can say that the product **528** is stable and therefore presumably cyclisation is not fast and side-reactions predominate under these conditions. Nevertheless, the successful formation of **528** has left the path open for inversion of the C-4 stereocentre and subsequent access of the target stereoisomer **490**.

4.7 Conclusion

In conclusion a synthesis of the key dehydroproline carbamate **502** has been achieved from *trans*-hydroxy-L-proline in 6 steps and overall good yield (38%) with minimal purification steps being required. Using this intermediate **502** the synthesis of three out of four possible stereoisomers has been accomplished using a range of synthetic chemistry (Scheme 163).



Scheme 163

Application of a tethered aminohydroxylation reaction on the homoallylic *N*-heterocyclic derivative **502** allowed access to the completely novel all *syn*-isomer **488**. Utilisation of a regio- and stereoselective intramolecular epoxide opening enabled the synthesis of the completely novel isomer **489** and the use of an unprecedented regioselective intermolecular epoxide opening provided a route to the isomer **491**, of which derivatives have been synthesised, but reports are limited. Lastly, an alternative synthetic route to the final isomer **490** has been established and in the process, the configurational stability of the aldehyde **568** was demonstrated, which could be further elaborated to produce analogues if

necessary, and future work could address this. Finally, we have also demonstrated selective deprotection protocols on the protected intermediates of **488**, **489** and **491**, providing a useful source of synthetic intermediates.

With reference to the biological activities of these compounds, their potential for displaying activity is significant and the final pyrrolidines **488** and **489** and numerous derivatives have been tested (in collaboration) against a range of glycosidase enzymes and the results are discussed in chapter 5

Chapter 5

Biological Testing and Conclusions

5.0 Biological Testing and Conclusions

5.1 Introduction

The final compounds whose syntheses have been discussed in the preceding chapters have been tested (in collaboration with Dr R. Nash at the Institute of Grassland and Environmental research, Aberystwyth,* see appendix for testing protocol) against 13 commercially available glycosidases.

5.2 Azadisaccharide results

The results obtained with the azadisaccharide compounds **269, 270, 271, 399, 400, 402, 403** (Figure 44) are shown below in Table 3. In an effort to identify if inhibition was due solely to the azasugar component of the azadisaccharide and not the attached aglycon, compounds **574** and **575** were also synthesised and tested against the same enzymes (Figure 44).

Enzyme	Compound	**269**	**270**	**271**	**400**	**399**	**402**	**403**	**574**	**575**
	Concentration in assay (μM)	442	442	227	442	442	442	443	904	458
α-D-glucosidase (yeast)		**20.6**	2.6	2.7	5.2	11.7	14.2	6.5	**95.2**	8.3
α-D-glucosidase (bacillus)		3.8	1.6	-2.7	-0.5	10.3	3.4	2.7	**24.0**	**26.7**
α-D-glucosidase (rice)		-7.5	-5.0	-5.6	-10.7	-2.5	-4.9	-6.2	**83.3**	9.3
β-D-glucosidase		0.2	5.0	4.8	2.1	-10.8	**32.4**	**30.3**	**24.3**	**36.9**
α-D-galactosidase		2.7	0.8	3.1	-0.5	4.7	5.1	2.6	7.7	0.0
β-galactosidase		-1.9	**29.7**	8.4	NT	NT	**36.2**	**55.3**	**24.8**	**27.6**
α-L-fucosidase		-2.5	5.5	-11.5	9.4	16.2	-8.5	-1.8	13.6	-0.9
α-D-mannosidase		9.7	**15.6**	-2.1	1.8	1.1	**29.3**	0.7	13.4	0.8
Naringinase		5.2	-4.4	0.2	-5.8	**40.6**	2.0	**17.5**	10.8	-4.3
N-acetyl-β-D-glucosaminidase (bovine kidney)		-0.3	8.9	**12.9**	6.2	12.0	-9.0	-1.2	**93.0**	-4.7
N-acetyl-β-D-glucosaminidase (Jack bean)		9.2	8.0	-0.5	-2.8	-4.9	9.5	9.7	14.3	3.6
N-acetyl-β-D-hexosaminidase		-1.1	6.9	-2.1	3.2	-0.4	-3.0	-3.1	5.7	-1.9
Amyloglucosidase		-1.9	0.2	8.7	-10.0	1.6	-12.1	-0.9	5.8	-2.9

Inhibition values are in %; NT = not tested; negative values indicate no inhibition

Table 3

* Actual experiments undertaken by E. Evinson at the Institute of Grassland and Environmental Research, Aberystwyth

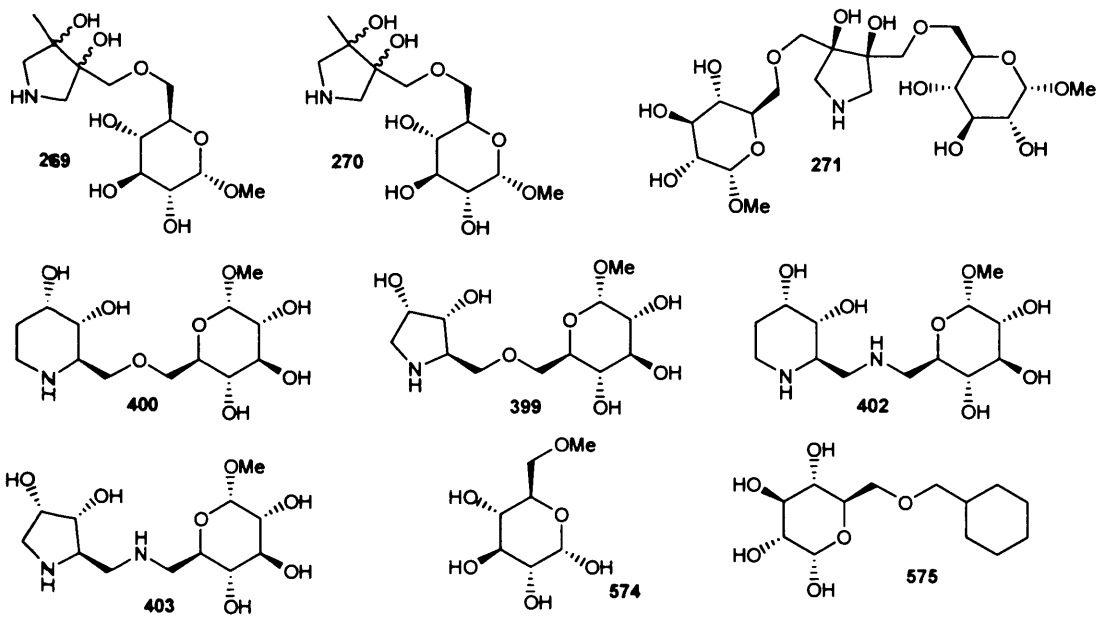


Figure 44

5.2.1 Analysis of the data

Unfortunately, due to time limitations, only values for percentage inhibition were determined against each enzyme for each of the azadisaccharide compounds. Hence the data in Table 3 is preliminary and further investigations would need to be undertaken to standardise these values (*i.e.* determination of IC_{50} and K_i). However, although we cannot compare the activities of the compounds accurately, (in Table 3 and to those of related compounds), these values do enable us to identify any interesting inhibitors and gauge a rough estimate of selectivity and potency.

From Table 3 we can see that the (2→6) linked compounds **269**, **270** and **271**, display no real significant inhibition of any of the enzymes examined in this study. Testing of these compounds **269**, **270** and **271** was carried out at 0.44mM, 0.44mM and 0.227mM respectively and at these concentrations no inhibition near 50% was observed thus, signifying the IC_{50} values would be greater than these concentrations. Interestingly the two diastereoisomers did seem to display some selective inhibition albeit at low levels. Thus, whereas **269** displayed weak inhibition (*ca.* 20%) of α-D-glucosidase from yeast, **270** was inactive against this enzyme and instead inhibited β-galactosidase at a similar level. This suggests these compounds are poor transition-state/substrate analogues, which could be attributed to the fact these compounds are linked at a hydroxylated carbon (rarely observed in nature).

As can be seen from Table 3, the results obtained for the (1→6) linked *O*-azadisaccharides **399** and **400** are a little more interesting. Disappointingly, the piperidine compound **400** exhibited no significant inhibition of any of the enzymes. This is somewhat surprising as the naturally occurring piperidine containing azadisaccharides **35**→**38** (Figure

45, also discussed previously in chapter 1, section 1.2.4) have been found to be potent selective inhibitors of α-glucosidases displaying IC_{50} values in the μM range (**35** = 0.95μM, **36** = 2.3μM, **37** = 30μM and **38** = 0.54mM)[16], and the synthetic azadisaccharide MDL 25637 **34** (discussed in Chapter 1, section 1.2.4) has been determined as an excellent inhibitor of intestinal glycosidases (**34** inhibits trehalase, isomaltase, sucrase, glucoamylase and maltase all with IC_{50} values between 0.25-9.6μM)[19] (Figure 45). The differences in inhibition could be ascribed to the fact that the azadisaccharide **400** contains the considerably less hydroxylated 3-*epi*-fagomine azasugar **333** compared to the nojirimycin analogue in **34** and 1-deoxynojirimycin in compounds **35**→**38**. Additionally, in our compound **400**, the azasugar is linked to C-6 of the aglycon where as in the biologically active compounds the linkage is always at C-1.

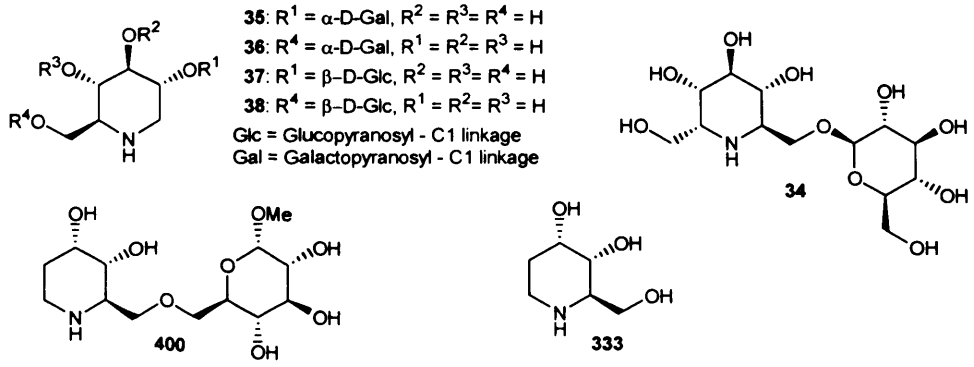


Figure 45

Comparison of the results obtained for **400** to those reported for the parent piperidine **333** seems to indicate that the attachment of an aglycon to 3-*epi*-fagomine abolishes any biological activity. Although **333** has only been reported as a weak inhibitor of α- and β-glucosidases (rice and almond respectively) (IC_{50} = 0.12mM),[16] the percentage inhibition observed with **400** for these enzymes at 0.4mM was 5.2% and -10.7% respectively (Table 3). This suggests this enzyme either cannot tolerate the increased steric bulk of the substrate **400**, or the 2-hydroxymethylene substituent maybe important for inhibition.

Analysis of the results in Table 3 also shows that the pyrrolidine azadisaccharide **399** exhibited no significant activity against many of the enzymes, although 40.6% inhibition of naringinase was observed (at 0.44mM). Although this activity is not impressive (approximate IC_{50} ~ 0.6mM assuming linear relationship), it is significant and demonstrates selectivity for this enzyme. Naringinase is an α-Rhamnosidase and processes L-rhamnose **576**,[171] although interestingly, compounds **577** and **578** have also been found to be weak inhibitors of this enzyme with similar activity to **399** (Figure 46).[172] If the structures of **577** and **578**, are compared to the disaccharide **399,** we can see that there are some structural similarities between these compounds thus, maybe explaining the activity observed.

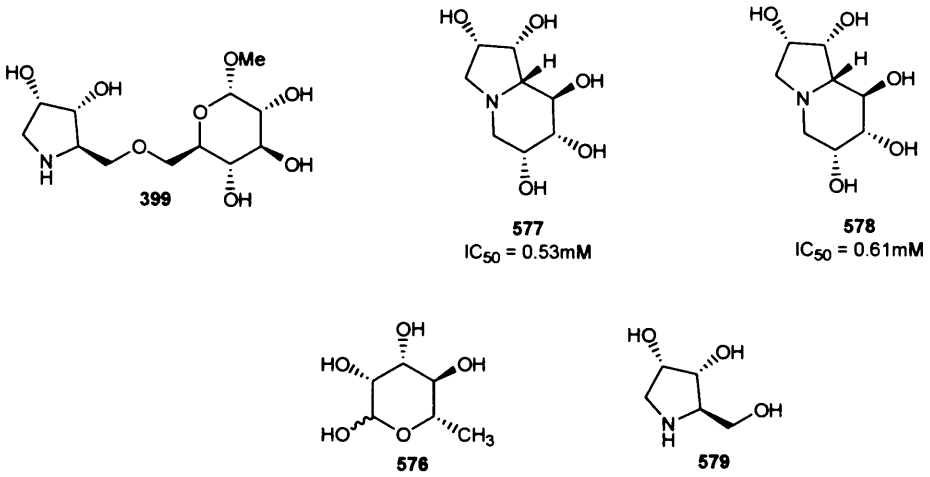


Figure 46

Again comparison of the activities observed with **399** to those reported for the parent pyrrolidine **579** (Figure 46) seems to suggest attachment of an aglycon does reduce the potency of the inhibitor. Although **579** has only been determined as a moderate inhibitor of most glycosidases (IC_{50} values in mM range),[17] attachment of the aglycon has not increased this activity as at 0.44mM (with the exception of naringinase) no significant inhibitory activity above 20% (suggesting IC_{50} values could be mM range) was observed for any of the glycosidases. However, by attaching an aglycon to **579** *i.e.* compound **399** a mM inhibitor of naringinase has been generated and is selective for this enzyme over others. Thus, **399** is more selective than **579** and we are very pleased with this preliminary finding.

Although slightly impure (~90% pure) samples of the diamines **402** and **403** (Figure 47) were tested, the results shown in Table 3 indicate that these compounds do exhibit some inhibitory activity.

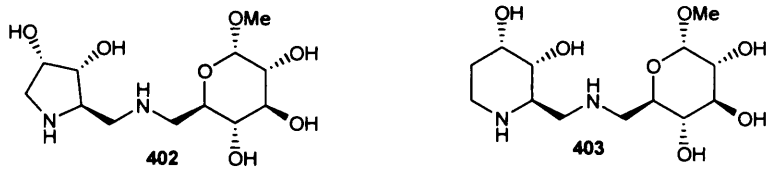


Figure 47

Interestingly, both **402** and **403** inhibited β-glucosidase with 32.4% and 30.3% inhibition respectively (at 0.44mM) and β-galactosidase with 36.2% and 55.3% inhibition respectively (at 0.44mM, *i.e.* IC_{50} < 0.46 mM for **403**). Again this inhibition is only modest, however due to the fact both **402** and **403** show increased inhibition for the same enzymes, seems to suggest some selectivity. However, we are aware that compounds **574** and **575** also exhibit similar activities towards these enzymes, hence the observed inhibition could only be due to the aglycon component of these inhibitors. Additionally because the tested samples were not completely pure, we cannot be confident that the activity observed has arisen solely from the

diamines **402** and **403**. Therefore accurate comparisons of these results to those reported for similar compounds can only be tentatively made at this point.

5.2.2 Summary

In general we have found the azadisaccharides **269**, **270**, **271**, **399**, **400**, **402** and **403**, to be not significantly active against 13 commercially available glycosidases; nonetheless some moderate inhibitors have been identified and perhaps more importantly some selective inhibition has been observed.

Comparison of our preliminary results to those reported for similar compounds seems to suggest that at least three hydroxyl groups on the azasugar ring (especially in piperidines) and attachment of the azasugar at position C-1 could possibly be important factors needed for activity. Our results also indicate that any biological activity exhibited by the azasugars **333** and **579** is diminished when the aglycon **273** is attached. Nonetheless, we have found the pyrrolidine disaccharide **399** to be a moderate selective inhibitor of naringinase and **402** and **403** to have moderate inhibitory activities for β-glucosidase and β-galactosidase (although the purity of **402** and **403** has to be taken into account).

Compounds **574** and **575** were synthesised and tested to enable us to make accurate conclusions regarding any potential activities observed for the tested azadisaccharides. We wanted to establish if any activity was due to the azasugar component of the disaccharide and not just the aglycon. Pleasingly, because compounds **574** and **575** did not show significant activity towards naringinase we can presume the presence of the azasugar ring is a considerable factor responsible for the activity displayed by **399**. However, as discussed above, because **574** and **575** displayed moderate activities towards β-glucosidase and β-galactosidase, accurate conclusions with regards to the activities of **402** and **403** could not be drawn.

5.3 Aminopyrrolidine results

The results obtained with the aminopyrrolidine compounds **488** and **489** are shown below in Table 4. Unfortunately due to the synthesis of compound **491** only being achieved towards the end of this project, time did not permit this compound to be tested. Also, to evaluate the importance of the hydroxyl and amino groups on activity, intermediates **504**, **522**, **513**, **514** and **526** were also tested.

Enzyme	Compound	489	488	513	514	522	526	504
	Concentration in assay (μM)	1082	1082	499	904	458	499	458
α-D-glucosidase (yeast)		**16.2**	**21.2**	5.8	10.7	-1.0	-5.8	**23.1**
α-D-glucosidase (bacillus)		9.6	8.2	-8.6	0.5	3.9	-8.9	-7.8
α-D-glucosidase (rice)		-2.1	3.9	4.0	-5.3	-9.3	-7.3	-21.0
β-D-glucosidase		0.6	11.8	-6.3	-8.1	9.6	7.6	-3.8
α-D-galactosidase		6.5	**36.3**	3.9	4.8	1.3	2.7	0.7
β-galactosidase		**51.7**	**56.1**	0.5	2.7	1.0	**20.7**	3.8
α-L-fucosidase		-5.6	-1.9	9.1	-2.1	11.5	7.7	17.8
α-D-mannosidase		2.6	-4.9	8.3	14.5	-3.6	4.0	-2.5
Naringinase		-0.6	-1.7	-3.7	4.4	-6.6	1.0	2.7
N-acetyl-β-D-glucosaminidase (bovine kidney)		-14.9	-13.7	5.5	11.0	6.6	-0.6	12.8
N-acetyl-β-D-glucosaminidase (Jack bean)		8.6	.114	7.9	-2.0	0.5	2.8	-4.9
N-acetyl-β-D-hexosaminidase		-2.3	-0.9	5.3	8.4	3.5	2.9	2.2
Amyloglucosidase		-1.6	-2.0	4.7	-9.3	-6.2	-0.7	-3.2

Inhibition values are in %; negative values indicate no inhibition

Table 4

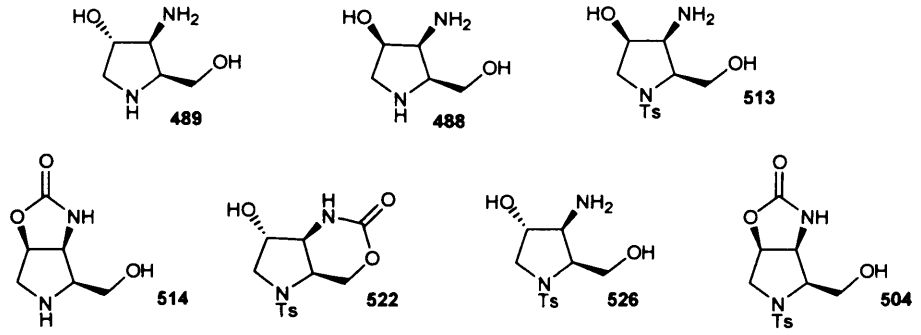


Figure 48

5.3.1 Analysis of results

Again, due to time restrictions, only percentage inhibition values were determined against each enzyme for each of the aminopyrrolidines. Therefore, the data in Table 4 is also preliminary and further investigations would again need to be undertaken to standardise these values (*i.e.* determination of IC_{50} and K_i). However, by comparing these results to those reported for the corresponding hydroxylated compounds, we can investigate how the replacement of a hydroxyl group by an amino group at C-3 in a pyrrolidine ring affects the biological activity of these compounds.

From Table 4 it can be seen, that the fully deprotected aminopyrrolidines **489** and **488** showed moderate inhibition of β-galactosidase (51.7% and 56.1% at 1.08mM respectively)

and **488** also showed slight inhibition of α-galactosidase (36.3% inhibition at 1.08mM). If these results are compared to those for the hydroxylated equivalents of **489** and **488** *i.e.* **580** and **581** we can see that the replacement of a hydroxyl group with an amino group at C-3 has a marked affect on the inhibitory activities displayed by these compounds (Figure 49).

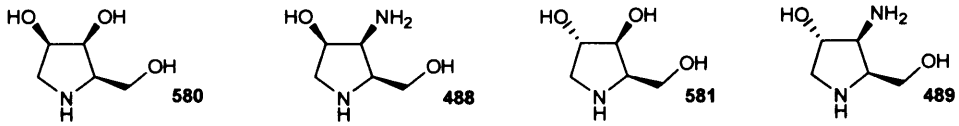


Figure 49

Thus, although only demonstrating moderate activity against most glycosidases (IC_{50} values 0.1-0.4mM), the triol **580** has been identified as a significant inhibitor of α-galactosidase (*asp. niger*) (IC_{50} = 0.2μM).[173] Interestingly although the aminopyrrolidine **488** has been shown to selectively inhibit α- and β-galactosidases, the strength of inhibition is very modest (IC_{50} ~1mM) and considerably less than observed for **580**, thus indicating the C-3-hydroxyl group on the pyrrolidine ring is important for the activity of **580**. In contrast the triol **581** has been identified as a moderate inhibitor of almond emulsion β-glucosidase (K_i = 7.1mM) and a weak non-specific inhibitor of various other glucosidases.[174, 175] In replacing the 3-hydroxyl in this compound *i.e.* to form the aminopyrrolidine **489**, the activity of this system has not increased, although in view of the minimal data reported for this compound, we can not accurately determine how this functionality has affected the biological activity of this compound.

Although the percentage inhibition values exhibited by **489** and **488** against β-galactosidase are only moderate, comparing these results to those obtained for the protected intermediates **504, 513, 514, 522** and **526** demonstrates the biological importance of the hydroxyl and amino functionalities (Figure 50).

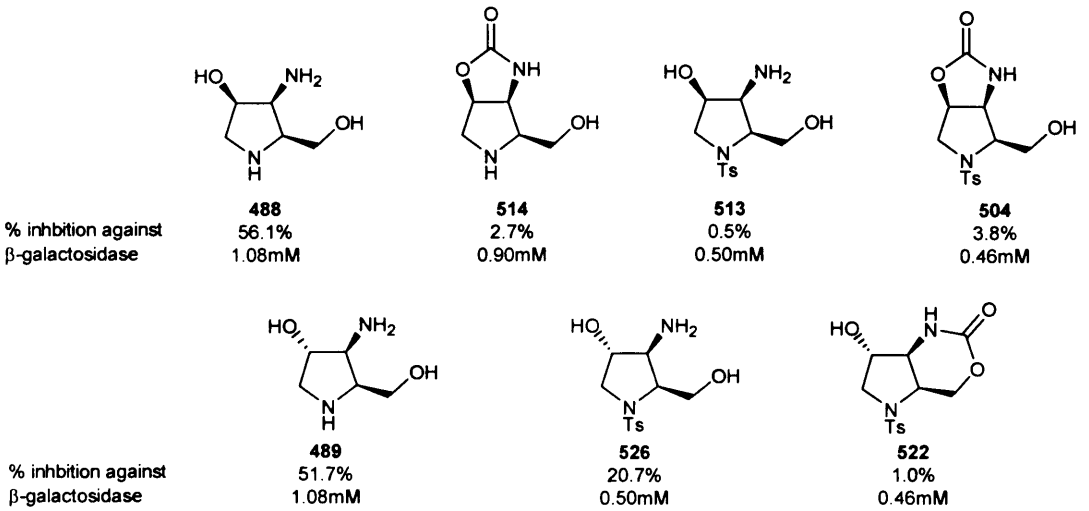


Figure 50

The (2*S*, 3*S*, 4*R*) isomer **488** exhibited 56.1% inhibition against β-galctosidase (at 1.08mM), however against the same enzyme, carbamate **514**, displayed 2.7% inhibition (at 0.90mM), the tosyl protected aminopyrrolidine **513** 0.5% inhibition (at 0.50mM) and the tosyl protected carbamate only 3.8% inhibition (at 0.46mM) (Figure 50). Similarly, the (2*S*, 3*S*, 4*S*) isomer **489**, exhibited 51.7% inhibition (at 1.08 mM), the tosyl protected compound **526** displayed 20.7% inhibition (at 0.50mM) and the tosyl protected carbamate **522** 1.0% inhibition (at 0.46mM) against β-galactosidase (Figure 50).

These results are obtained at different concentrations and hence can be only roughly compared, but they do suggest that both the secondary and primary amino as well as the hydroxyl groups are essential for the moderate activity exhibited against β-galactosidase. Interestingly, although not significant inhibitors, comparing the values observed for the tosyl protected intermediates **513** and **526** against β-galactosidase (0.5% vs. 20.7% both at 0.50mM), does reveal a large activity difference between the two compounds, thus implying the stereochemistry of the C-4 hydroxyl maybe important for activity.

5.3.2 Summary

Testing of the aminopyrrolidine compounds has revealed that although these compounds are not potent inhibitors for any of the tested glycosidases they do display some interesting activity patterns. Thus, compounds **489** and **488** were both found to be moderate inhibitors of β-galactosidase and **488** a modest inhibitor of α-galactosidase although the percentage inhibitions observed were considerably less than those for the hydroxylated compounds **580** and **581** against the same enzymes. These results suggest the presence of a hydroxyl group at C-3 of the pyrrolidine ring is important for activity in these systems.

Testing of the protected intermediates **513**, **514**, **504**, **522** and **526** did demonstrate the importance of the pyrrolidine primary and secondary amino and hydroxyl groups for the moderate inhibition observed against β-galactosidase and comparison of the tosyl protected intermediates **526** and **513** suggested the stereochemistry of the C-4 hydroxyl group maybe significant for activity.

5.4 Thesis Conclusions

In an attempt to produce glycosidase inhibitors with increased selectivity and potency, a range of novel *N*-heterocyclic compounds have been successfully synthesised.

Utilising a simple and flexible synthetic route involving a samarium diiodide pinacol coupling, the stereoselective synthesis of two (2→6) linked diastereomeric homoaza-*O*-disaccharides **269** and **270** has been achieved. Subsequent adaptation of this synthesis has

additionally allowed the (2→6) linked azatrisaccharide **271** to be accessed, demonstrating the synthesis of a completely novel class of compound (Figure 51).

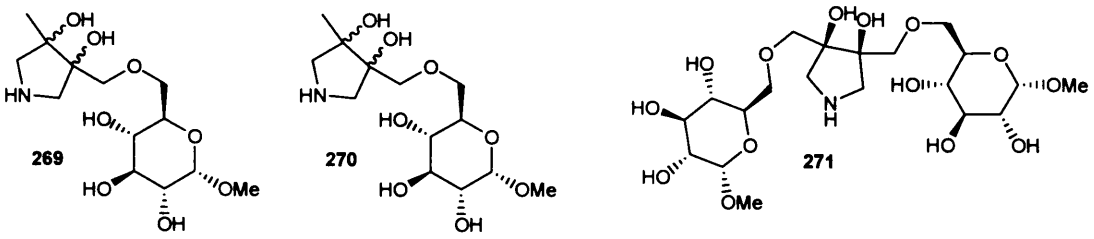


Figure 51

Complementary to this, utilising an alternative synthetic route employing RCM and stereoselective dihydroxylation, the synthesis of novel (1→6) linked azadisaccharides has been accomplished. Due to the flexibility of the synthetic route, access to both *O*- and *N*-linked pyrrolidine and piperidine azadisaccharides was enabled and four new compounds **399**, **400**, **402** and **403** were synthesised (Figure 52).

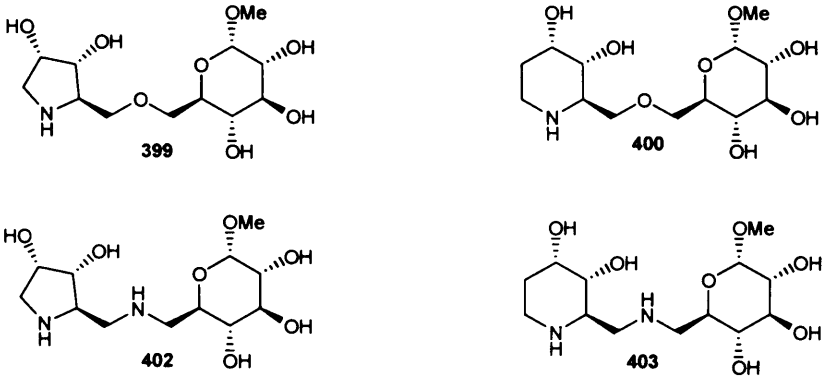


Figure 52

Subsequent screening of these disaccharides against 13 commercially available glycosidases (in collaboration) has identified **399** as a moderate selective inhibitor of naringinase and **402** and **403** to be potentially weak inhibitors of β-glucosidase and β-galactosidase.

An investigation into aminopyrrolidine synthesis was initially encouraged by the successful application of a tethered aminohydroxylation reaction on a key dehydroproline derivative **492** (initially employed in the synthesis of the (1→6) linked azadisaccharides **399**, **400**, **402** and **403**) (Figure 53). This subsequently, enabled the first synthesis of the novel (2*S*, 3*S*, 4*R*) aminopyrrolidine **488** to be achieved and led to further investigations into the synthesis of three additional isomeric aminopyrrolidines **489**, **490** and **491** using the same intermediate **492** (Figure 53).

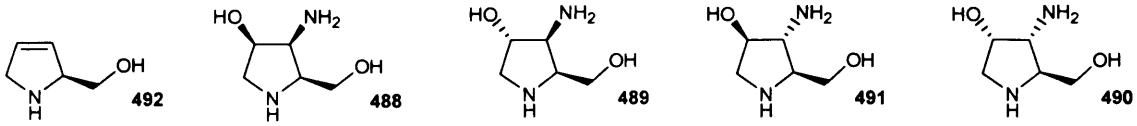


Figure 53

Pleasingly two of these isomers were also synthesised, the completely novel (2*S*, 3*S*, 4*S*) isomer **489** and the (2*S*, 3*R*, 4*R*) isomer **491** and significant progress was made in accessing the final (2*S*, 3*R*, 4*S*) isomer **490**. The biological activities of **488** and **489** were assessed by screening against 13 commercially available glycosidases, identifying both as moderate selective inhibitors of β-galactosidase.

5.5 Possible Future Studies

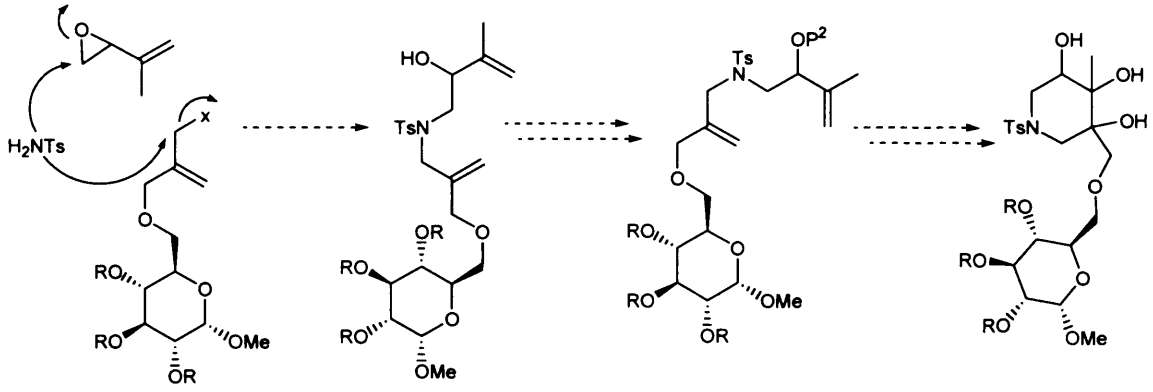
5.5.1 Disaccharide synthesis

5.5.1.1 Pinacol coupling approach

As discussed previously (chapter 2, section 2.5.8), regrettably, due to time restrictions, the absolute stereochemistries of the disaccharides **269** and **270**, were not identified and concrete proof regarding the *cis* stereochemistry of the trisaccharide **271** was not obtained. Consequently, these factors do need to be ascertained, and hopefully immediate future work in this area will involve this.

Unfortunately, preliminary results seem to suggest that none of the (2→6) linked oligosaccharide mimics display any significant biological activity against the glycosidases examined in our study. We initially suspected this lack of activity may have been because the aglycon was linked to a hydroxylated carbon on the azasugar ring. However, by comparing the structures of our compounds to those reported in the literature, it is apparent that most active azadisaccharide inhibitors contain a piperidine ring with at least three hydroxyl groups and that the azasugar is usually attached to the anomeric position of the aglycon. Therefore, to ascertain whether the lack of inhibition we have observed is due to the linkage of the disaccharide, future work could involve the adaptation of the pinacol synthetic route (*e.g.* using a route shown in Scheme 164) to enable the synthesis of increased hydroxylated piperidine disaccharides. Additionally, as this proposed route would utilise both protected and unprotected hydroxylated dicarbonyl precursors, investigations into a more diastereoselective pinacol coupling could also be carried out.

Furthermore, because of the commercial availability of the Grubbs second generation catalyst and in view of its successful application in the formation of the pinacol derived diols **266** and **267** (chapter 2, section 2.5.8) an RCM-dihydroxylation approach could additionally be explored to these target compounds.

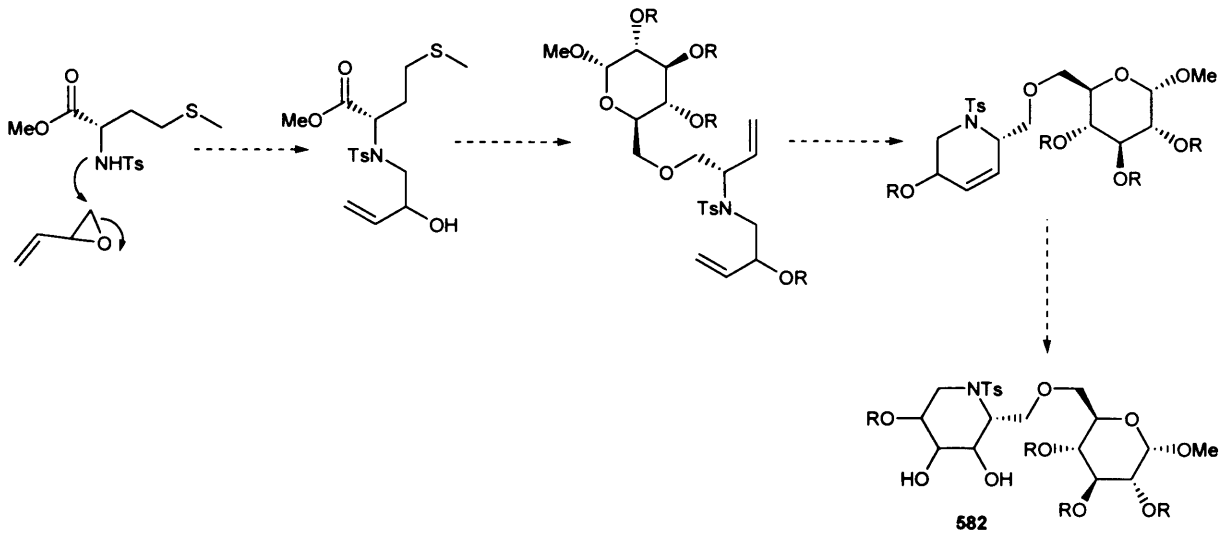


Scheme 164

5.5.1.2 The RCM-dihydroxylation approach

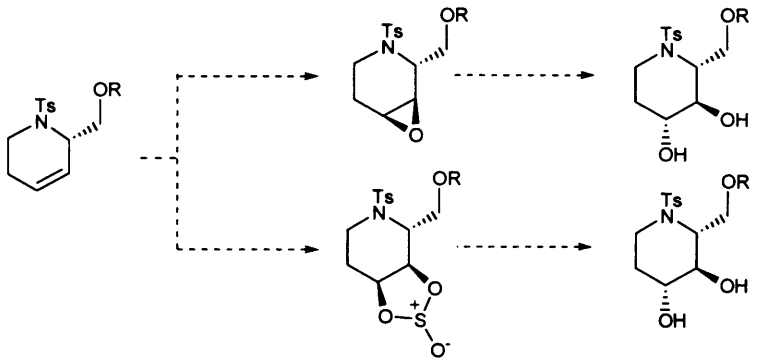
Unfortunately, because of problems encountered in the isolation of the fully deprotected amine linked azadisaccharides **402** and **403** (chapter 3, section 3.4.1), these compounds were not fully characterised and thus, these compounds will need to be resynthesised. In attempting this, the use of alternative nitrogen protecting groups could be investigated as the tosyl group was used originally, as at the outset of the project, the pinacol coupling was going to be investigated (strongly electron withdrawing groups encourage this reaction). However, because a RCM-dihydroxylation route has now been established to these compounds alternative, easier to remove, protecting groups (*e.g.* CBz or Boc) could be used to achieve the synthesis and isolation of **402** and **403**.

Interestingly, preliminary results from testing of the (1→6) linked azadisaccharides, suggests a moderate selective inhibitor of naringinase has been discovered. Using this compound **399** as a starting point, future work could involve modification of this compound in an attempt to generate a more potent inhibitor of naringinase. We believe the synthetic route we have established for the synthesis of the (1→6) compounds, would easily permit such structural variations, and allow the generation of more active compounds. For example, following the route outlined in Scheme 165 the synthesis of the deoxynojirimycin azadisaccharide **582** could be achieved.



Scheme 165

Additionally because this general synthesis utilises a RCM generating a cyclic alkene, access to the *trans*-diols could be investigated, either by the formation and opening of an epoxide, or by the synthesis and opening of a cyclic sulfite (Scheme 166).[176]



Scheme 166

5.5.2 Aminopyrrolidine synthesis

The synthesis of three novel diastereomeric aminopyrrolidines **488**, **489** and **491** has been achieved, and although a viable synthetic route to a fourth aminopyrrolidine isomer **490** was demonstrated, due to limited time allowances, scale-up and repetition of the route and further investigations into the final steps was not accomplished. Therefore, to understand the full effect of the replacement of a hydroxyl group with an amino group at C-3 in these compounds, future work could involve the synthesis of the (2*S*, 3*R*, 4*S*) isomer **490** and the testing of this compound together with the (2*S*, 3*R*, 4*R*) isomer **491**.

On the basis of the preliminary results, compounds **488** and **489** were identified to not be particularly good glycosidase inhibitors, only displaying moderate inhibition of β-galactosidase. This activity was also found to be considerably less than that by their hydroxylated equivalents for the same enzyme and for other glycosidases. However, in an

effort to generate new inhibitors and investigate the effect of substituting a hydroxyl group with an amino group, the synthesis of amino analogues of additional biological active compounds could be investigated.

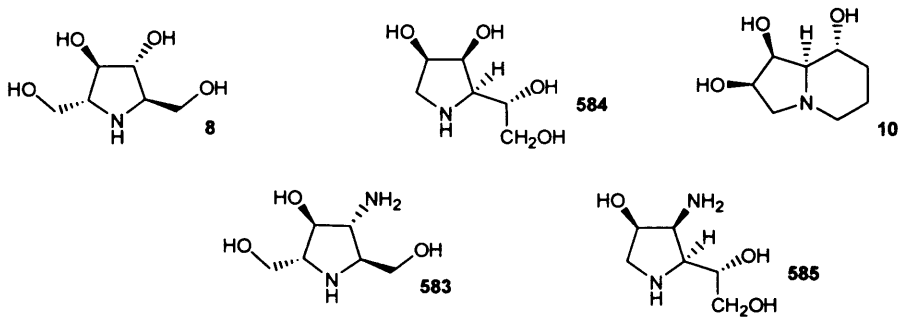
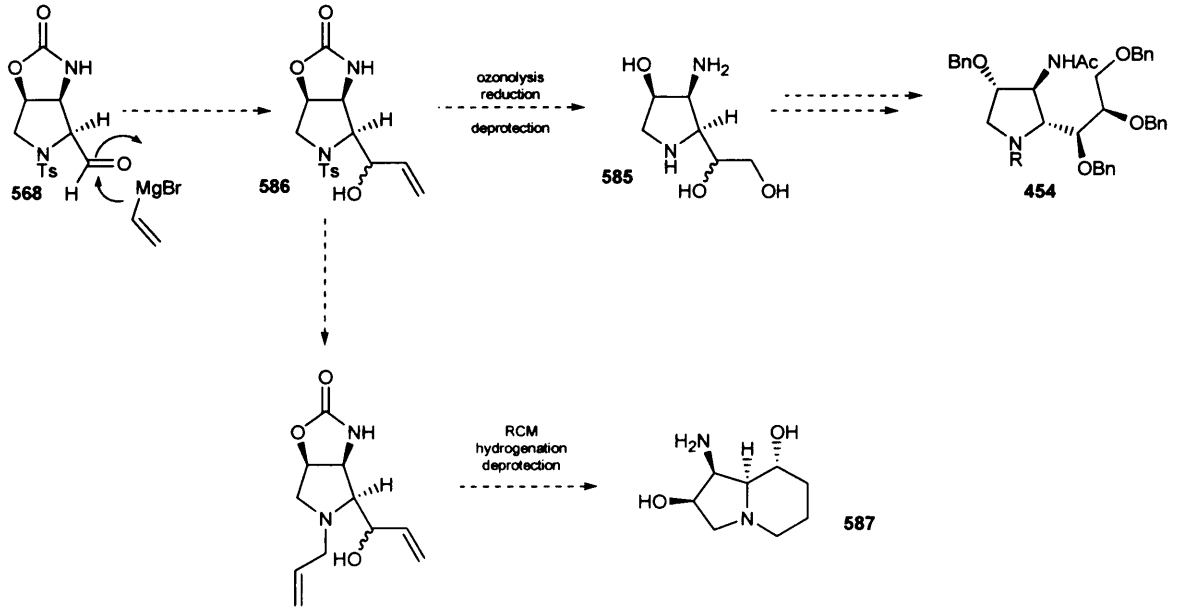


Figure 54

For example, the 2,5-dihydroxymethyl-pyrrolidine **8** (Figure 54) has been reported[174] as a particularly active glycosidase inhibitor (IC_{50} = 3.3μM and 7.8μM against α-glucosidase (yeast) and β-glucosidase (emulsion) respectively) and therefore it would be quite interesting to synthesise the C-3 amino equivalent **583**, to examine the affect on biological activity (Figure 54). Additionally the open chain swainsonine derivative **584** has been identified as a very selective α-mannosidase (Jack bean) inhibitor (IC_{50} = 0.5μM, in agreement with the naturally occurring mannosidase inhibitor swainsonine **10**),[174] and so again it would be interesting to see how the C-3 amino equivalent **585** would affect the selectivity and the potency of inhibition.

Access to the latter compound **585** could be achieved using the aldehyde **568** (synthesis already established, see chapter 4, section 4.6.3.1) (Scheme 167).



Scheme 167

Thus, treatment of the aldehyde **568** with a vinyl Grignard could enable access to the alcohol **586**. A subsequent ozonolysis and reduction could then yield diol **585**, possibly opening a pathway to the synthesis of compounds such as **454**, which are known sialidase inhibitors.[140] Alternatively, if alcohol **586** underwent a tosyl deprotection followed by alkylation, a RCM followed by double bond reduction and carbamate deprotection, the synthesis of the C-3 amino swainsonine derivative **587** could be realised, whose biological activity would be very interesting to investigate.

Chapter 6
Experimental

6.0 Experimental

General

NMR spectra were recorded on a Bruker ARX 250 (^{1}H, 250.13 MHz; ^{13}C, 62.90 MHz) spectrometer, a Bruker DPX 300 (^{1}H, 300.13 MHz; ^{13}C, 75.47 MHz) spectrometer and a Bruker DRX 400 (^{1}H, 400.13 MHz; ^{13}C, 100.62 MHz) spectrometer. Chemical shifts for ^{1}H and ^{13}C NMR spectra are expressed in ppm on the δ scale relative to an internal standard (TMS) or residual solvent and the following abbreviations are used (app-apparent, s-singlet; d-doublet; t-triplet; q-quartet; m-multiplet; dd; doublet of doublets; ddd; doublet of doublet of doublets; h-heptet; br-broad; Ar-aryl; Ph-phenyl; *J*-coupling constant (Hz). Assignments of chemical shifts for ^{13}C resonances was assisted by DEPT.

Electron Impact (EI) mass spectra were recorded on Kratos Concept 1H. Chemical Ionization (CI) mass spectra were recorded on a Kratos Concept 1H using ammonia as the reagent gas. Fast Atom Bombardment (FAB) mass spectra were recorded on a Kratos Concept 1H using xenon and *m*-nitrobenzyl alcohol as the matrix. Electrospray (ES) mass spectra were recorded on a Micromass Quattro LC spectrometer. High Resolution Mass Spectrometry (HRMS) was measured on a Kratos Concept 1H spectrometer using peak matching to stable reference peaks, depending on the technique used.

Flash column chromatography was performed using silica gel (Sorbsil C-60 silica gel 40-60M). Column fractions were collected and monitored by Thin Layer Chromatography (TLC) and carried out on precoated aluminium backed silica gel plates supplied by E. Merck, A.G. Darmstadt, Germany (Silica gel 60 F_{254}, thickness 0.2 mm) or on precoated glass plates supplied by Merck (Silica gel 60 F_{254}). The compounds were visualized using UV light, potassium permanganate, *p*-anisaldehyde, 2,4-dinitrophenolhydrazine (DNP) or phosphomolybdic acid (PMA).

Microwave experiments were carried on a CEM Discover (variable power, max. 300W). IR spectra of crystalline compounds and liquids were recorded as solutions in dichloromethane or chloroform and of other liquids as thin films using a Perkin Elmer 1600 series FT-IR spectrometer and using a Perkin Elmer FT-IR with ATR attachment (solid-phase only). IR spectra were measured in units of cm^{-1} and the following abbreviations used: s-strong, m-medium and w-weak.

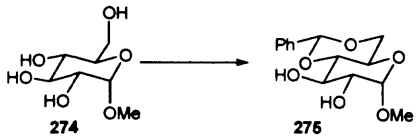
Light petroleum refers to the fraction boiling between 40-60 °C. Tetrahydrofuran (THF) was dried by refluxing with benzophenone over sodium wire under an atmosphere of nitrogen, and was distilled and collected by syringe as required. Dichloromethane, toluene and acetonitrile were dried by refluxing with calcium hydride. All chemicals (and other

solvents) were used as received without any further purification. Samples were routinely freed from traces of solvent using an oil pump (~ 2mm Hg) before carrying out spectroscopic measurements.

A Pinacol coupling approach to (2→6)-*O*-linked sugar mimics

6.1 Synthesis of the protected sugar moiety 273

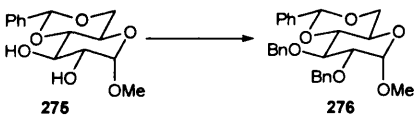
6.1.1 Methyl-[O^4, O^6-((*R*)-benzylidene)-α-D-glucopyranoside] 275[69]



274 275

Methyl-α-D-glucopyranoside **274** (51.0 g, 260 mmol), benzaldehyde dimethylacetal (39.2 g, 38.6 ml, 260 mmol), DMF (300 ml) and *p*-toluene sulphonic acid monohydrate (0.15 g, 0.8 mmol) were placed in a flask and the mixture refluxed under vacuum (20 mmHg, 65 °C) for 3 h. After this time DMF was removed under reduced pressure and the resulting white solid dispersed in a 1% solution of sodium hydrogen carbonate (500 ml) on a water bath whilst heating. After cooling the product was filtered, washed with water (250 ml) and dried under vacuum and over phosphorous pentoxide overnight. The resulting white solid was recrystallised from a mixture of isopropanol (132.5 ml) and pyridine (2.25 ml) to yield the *benzylidene acetal* **275** (51.14 g, 69%) as a white solid. Mp: 168-169 °C (from isopropanol-pyridine) (Lit.[177],168-169 °C); $[\alpha]^{20}_D$: +92.3 (c=1.81 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3053s, 2985s, 1421m, 1265s, 1067w, 1028w, 895m, 760s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.46 (3H, s, OMe), 3.49 (1H, app t obscured, *J*9.2, H-4), 3.62 (1H, dd, *J*9.2, 3.9, H-2), 3.74 (1H, app t, *J*10.0, H-6_{ax}), 3.77-3.86 (1H, m, H-6_{eq}), 3.93 (1H, app t, *J*9.2, H-3), 4.20-4.36 (1H, m, H-5), 4.80 (1H, d, *J*3.9, H-1), 5.53 (1H, s, H-7), 7.34-7.42 (3H, m, 3xPh-H), 7.46-7.51 (2H, m, 2xPh-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 55.52 (CH_3), 62.34 (CH), 68.91 (CH_2), 71.67 (CH), 72.83 (CH), 80.9 (CH), 99.77 (CH), 101.92 (CH), 126.30 (2 × CH), 128.30 (2 × CH), 129.22 (CH), 137.03 (C); MS (ES) m/z: 283 ($M+H^+$, 40%), 251 (M-OMe); Accurate mass (FAB): Found 283.1181 ($M+H^+$ $C_{14}H_{19}O_6$ requires 283.1181).

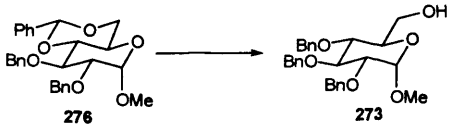
6.1.2 Methyl-[O^2, O^3-dibenzyl-O^4, O^6-((*R*) -benzylidene)-α-D-glucopyranoside] 276[70]



275 276

Sodium hydride (95%, 3.23 g, 126 mmol) was added to a solution of acetal **275** (8.0 g, 28 mmol) in DMF (150 ml) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 0.5 h. Benzyl bromide (14.5 g, 10.1 ml, 85.0 mmol) was added dropwise to the stirring solution and the mixture was allowed to stir for 26 h before being quenched with water. The organic product was extracted with ethyl acetate (3x50 ml). The organic layers were combined, dried ($MgSO_4$) and solvent was removed under reduced pressure to give a crude off-white solid. This material was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to yield the *fully protected sugar* **276** (10.6 g, 81%) as a white solid. Mp: 96-97 °C (from petroleum 40-60) (Lit.[70], 96 °C (from hexane)); $[\alpha]^{20}_D$: +12.2 (c=1.80 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3053m, 2985w, 2928w, 1496s, 1421s, 1262s, 1088m, 1053m, 895m, 747s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.44 (3H, s, OMe), 3.59 (1H, dd obscured, *J*9.2, 3.7, H-2), 3.63 (1H, app t obscured, *J*9.2, H-4), 3.74 (1H, app t, *J*9.2, H-6_{ax}), 3.86 (1H, app td, *J*9.2, 4.3, H-5), 4.08 (1H, app t, *J*9.2, H-3), 4.30 (1H, dd, *J*9.4, 4.3, H-6_{eq}), 4.63 (1H, d, *J*3.7, H-1), 4.73 (1H, d, *J*12.0, PhC*H*HO), 4.86 (1H, d, *J*11.2, PhCH*H*O), 4.89 (1H, d, *J*12.0, PhCH*H*O), 4.95 (1H, d, *J*11.2, PhCH*H*O), 5.59 (1H, s, H-7), 7.29-7.37 (13H, m, 13xPh-H), 7.40-7.44 (2H, m, 2xPh-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 55.32 (CH_3), 62.31 (CH), 69.05 (CH_2), 73.76 (CH_2), 75.31 (CH_2), 78.59 (CH), 79.18 (CH), 82.13 (CH), 99.23 (CH), 101.13 (CH), 126.01 (2 × CH), 127.55 (CH), 127.89 (CH), 128.00 (2 × CH), 128.09 (2 × CH), 128.19 (2 × CH), 128.28 (2 × CH), 128.42 (2 × CH), 128.87 (CH), 137.41 (C), 138.15 (C), 138.72 (C); MS (ES) m/z (%): 480 ($M+NH_4^+$, 100%), 463 (42, $M+H^+$); Accurate mass (FAB): Found 463.2120 ($M+H^+$ $C_{28}H_{31}O_6$ requires 463.2120).

6.1.3 Methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside] 273[71]

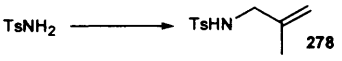


The fully protected sugar **276** (1.03 g, 2.2 mmol) was added to a stirring suspension of $LiAlH_4$ (0.1 g, 2.75 mmol) in dry diethyl ether (10 ml) and the mixture was stirred for 1.5 h at room temperature. Dichloromethane (~40 ml) was then added to the reaction and the solution was stirred a further 20 min. After this time the solution was cooled to 0 °C and $AlCl_3$ (0.36 g, 2.75 mmol) was added. The reaction was allowed to warm to room temperature and then refluxed (50 °C) for 22 h. The reaction mixture was carefully poured into cold water and the product extracted into ethyl acetate (3x5 ml). The organic layers were combined, dried ($MgSO_4$) and solvent was removed under reduced pressure. The product was purified by flash column chromatography on silica (25:75, ethyl acetate: petroleum-ether) to give the *alcohol* **273** (0.8 g, 79%) as a white solid. Mp: 66-67 °C (Lit.[178], 66.5-67 °C); $[\alpha]^{20}_D$: +44.6 (c=1.67

MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3595w (OH), 3054m, 2986w, 2928w, 1603w, 1497w, 1269s, 895m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.37 (3H, s, OMe), 3.46-3.55 (2H, m, H-2, H-4), 3.63-3.81 (3H, m, H-5, 2xH-6), 4.02 (1H, app t, *J*9.2, H-3), 4.58 (1H, d, *J*3.4, H-1), 4.65 (1H, d, *J*11.3, PhC*H*HO) 4.67 (1H, d, *J*12.2, PhC*H*HO), 4.81 (1H, d, *J*11.3, PhCH*H*O), 4.84 (1H, d, *J*11.0, PhC*H*HO), 4.89 (1H, d, *J*12.2, PhCH*H*O), 5.00 (1H, d, *J*11.0, PhCH*H*O), 7.27-7.36 (15H, m,15xPh-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 55.13 (CH_3), 61.81 (CH_2), 70.64 (CH), 73.36 (CH_2), 74.97 (CH_2) 75.68 (CH_2), 77.51 (CH), 79.96 (CH), 81.91 (CH), 98.14 (CH), 127.55 (CH), 127.81 (CH), 127.90 (3 × CH), 127.97 (2 × CH), 128.06 (2 × CH), 128.35 (2 × CH), 128.42 (4 × CH), 138.09 (C), 138.12 (C), 138.71 (C); MS (ES) m/z (%): 482 ($M+NH_4^+$, 100%), 483 (32); Accurate mass (FAB): Found 487.2097 ($M+Na^+$ $C_{28}H_{32}O_6Na$ requires 487.2097).

6.2 Synthesis of the (2→6) linked homoaza-*O*-disaccharides 266 and 267[68]

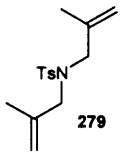
6.2.1 *N*-(2-Methallyl)-4-methylbenzenesulfonamide 278



Potassium carbonate (16.1 g, 117 mmol) was added to a stirring solution of toluene sulfonamide (20.0 g, 117 mmol) in acetone (200 ml) and the reaction was stirred for 0.5 h. 3-Chloro-2-methyl-propene (11.6 ml, 10.6 g, 117.0 mmol) was then added and the reaction was refluxed for 66 h. The reaction mixture was filtered and the solvent removed under vacuum. The crude solid was dissolved in ethyl acetate (100 ml) and washed with water (2x100 ml). The aqueous extract was back extracted into ethyl acetate (3x50 ml). The organic extracts were combined, dried ($MgSO_4$) and solvent was removed under reduced pressure. The crude material was purified by flash column chromatography on silica (60:40, diethyl ether: petroleum-ether) to give the *mono-substituted sulfonamide* **278** (9.79 g, 37%) as a white solid together with the *di-substituted product* **279** (1.82 g, 7%) as a yellow oil. Data for **278**: Mp: 48-49 °C (Lit.[179], 50-52 °C (from CH_2Cl_2:hexane)); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3384m (NH), 3053s, 2985w, 1658w, 1598w (aromatic), 1495w, 1421m, 1332m, 1289s, 1126s, 1094m, 896m, 895m, 747s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.68 (3H, s, Me), 2.43 (3H, s, Ar 4-Me), 3.48 (2H, d, *J*6.4, NCH_2), 4.78 (1H, br t, *J*6.4, NH), 4.80 (1H, br s, C=C*H*H), 4.86 (1H, br s, C=CH*H*), 7.30 (2H, d, *J*8.5, 2xAr H-3), 7.76 (2H, d, *J*8.5, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 20.04 (CH_3), 21.45 (CH_3), 48.91 (CH_2), 112.69 (CH_2), 127.00 (2 × CH), 129.63 (2 × CH), 136.44 (C), 140.46 (C), 143.36 (C); MS (ES) m/z (%): 226 ($M+H^+$, 100%), 243 (78, $M+NH_4^+$), 204 (45); Accurate mass (FAB): Found 226.0901 ($M+H^+$ $C_{11}H_{16}NO_2$ requires

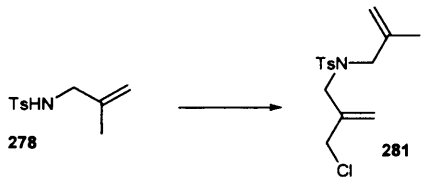
226.0901). Anal. Found: C, 58.63; H, 6.53; N, 5.99; $C_{11}H_{15}O_2NS$ requires: C, 58.67; H, 6.71; N, 6.22.

Data for **279**: *N, N-Bis*-(2-methylallyl)-4-methylbenzenesulfonamide



δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.61 (6H, s, 2xMe), 2.41 (3H, s, Ar 4-Me), 3.71 (4H, s, 2xNCH_2), 4.78 (2H, s, 2xC=C*H*H), 4.86 (2H, s, 2xC=CH*H*), 7.28 (2H, d, *J*8.3, 2xAr H-3), 7.71 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 19.90 (2 × CH_3), 21.39 (CH_3), 53.48 (2 × CH_2), 114.40 (2 × CH_2), 127.17 (2 × CH), 129.41 (2 × CH), 137.41 (C), 140.02 (2 × C), 142.99 (C).

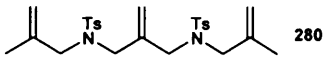
6.2.2 *N*-(2-Chloromethylallyl)-*N*-(2-methallyl)-4-methylbenzenesulfonamide 281



A solution of the mono-substituted sulfonamide **278** (5.0 g, 22 mmol) in DMF (100 ml) was added dropwise to a 0 °C solution sodium hydride (0.97 g of a 60% dispersion in oil, 40 mmol) in DMF (2 ml). The solution was allowed to warm to room temperature and was stirred for 0.5 h. 3-Chloro-2-chloromethyl-1-propene (4.5 ml, 4.86 g, 39.0 mmol) was then added to the stirring solution and the reaction was stirred a further 15 h before being quenched with 50:50 solution of water and saturated aqueous ammonium chloride (100 ml). The aqueous layer was extracted with ethyl acetate (3x50 ml), the organic layers were combined, dried ($MgSO_4$) and solvent was removed under reduced pressure. The crude material was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *chloride* **281** (4.77 g, 68%) as a colourless oil. ν_{max} (CH_2Cl_2)/ cm^{-1}: 3053s, 2984w, 2923w, 2856w, 1654w, 1598w, 1495w, 1421m, 1442w, 1421w, 1340m, 1264s, 1160s, 1097s, 896m, 895m, 747s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.63 (3H, s, CH_3), 2.43 (3H, s, Ar 4-Me), 3.72 (2H, s, $NCH_2C(CH_2Cl)$), 3.84 (2H, s, NCH_2CCH_3), 3.96 (2H, s, CH_2Cl), 4.80 (1H, s, CH_3C=C*H*H), 4.89 (1H, s, CH_3C=CH*H*), 5.11 (1H, s, $ClCH_2C$=C*H*H), 5.29 (1H, s, $ClCH_2C$=CH*H*), 7.30 (2H, d, *J*8.0, 2xAr H-3), 7.71 (2H, d, *J*8.0, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 19.90 (CH_3), 21.46 (CH_3), 45.40 (CH_2), 49.35 (CH_2), 53.99 (CH_2), 115.08 (CH_2), 118.51 (CH_2), 127.25 (2 × CH), 129.59 (2 × CH), 136.80 (C), 139.84 (C), 140.06 (C), 143.38 (C); MS (ES) m/z (%): 314 ($M+H^+$, 100%), 331 (40, $M+NH_4^+$), 644 (30). Accurate

mass (FAB): Found 314.0981 ($M+H^+$ $C_{15}H_{21}NO_2SCl$ requires 314.0982). Anal. Found: C, 57.29; H, 6.59; N, 4.32; $C_{15}H_{20}O_2NSCl$ requires: C, 57.41; H, 6.42; N, 4.46.

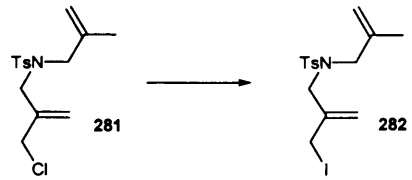
A small amount of the side product **280** (1.65 g, 24%) was also isolated:



280

δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.56 (6H, s, 2xCH_3), 2.42 (6H, s, 2xAr 4-Me), 3.62 (4H, s, 2xNC*H*$_2$CCH$_3$), 3.69 (4H, s, 2xNCH$_2$C(CH$_2$)), 4.73 (2H, s, 2xCH$_3$C=C*H*H), 4.84 (2H, s, 2xCH$_3$C=CH*H*), 4.89 (2H, s, C=CH$_2$), 7.28 (4H, d, *J*8.3, 4xAr H-3), 7.67 (4H, d, *J*8.3, 4xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 19.96 (2 × CH_3), 21.48 (2 × CH_3), 49.95 (2 × CH_2), 53.63 (2 × CH_2), 114.87 (2 × CH_2), 116.49 (CH_2), 127.24 (4 × CH), 129.58 (4 × CH), 137.10 (2 × C), 138.35 (2 × C), 139.86 (C), 143.24 (2 × C).

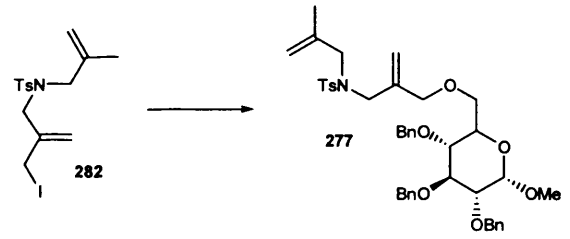
6.2.3 *N*-(2-Iodomethallyl)-*N*-(2-methylallyl)-4-methylbenzenesulfonamide 282



281 **282**

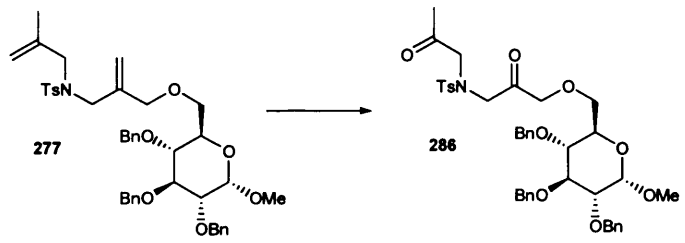
Sodium iodide (0.55 g, 1.82 mmol) and potassium carbonate (0.13 g, 0.9 mmol) were added to a stirring solution of the chloride **281** (0.57 g, 1.82 mmol) in acetone (50 ml). The solution was refluxed for 4.5 h before being filtered. The organic filtrate was concentrated under reduced pressure and the resulting crude residue was dissolved into diethyl ether (25 ml) and was washed with water (3x10 ml). The aqueous washings were back extracted with diethyl ether (3x10 ml), the organic extracts were combined, dried ($MgSO_4$) and solvent was removed under reduced pressure to give the *iodide* **282** (0.62 g, 83%) as a colourless oil which was taken through to the next step without purification. δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.64 (3H, s, Me), 2.43 (3H, s, Ar 4-Me), 3.74 (2H, s, NC*H*$_2$C(CH$_2$I)), 3.84 (2H, s, NC*H*$_2$CCH$_3$), 3.89 (2H, s, CH$_2$I), 4.82 (1H, s, CH$_3$C=C*H*H), 4.89 (1H, s, CH$_3$C=CH*H*), 5.04 (1H, s, ICH$_2$C=C*H*H), 5.36 (1H, s, ICH$_2$C=CH*H*), 7.30 (2H, d, *J*8.3, 2xAr 3-H), 7.73 (2H, d, *J*8.3, 2xAr 2-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 6.22 (CH_2), 19.98 (CH_3), 21.50 (CH_3), 49.88 (CH_2), 54.05 (CH_2), 115.12 (CH_2), 117.68 (CH_2), 127.31 (2 × CH), 129.64 (2 × CH), 136.88 (C), 139.85 (C), 141.56 (C), 143.38 (C).

6.2.4 *N*-(2-Methallyl)-*N*-[2-[(methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside])allyl]-4-methylbenzenesulfonamide 277



The alcohol **273** (0.66 g, 1.43 mmol) dissolved in THF (15 ml) was added dropwise to sodium hydride (0.09 g of a 60% dispersion in oil, 3.58 mmol) in THF (5 ml) at 0 °C. The reaction was warmed to room temperature and stirred for 0.5 h before the iodide **282** (0.58 g, 1.43 mmol) in THF (5 ml) was transferred to the reaction mixture. The reaction was heated at 40 °C for 24 h before being quenched with saturated aqueous ammonium chloride (10 ml). The aqueous layer was washed with diethyl ether (3x10 ml), the organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product was purified using flash column chromatography on silica (20:80, ethyl acetate: petroleum-ether) to give the *diene* **277** (0.77 g, 73%) as a colourless oil. $[\alpha]^{20}_D$: +31.5 (c=1.20 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3052s, 2985m, 2918m, 1654w, 1598w, 1496w, 1454m, 1421w, 1340m, 1261s, 1209w, 1198w, 1159s, 1097s, 1071s, 1047s, 1028m, 911m, 855m, 816w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.50 (3H, s, CH_3C), 2.38 (3H, s, Ar 4-Me), 3.36 (3H, s, OMe), 3.46-3.57 (4H, m, H-4, H-2, 2xH-6), 3.69-3.83 (7H, m, 2 x CH_2N, H-5, CH_2O), 3.97 (1H, app t, *J*9.1, H-3), 4.57 (1H, d, *J*11.7, PhC*H*HO), 4.61 (1H, d, *J*3.4, H-1), 4.68 (1H, s, C=C*H*H), 4.71 (1H, d, *J*11.2, PhC*H*HO), 4.74 (1H, s, C=CH*H*), 4.79 (1H, d, *J*11.7, PhCH*H*O), 4.81 (1H, d, *J*11.0, PhC*H*HO), 4.81 (1H, s, C=C*H*H), 4.86 (1H, d, *J*11.2, PhCH*HO*), 4.98 (1H, d, *J*11.0, PhCH*H*O), 5.16 (1H, s, C=CH*H*), 7.21-7.37 (17H, m, 15xPh-H, 2xAr H-3), 7.66 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 19.93 (CH_3), 21.44 (CH_3), 45.49 (CH_2), 53.47 (CH_2), 55.09 (CH_3), 68.96 (CH_2), 70.03 (CH), 72.07 (CH_2), 73.34 (CH_2), 74.98 (CH_2), 75.72 (CH_2), 77.86 (CH), 79.92 (CH), 82.05 (CH), 98.05 (CH), 114.60 (CH_2), 115.29 (CH_2), 127.24 (2 × CH), 127.56 (CH), 127.70 (CH), 127.77 (2 × CH), 127.87 (CH), 127.95 (2 × CH), 128.09 (2 × CH), 128.35 (2 × CH), 128.39 (2 × CH), 128.42 (2 × CH), 129.48 (2 × CH), 137.27 (C), 138.16 (C), 138.33 (C), 138.78 (C), 140.00 (C), 140.23 (C), 143.09 (C); MS (ES) m/z (%): 759 ($M+NH_4^+$, 100%), 760 (50, $M+H_3O^+$); Accurate mass (FAB): Found 710.3152 (M-OMe $C_{42}H_{48}NO_7S$ requires 710.3152).

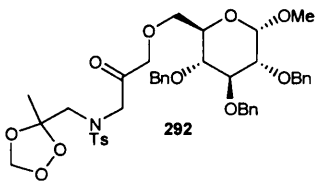
6.2.5 *N*-(2-Oxopropyl)-*N*-[2-oxo-3-(methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside])propyl]-4-methylbenzenesulfonamide 286[78]



General ozonolysis procedure:

Nitrogen was first bubbled through a solution of the diene **277** (0.50 g, 0.68 mmol) in dichloromethane (20 ml) for 2 min. The solution was cooled to –78 °C and ozone was bubbled through the solution until a blue / purple colour was observed. Nitrogen was then bubbled through the solution until it turned colourless. The reaction was warmed to room temperature and dimethyl sulfide (0.17 g, 0.2 ml, 2.72 mmol) was added and the reaction was stirred at room temperature for a further 18 hours. The solvent was removed under vacuum to give the crude *diketone* **286** (0.16 g, 55%) as a colourless oil which was taken through to the next step without purification. ν_{max} (CH_2Cl_2)/ cm^{-1}: 3052s, 2986s, 2923s, 1730m (C=O), 1454s, 1422s, 1357s, 1306m, 1279s, 1252s, 1193m, 1162s, 1093s, 1072s, 1047s, 1028s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.13 (3H, s, Me), 2.38 (3H, s, Ar 4-Me), 3.36 (3H, s, OMe), 3.48-3.78 (5H, m, H-2, H-4, H-5, 2xH-6), 3.99 (1H, app t, *J*9.4, H-3), 4.08-4.11 (4H, m, 2xNCH_2), 4.23-4.25 (2H, m, CH_2O), 4.60 (1H, d, *J*3.4, H-1), 4.64 (1H, d, *J*11.0, PhC*H*HO), 4.66 (1H, d, *J*11.4, PhC*H*HO), 4.80 (1H, d, *J*11.0, PhCH*H*O), 4.82 (1H, d, *J*11.0, C*H*HO), 4.89 (1H, d, *J*11.4, PhCH*H*O), 4.99 (1H, d, *J*11.0, PhCH*H*O), 7.09-7.35 (17H, m, 15xPh-H, 2xAr H-3), 7.65 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.40 (CH_3), 26.85 (CH_3), 54.05 (CH_2), 55.13 (CH_3), 56.85 (CH_2), 69.94 (CH), 70.38 (CH_2), 72.14 (CH_2), 73.30 (CH_2), 74.87 (CH_2), 75.37 (CH_2), 79.87 (CH), 81.87 (2 × CH), 97.97 (CH), 127.25-129.59 (19 × CH), 135.69 (C), 138.00 (2 × C), 138.62 (C), 143.77 (C), 203.1 (C), 203.3 (C); MS (ES) m/z (%): 795 ($M+NH_4^+$+MeOH, 100%), 763 (80, $M+NH_4^+$).

A small amount of the side product **292** (0.03 g, 19%) was also isolated:



δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.62 (3H, s, Me), 2.41 (3H, s, Ar 4-Me), 3.21 (1H, app t, *J*9.4, H-4), 3.36 (3H, s, OMe), 3.40-3.80 (4H, m, H-2, H-5, 2xH-6), 4.0 (1H, app t, *J*9.4, H-3), 4.08-4.30 (6H, m, 2xNCH_2, CH_2O), 4.60-4.99 (8H, m, H-1, 6xPhC*H*HO, C*H*HO), 5.13 (1H, d, *J*10.3, CH*H*O), 7.10-7.41 (17H, m, 15xPh-H, 2xAr H-3), 7.71 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 17.93 (CH_3), 20.46 (CH_3), 51.38 (CH_2), 54.13 (CH_3), 54.15 (CH_2), 68.97 (CH), 69.25 (CH_2), 72.33 (CH_2), 73.97 (CH_2), 74.41 (CH_2), 74.72 (CH_2), 76.38 (CH), 78.78 (CH), 80.95 (CH), 92.94 (CH_2), 97.04 (CH), 107.67 (CH_2), 126.40-128.59 (19 × CH), 134.77 (C), 137.01 (C), 137.18 (C), 137.61 (C), 142.76 (C), 201.72 (C); MS (ES) m/z (%): 809 ($M+NH_4^+$+MeOH, 100%).

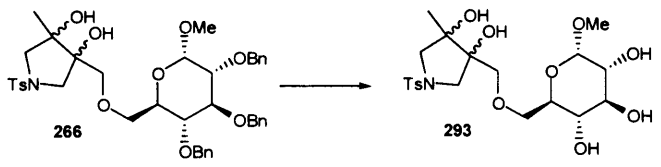
6.2.6 (3*S*, 4*S*) and (3*R*, 4*R*)-3-Methyl-1-(toluene-4-sulfonyl)-4-(methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3,4-diol 266 and 267[68,73]

General pinacol coupling procedure:

Samarium metal (0.61 g, 4.07 mmol, weighed out in a glove box) was added to a flame dried reaction vessel. The vessel was subjected to 3 cycles of freeze-pump-thaw (purging with argon). Meanwhile THF (34 ml) was degassed (three cycles) and added to the reaction vessel. To the stirring solution freshly distilled diiodomethane (0.90 g, 0.27 ml, 3.39 mmol) was added. After a short induction period the reaction turned yellow, green and finally deep blue. The reaction mixture was left stirring for 2 h. The diketone **286** (0.50 g, 0.67mmol) was dissolved in THF (2 ml) and this solution was degassed (2 cycles of freeze-pump-thaw). ^{t}BuOH (0.30 g, 0.38 ml, 4.07 mmol) was added to the solution and the mixture was degassed a further two times. This solution was then added to the SmI_2 at –78 °C. The reaction was left stirring 18 h and allowed to warm to room temperature before being quenched with saturated aqueous sodium hydrogen carbonate (10 ml). The product was extracted into ethyl acetate (3x20 ml) and the organic layers were washed with a 10% aqueous solution of sodium thiosulphate (20 ml). The organic layer was dried ($MgSO_4$) and solvent was removed under vacuum to give crude product. The crude material was purified using flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *diols* **266** and **267** (1:1 ratio of diastereoisomers) as colourless oils; (diastereoisomer 1 (1st eluted) 0.0695 g, diastereoisomer 2 (2nd eluted) 0.078 g, mixed fraction 0.0414 g, 37% total yield over two steps from **277**). Data for diastereoisomer 1 **266**: $[\alpha]^{20}_D$: +42.5 (c= 1.0 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3689w (OH), 3053s, 2986m, 1422s, 1276s, 1253s, 896m; δ_H (400 MHz;

$CDCl_3$; Me_4Si): 1.15 (3H, s, CCH_3), 2.40 (3H, s, Ar 4-Me), 3.26 (2H, s, CH_2N), 3.30-3.32 (2H, m, NCH_2), 3.32-3.41 (1H, m, H-4), 3.34 (3H, s, OMe), 3.44 (2H, s, CH_2O), 3.49 (1H, dd, *J*9.6, 3.7, H-2), 3.55 (1H, dd, *J*10.7, 5.5, H-5), 3.63-3.67 (1H, m, H-6), 3.70-3.74 (1H, m, H-6), 3.97 (1H, app t, *J*9.6, H-3), 4.53 (1H, d, *J*12.1, PhC*H*HO), 4.58 (1H, d, *J*3.7, H-1), 4.66 (1H, d, *J*12.1, PhC*H*HO), 4.78 (1H, d, *J*12.1, PhCH*H*O), 4.79 (1H, d, *J*11.0, PhC*H*HO), 4.87 (1H, d, *J*12.1, PhCH*H*O), 4.89 (1H, d, *J*11.0, PhCH*H*O), 7.19-7.37 (17H, m, 15xPh-H, 2xAr H-3), 7.69 (2H, d, *J*8.3, 2xAr H-2); δ_C (100.6 MHz; $CDCl_3$; Me_4Si): 21.28 (CH_3), 21.45 (CH_3), 55.05 (CH_2), 55.26 (CH_3), 57.49 (CH_2), 69.96 (CH), 71.09 (CH_2), 72.76 (CH_2), 73.31 (CH_2), 74.84 (CH_2), 75.73 (CH_2), 77.50 (C), 77.57 (CH), 79.06 (C), 79.98 (CH), 81.78 (CH), 97.95 (CH), 127.42 (2 × CH), 127.62 (CH), 127.78 (3 × CH), 127.88 (CH), 127.95 (4 × CH), 128.37 (2 × CH), 128.41 (2 × CH), 128.435 (2 × CH), 129.54 (2 × CH), 133.80 (C), 137.94 (2 × C), 138.47 (C), 143.41 (C); MS (ES) m/z (%): 765 ($M+NH_4^+$, 100%), 766 (50), 767 (15); Accurate mass (FAB): Found 746.3001 ($M-H^+$ $C_{41}H_{48}NO_{10}S$ requires 746.2999). Data for diastereoisomer 2 **267**: $[\alpha]^{20}_D$: +55.5 (c= 1.0 CH_2Cl_2); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3686w (OH), 2982m, 2688m, 1642w, 1545w, 1435s, 1283s, 1251s, 1165m; δ_H (400 MHz; $CDCl_3$; Me_4Si): 1.15 (3H, s, CH_3), 2.40 (3H, s, Ar 4-Me), 3.23-3.31 (4H, m, 2xCH_2N), 3.35 (3H, s, OMe), 3.38-3.56 (5H, m, CH_2O, H-2, H-4, H-5), 3.63-3.73 (2H, m, H-6), 3.97 (1H, app t, *J*9.2, H-3), 4.54 (1H, d, *J*11.0, PhC*H*HO), 4.57 (1H, d, *J*3.0, H-1), 4.65 (1H, d, *J*11.9, PhC*H*HO), 4.78 (1H, d, *J*11.0, PhCH*H*O), 4.80 (1H, d, *J*11.0, PhC*H*HO), 4.88 (1H, d, *J*11.9, PhCH*H*O), 4.97 (1H, d, *J*11.0, PhC*H*HO), 7.19-7.37 (17H, m, 15xPh-H, 2xAr H-3), 7.69 (2H, d, *J*8.3, 2xAr H-2); δ_C (100.6 MHz; $CDCl_3$; Me_4Si): 21.34 (CH_3), 21.47 (CH_3), 55.00 (CH_2), 55.31 (CH_3), 57.47 (CH_2), 69.91 (CH), 70.70 (CH_2), 72.77 (CH_2), 73.40 (CH_2), 75.00 (CH_2), 75.76 (CH_2), 77.43 (CH), 77.49 (C), 79.05 (C), 80.03 (CH), 81.79 (CH), 98.08 (CH), 127.43 (2 × CH), 127.63 (CH), 127.75 (3 × CH), 127.94 (3 × CH), 128.00 (2 × CH), 128.37 (2 × CH), 128.40 (2 × CH), 128.43 (2 × CH), 129.55 (2 × CH), 133.83 (C), 137.95 (C), 138.01 (C), 138.47 (C), 143.42 (C); (ES) m/z (%): 765 ($M+NH_4^+$, 100%), 766 (50), 767 (20); Accurate mass (FAB): Found 746.3001 ($M-H^+$ $C_{41}H_{48}NO_{10}S$ requires 746.2999).

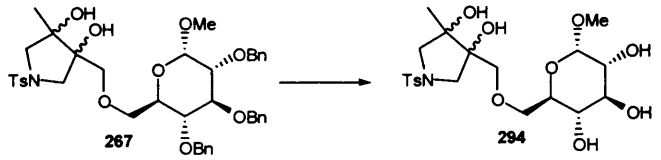
6.2.7 3-Methyl-1-(toluene-4-sulfonyl)-4-(methyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3,4-diol 293



General hydrogenolysis procedure:

The diol **266** (0.16g, 0.23 mmol) was dissolved in ethanol (16 ml) and acetic acid (1.71 ml). $Pd(OAc)_2$ (0.045 g, 0.2 mmol) was added to the solution, which was then evacuated and filled with nitrogen (three cycles) before being put under an atmosphere of hydrogen (balloon). The reaction mixture was then stirred under hydrogen for 6 h before being filtered. The solvent was removed under vacuum to give the crude product which was purified by flash column chromatography on silica (20:80, methanol: ethyl acetate) to give the *pentol* **293** (0.055 g, 51%) as a foam. $[\alpha]^{20}_D$: +44.6 (c= 1.12 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3420.2br (OH), 1732w, 1603w, 1344m, 1271s, 1262s, 1157m, 1051m; δ_H (400 MHz; D_2O): 1.01 (3H, s, CH_3), 2.40 (3H, s, Ar 4-Me), 3.04 (1H, d, *J*10.4, NC*H*H), 3.23 (1H, d, *J*10.8, NC*H*H), 3.28 (1H, d, *J*10.6, C*H*HO), 3.30 (1H, d, *J*10.4, NCH*H*), 3.33 (1H, dd, *J*10.0, 9.0, H-4), 3.37 (3H, s, OMe), 3.41 (1H, d, *J*10.8, NCH*H*), 3.45 (1H, dd, *J*11.3, 2.6, H-6), 3.46-3.52 (1H, m overlapping, H-6), 3.50 (1H, dd, *J*9.0, 3.7, H-2), 3.54 (1H, d, *J*10.6, CH*H*O), 3.62 (1H, app t, *J*9.0 H-3), 3.63-3.65 (1H, m overlapping, H-5), 4.74 (1H, d, *J*3.7, H-1), 7.45 (2H, d, *J*8.0, 2xAr H-3), 7.71 (2H, d, *J*8.0, 2xAr H-2); δ_C (100.6 MHz; D_2O): 19.11 (CH_3), 21.14 (CH_3), 53.33 (CH_2), 55.61 (CH_3), 58.02 (CH_2), 70.09 (CH), 70.46 (CH_2), 70.83 (CH), 71.60 (CH), 72.15 (CH_2), 73.43 (CH), 77.54 (C), 80.21 (C), 99.70 (CH), 127.73 (2 × CH), 130.59 (2 × CH), 131.93 (C), 145.95 (C); (ES) m/z (%): 478 ($M+H^+$, 30%), 495 (20), 496 (10); Accurate mass (FAB): Found 478.1746 ($M+H^+$ $C_{20}H_{32}NO_{10}S$ requires 478.1747).

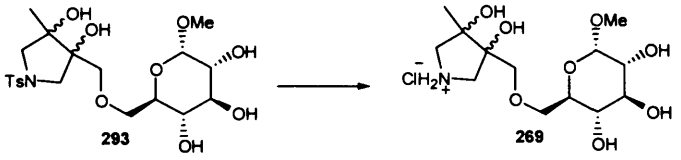
6.2.8 3-Methyl-1-(toluene-4-sulfonyl)-4-(methyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3,4-diol 294



The diol **267** (0.10g, 0.14 mmol) was dissolved in ethanol (8.50 ml) and acetic acid (1.05 ml) and was subjected to the general hydrogenolysis procedure (outlined in experiment 6.2.7) using $Pd(OAc)_2$ (0.028 g, 0.13 mmol). The reaction mixture was left stirring under a hydrogen atmosphere for 3 h before being filtered. The filtrate was concentrated under vacuum and the resulting crude material was purified by flash column chromatography on silica (10:90, methanol: ethyl acetate) to give the *pentol* **294** (0.039g, 60%) as a white foam. $[\alpha]^{20}_D$: +68.2 (c= 0.93 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3683br (OH), 1602.3w, 1344m, 1272s, 1157m, 1052m, 753s; δ_H (400 MHz; D_2O): 1.01 (3H, s, CH_3), 2.40 (3H, s, Ar 4-Me), 3.12 (1H, d, *J*10.3, NC*H*H), 3.22 (1H, d, *J*10.6, NC*H*H), 3.29 (1H, d, *J*10.4, C*H*HO), 3.37 (3H, s, OMe), 3.37 (1H, app t overlapping, *J*9.7, H-4), 3.38 (1H, d, *J*10.6, NCH*H*), 3.39 (1H, d, *J*10.3, NCH*H*),

3.49 (1H, dd, *J*9.7, 3.7, H-2), 3.54 (1H, d, *J*10.4, CH*H*O), 3.54-3.63 (3H, m overlapping, H-5, 2xH-6), 3.59 (1H, app t overlapping, *J*9.7, H-3), 4.73 (1H, d, *J*3.7, H-1), 7.46 (2H, d, *J*8.0, 2xAr H-3), 7.73 (2H, d, *J*8.0, 2xAr H-4); δ_C (100.6 MHz; D_2O): 19.40 (CH_3), 21.14 (CH_3), 53.80 (CH_2), 55.60 (CH_3), 58.00 (CH_2), 70.09 (CH), 70.63 (CH_2), 70.82 (CH), 71.57 (CH), 72.25 (CH_2), 73.53 (CH), 77.58 (C), 80.19 (C), 99.68 (CH), 127.77 (2 × CH), 130.59 (2 × CH), 131.93 (C), 145.95 (C); (ES) m/z (%): 478 ($M+H^+$, 100%); Accurate mass (FAB): Found 478.1747 ($M+H^+$ $C_{20}H_{32}NO_{10}S$ requires 478.1747).

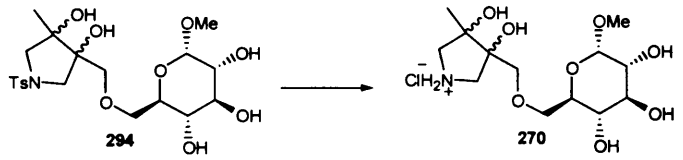
6.2.9 3-Methyl-4-(methyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3, 4-diol hydrochloride 269



General tosyl deprotection procedure:

The pentol **293** (0.021 g, 0.042 mmol) in a three necked flask fitted with a dry ice condenser was dissolved in a minimal amount of liquid ammonia (5-10 ml) at -78 °C. Sodium metal (0.0025 g, 0.11 mmol) was then added portion-wise to the stirring reaction mixture. After the addition of sodium was complete the reaction was warmed to room temperature and the liquid ammonia was allowed to evaporate. The crude residue was dissolved into water and transferred to a round bottom flask and the water was removed under reduced pressure. The resulting material was purified by flash column chromatography on silica (100% methanol) to give *the amine* **269** (0.0071 g, 51%) as a colourless oil. To aid analysis dilute HCl (aq) was added to the amine in water until the solution was approximately pH 2~3 yielding the corrponding hydrochloride salt of **269**. $[\alpha]^{20}_D$: +29.8 (c= 0.19 MeOH); ν_{max} (solid state)/ cm^{-1}: 3126br (OH), 2946w, 1717w, 1597w, 1455m, 1390m, 1103m, 1036s, 1005s, 903m; δ_H (400 MHz; D_2O): 1.40 (3H, s, CH_3), 3.43 (1H, d, *J*12.8, C*H*HN), 3.48 (3H, s, OMe), 3.51 (1H, app t, *J*9.6, H-4), 3.61 (1H, dd, *J*9.6, 3.6, H-2), 3.66 (1H, d, *J*12.8, CH*H*N), 3.69 (1H d, *J*12.8, C*H*HN), 3.70 (1H, d, *J*10.0, C*H*HO), 3.73 (1H, app t, *J*9.6 H-3), 3.75 (1H, d, *J*10.0, CH*H*O), 3.81-3.86 (3H, m, H-5, H-6, CH*H*N), 3.90 (1H, dd, *J*11.6, 5.2, H-6), 4.71 (1H, d, *J*3.6, H-1); δ_C (100.6 MHz; D_2O): 18.63 (CH_3), 50.81 (CH_2), 54.61 (CH_2), 55.29 (CH_3), 69.59 (CH), 70.27 (CH_2), 70.54 (CH), 71.19 (CH), 72.32 (CH_2), 73.00 (CH), 77.22 (C), 79.46 (C), 99.42 (CH); (ES) m/z (%): 324 ($M+H^+$, 100%), 441 (92%); (ES) m/z (%): 324 ($M+H^+$, 100%); Accurate mass (FAB): Found 324.1659 ($M+H^+$ $C_{13}H_{26}NO_8$ requires 324.1658).

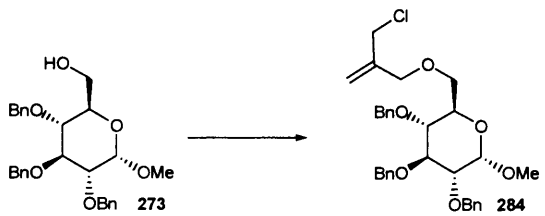
6.2.10 3-Methyl-4-(methyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3, 4-diol hydrochloride 270



The pentol **294** (0.023 g, 0.047 mmol) was subjected to the general tosyl deprotection procedure (outline in experiment 6.2.9) using sodium metal (0.0027 g, 0.117 mmol) in liquid ammonia (~5 ml). After the addition of sodium was complete the reaction was warmed to room temperature and the liquid ammonia was allowed to evaporate. The crude residue was dissolved into water, transferred to a round bottom flask and the water was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (100% methanol) to give the *amine* **270** (0.0078 g, 50%; contaminated *ca.* 10% unidentified impurity by ^{1}H-NMR) as a colourless oil. To aid analysis dilute HCl (aq) was added to the amine in water until the solution was approximately pH 2~3 yielding the corresponding hydrochloride salt of **270**. $[\alpha]^{20}_D$: + 34.7 (c= 0.12 MeOH); ν_{max} (solid state)/ cm^{-1}: 3305br (OH), 2976w, 1629m, 1451m, 1142m, 1099m, 1038s, 1004s, 903w, 754w; δ_H (400 MHz; D_2O) (assigned major peaks as ~90% pure) : 1.39 (3H, s, CH_3), 3.41 (1H, d, *J*12.4, C*H*HN), 3.46 (3H, s, OMe), 3.49 (1H, app t, *J*9.6, H-4), 3.60 (1H, dd, *J*9.6, 3.6, H-2), 3.65 (1H, d, *J*12.4, CH*H*N), 3.67 (1H d, *J*10.4, C*H*HO), 3.69 (1H, d, *J*10.8, C*H*HN), 3.68-3.72 (1H, m overlapping, H-6), 3.70 (1H, d, *J*10.4, CH*H*O), 3.77 (1H, d, *J*10.8 CH*H*N), 3.82-3.91 (3H, m, H-5, H-6, H-3), 4.85 (1H, d, *J*3.6, H-1); δ_C (100.6 MHz; D_2O): 18.56 (CH_3), 50.78 (CH_2), 54.68 (CH_2), 55.27 (CH_3), 69.62 (CH), 70.12 (CH_2), 70.40 (CH), 71.17 (CH), 72.28 (CH_2), 73.08 (CH), 77.19 (C), 79.43 (C), 99.41 (CH); (ES) m/z (%): 324 (M+H$^+$, 100%), 346 (92%, M+Na); Accurate mass (FAB): Found 324.1658 (M+H$^+$ $C_{13}H_{26}NO_8$ requires 324.1658).

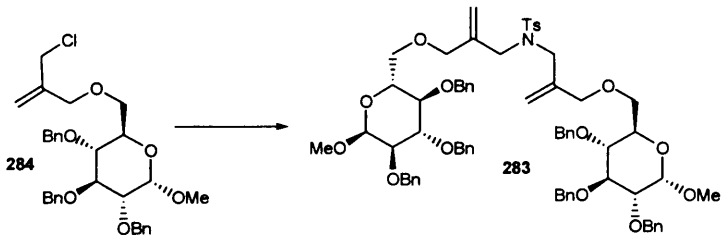
6.3 Synthesis of the (2→6) linked homoaza-O-trisaccharide 271[68]

6.3.1 Methyl-[O^2, O^3, O^4-tribenzyl-O^6-(2-chloromethyl-1-propene)-α-D-glucopyranoside] 284



To a 0 °C solution of petrol washed sodium hydride (0.46 g of a 60% dispersion in oil, 19.0 mmol) in THF (20 ml), was added the alcohol **273** (5.0 g, 11.0 mmol) in THF (80 ml). The solution was stirred for 0.5 h and then warmed to room temperature and 3-chloro-2-chloro methyl propene (6.7 g, 6.2 ml, 50.0 mmol) was added. The reaction was refluxed for 26 h before being quenched with a 50:50 solution of saturated aqueous ammonium chloride and water. The aqueous layer was extracted with diethyl ether (3x20 ml), the organic layers were combined, dried ($MgSO_4$) and solvent removed under vacuum. Purification by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) gave the *chloride* **284** (2.94 g, 50%, 70% based on recovered **273**) as a white solid. Mp. 39-41 °C; $[\alpha]^{20}_D$: +54.2 (c= 1.44 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3054s, 2985m, 2911m, 1604m (C=C), 1497w, 1454m, 1358m, 1271s, 1193w, 1160m, 1072w, 1048m, 1028m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.39 (3H, s, OMe), 3.52-3.58 (2H, m, H-2, H-4), 3.59-3.66 (2H, m, H-5, H-6), 3.69-3.78 (1H, m, H-6), 4.00 (1H, app t, *J*9.4, H-3), 4.08-4.11 (4H, m, CH_2Cl, CH_2O), 4.61 (1H, d, *J*11.0, PhC*H*HO), 4.62 (1H, d, *J*3.4, H-1), 4.66 (1H, d, *J*12.1, PhC*H*HO), 4.80 (1H, d, *J*11.0, PhCH*H*O), 4.81 (1H, d, *J*11.0, PhC*H*HO), 4.90 (1H, d, *J*12.1, PhCH*H*O), 4.99 (1H, d, *J*11.0, PhCH*H*O), 5.22 (1H, d, *J*1.2, C=C*H*H), 5.26 (1H, br s, C=CH*H*), 7.26-7.36 (15H, m, 15xPh-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 45.12 (CH_2), 55.14 (CH_3), 69.09 (CH_2), 70.02 (CH), 71.42 (CH_2), 73.36 (CH_2), 75.03 (CH_2), 75.76 (CH_2), 77.76 (CH), 79.90 (CH), 82.09 (CH), 98.13 (CH), 116.80 (CH_2), 127.58 (CH), 127.69 (CH), 127.88 (2 × CH), 127.97 (CH), 127.98 (2 × CH), 128.09 (2 × CH), 128.40 (2 × CH), 128.43 (2 × CH), 128.61 (2 × CH), 138.14 (C), 138.33 (C), 138.74 (C), 141.72 (C); MS (ES) m/z (%): 551 ($M+H^+$, 50%), 552 (40, $M+2H^+$); Accurate mass (FAB): Found 551.2200 ($M+H^+$ $C_{32}H_{36}O_6Cl$ requires 551.2200). Anal. Found: C, 69.29; H, 6.91; $C_{32}H_{37}O_6Cl$ requires: C, 69.49; H, 6.74.

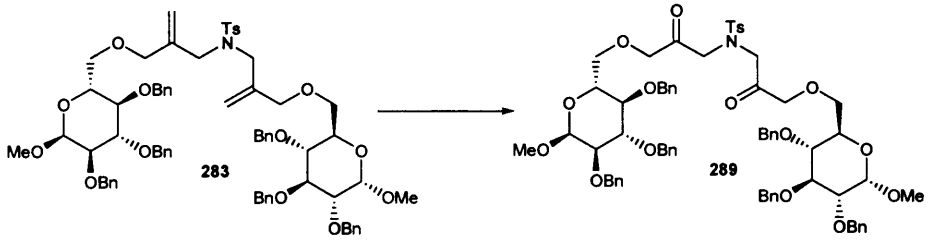
6.3.2 *N,N-Bis*-[2-(methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside])-l-allyl]-4-methylbenzensulfonamide 283



p-Toluenesulfonamide (0.39 g, 2.30 mmol) in DMF (40 ml) was added to a 0 °C solution of petrol washed sodium hydride (0.20 g, of a 60% dispersion in oil, 8.30 mmol) in DMF (10 ml). The solution was stirred for 0.5 h and then warmed to room temperature and stirred a further 0.5 h before the chloride **284** (2.9 g, 5.3 mmol) was added together with a catalytic amount of $Bu_4N^+I^-$. The reaction was refluxed for 18 h before being quenched with a 50:50

solution of saturated aqueous ammonium chloride and water. The aqueous layer was extracted with ethyl acetate (3x20 ml), the organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum. Purification of the resulting crude material by flash column chromatography on silica (50:50, ethyl acetate: petroleum-ether) gave the *diene* **283** (2.32 g, 84%) as a colourless oil. $[\alpha]^{20}_D$: +51.3 (c= 1.00 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3053s, 2985m, 2915m, 2869m, 1656w, 1599w, 1497w, 1454m, 1421m, 1341m, 1268s, 1193w, 1162m, 1135m, 1095w, 1071m, 1048m, 1028m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.3 (3H, s, Ar 4-Me), 3.35 (6H, s, 2xOMe), 3.47-3.54 (8H, m, 2xH-2, 2xH-4, 2xH-5, 2xH-6), 3.64-3.83 (2H, m, 2xH-6), 3.74 (4H, br s, 2xCH_2O), 3.77 (4H, br s, 2xCH_2O), 3.97 (2H, app t, *J*9.2, 2xH-3), 4.55 (2H, d, *J*11.0, 2xPhC*H*HO), 4.58 (2H, d, *J*3.4, 2xH-1), 4.65 (2H, d, *J*12.1, 2xPhC*H*HO), 4.78 (2H, d, *J*11.0, 2xPhCH*H*O), 4.81 (2H, d, *J*11.0, 2xPhCH*H*O), 4.85 (2H, d, *J*12.1, 2xPhCH*H*O), 4.94 (2H, br s, 2xC=C*H*H), 4.97 (2H, d, *J*11.0, 2xPhCH*H*O), 5.12 (2H, d, *J*1.2, 2xC=C*H*H), 7.2 (2H, d, *J*8.0, 2xAr H-3), 7.26-7.36 (30H, m, 30xPh-H), 7.63 (2H, d, *J*8.0, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.38 (CH_3), 49.64 (2 × CH_2), 55.05 (2 × CH_3), 68.94 (2 × CH_2), 69.97 (2 × CH), 71.89 (2 × CH_2), 73.27 (2 × CH_2), 74.92 (2 × CH_2), 75.66 (2 × CH_2), 77.81 (2 × CH), 79.91 (2 × CH), 82.02 (2 × CH), 97.96 (2 × CH), 114.81 (2 × CH_2), 127.18 (2 × CH), 127.53 (2 × CH), 127.71 (2 × CH), 127.80 (4 × CH), 127.90 (2 × CH), 127.96 (4 × CH), 128.03 (4 × CH), 128.36 (4 × CH), 128.41 (4 × CH), 128.43 (4 × CH), 129.66 (2 × CH), 138.09 (C), 138.27 (2 × C), 138.71 (2 × C), 138.83 (2 × C), 140.12 (2 × C), 143.41 (C); MS (ES) m/z (%): 1204 ($M+H^+$, 100%), 1016 (60), 1173 (55); Anal. Found: C, 70.55; H, 6.53; N, 1.14; $C_{71}H_{81}O_{14}NS$ requires: C, 70.80; H, 6.78; N, 1.16.

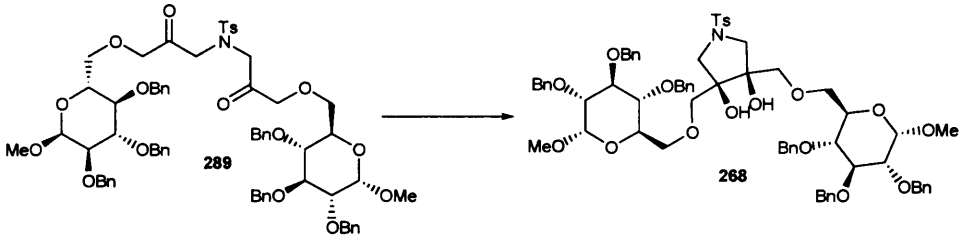
6.3.3 *N,N-Bis*-[2-oxo-3-(methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside]ylemethoxy) propyl]-4-methylbenzenesulfonamide 289



The general ozonolysis procedure was followed (see experiment 6.2.5) using the diene **283** (0.33 g, 0.27 mmol) in dichloromethane (25 ml). The reaction was quenched with dimethylsulfide (0.069 g, 0.08 ml, 1.11 mmol) and was stirred for 18 h. Removal of solvent under reduced pressure yielded the *diketone* **289** (0.23 g, 61%) as a colourless oil which was taken through to the next step without further purification. ν_{max} (CH_2Cl_2)/ cm^{-1}: 3055s, 3053s, 2986s, 1730s (C=O), 1599m, 1550m, 1497m, 1454m, 1422m, 1357m, 1306w, 1278s, 1162s,

1254s, 1049s, 1028s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.38 (3H, s, Ar 4-Me), 3.17-3.30 (4H, m, 2xCH_2N), 3.35 (6H, s, 2xOMe), 3.36 -3.43 (6H, m, 2xH-4, 2xCH_2O), 3.48-3.55 (4H, m, 2xH-2, 2xH-5), 3.58-3.73 (4H, m, 4xH-6), 3.99 (2H, app t, *J*9.2, 2xH-3), 4.57 (2H, d, *J*11.0, 2xPhC*H*HO), 4.60 (2H, d, *J*3.4, 2xH-1), 4.64 (2H, d, *J*11.0, 2xPhC*H*HO), 4.86 (2H, d, *J*11.0, 2xPhCH*H*O), 4.90 (2H, d, *J*11.0, 2xPhC*H*HO), 4.99 (2H, d, *J*11.0, 2xPhCH*H*O), 5.0 (2H, d, *J*11.0, 2xPhCH*H*O), 7.25-7.35 (32H, m, 30xPh-H, 2xAr H-3), 7.69 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.44 (CH_3), 54.35 (2 × CH_2), 55.21 (2 × CH_3), 70.05 (2 × CH), 70.46 (2 × CH_2), 73.48 (2 × CH_2), 74.96 (2 × CH_2), 75.45 (2 × CH_2), 75.71 (2 × CH_2), 77.71 (2 × CH), 79.98 (2 × CH), 82.00 (2 × CH), 98.05 (2 × CH), 127.35 (2 × CH), 127.61 (2 × CH), 127.75 (2 × CH), 127.94 (4 × CH), 128.07 (4 × CH), 128.12 (4 × CH), 128.35 (4 × CH), 128.43 (4 × CH), 129.68 (8 × CH), 134.52 (C), 138.09 (2 x C), 138.22 (2 × C), 138.73 (2 × C), 142.42 (C), 203.38 (2 × C); MS (ES) m/z (%): 1227 ($M+NH_4^+$, 30%), 1228 (32), 1229 (25).

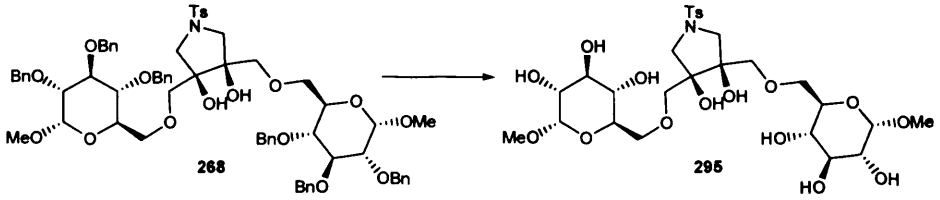
6.3.4 (3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-3,4-*bis*-(methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3,4-diol 268



The general pinacol coupling procedure was used (see experiment 6.2.6) using the diketone **289** (0.33 g, 0.28 mmol), samarium metal (0.25 g, 1.66 mmol), diiodomethane (0.11 ml, 0.37g, 1.39 mmol), and ^{t}BuOH (0.12g, 0.156 ml, 1.66 mmol) in THF (14 ml). The reaction was stirred for 18 h before being worked up and crude product was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *diol* **268** (0.16 g, 48% from **283**) as a colourless oil. $[\alpha]^{20}_D$: +38.0 (c= 1.16 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3425w (OH), 3053s, 2986s, 2305m, 1605w, 1550m, 1422m, 1276s, 1253s, 896s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.38 (3H, s, Ar 4-Me), 3.24 (2H, d, *J*10.0, 2xC*H*HN), 3.35-3.43 (8H, m, 2xCH*H*N, 2xCH_2O, 2xH-4), 3.35 (6H, s, 2xOMe), 3.36 -3.43 (6H, m, 2xH-4, 2xCH_2O), 3.48-3.55 (4H, m, 2xH-2, 2xH-5), 3.58-3.73 (4H, m, 4xH-6), 3.99 (2H, app t, *J*9.2, 2xH-3), 4.57 (2H, d, *J*11.0, 2xPhC*H*HO), 4.60 (2H, d, *J*3.9, 2xH-1), 4.64 (2H, d, *J*11.0, 2xPhC*H*HO), 4.86 (2H, d, *J*11.0, 2xPhCH*H*O), 4.90 (2H, d, *J*11.0, 2xPhC*H*HO), 4.99 (2H, d, *J*11.0, 2xPhCH*H*O), 5.0 (2H, d, *J*11.0, 2xPhCH*H*O), 7.25-7.35 (32H, m, 30xPh-H, 2xAr H-3), 7.69 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.41 (CH_3), 54.46 (2 × CH_2), 55.22

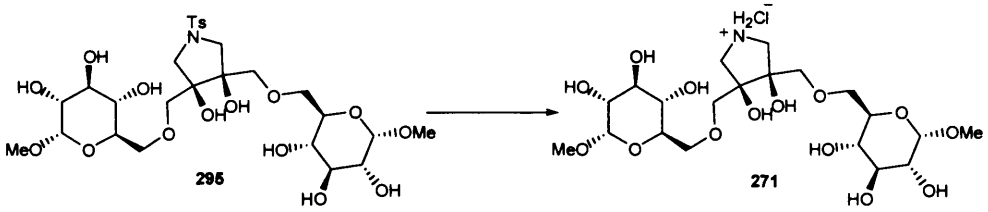
(2 × CH_3), 69.95 (2 × CH), 70.64 and 70.88 (2 × CH_2), 72.40 and 72.50 (2 × CH_2), 73.30 and 73.34 (2 × CH_2), 74.84 and 74.98 (2 × CH_2), 75.68 and 75.74 (2 × CH_2), 77.57 (2 × CH), 79.92 (2 × C), 80.79 (2 × CH), 81.78 (2 × CH), 98.00 (2 × CH), 127.44 (2 × CH), 127.57 (2 × CH), 127.61 (2 × CH), 127.70 (4 × CH), 127.88 (4 × CH), 127.91 (4 × CH), 127.93 (4 × CH), 128.36 (10 × CH), 129.49 (2 × CH), 133.63 (C), 137.91 and 137.94 (2 × C), 138.02 (2 × C), 138.43 and 138.50 (2 × C), 143.31 (C); MS (FAB) m/z (%): 1229 ($M+NH_4^+$, 100%), 1210 (80, $M+H^+$), 1016 (60), 1173 (55).

6.3.5 (3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-3,4-*bis*-(methyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3,4-diol 295



The diol **268** (0.034g, 0.028 mmol) was dissolved in ethanol (2.0 ml) and acetic acid (0.21 ml) and was subjected to the general hydrogenolysis procedure (outlined in experiment 6.2.7) using $Pd(OAc)_2$ (0.011 g, 0.049 mmol). The reaction mixture was left stirring under hydrogen for 5 h before being filtered. Solvent was removed under vacuum and the resulting crude product was purified by flash column chromatography on silica (20:80, methanol: dichloromethane) to give the *octol* **295** (0.015g, 81%) as a foam. $[\alpha]^{20}_D$: +65.1 (c= 1.57 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3623br (OH), 3399br (OH), 2945s, 1449m, 1338m, 1274s, 1270m, 1260m, 1015s, 814w; δ_H (400 MHz; D_2O): 2.50 (3H, s, Ar 4-Me), 3.22 (1H, d, *J*10.4, C*H*HN), 3.26 (1H, d, *J*10.6, CH*H*N), 3.40 (2H, app t, *J*9.2, 2xH-4), 3.41 (1H, d, *J*10.4, CH*H*N), 3.00-3.42 (2H, m overlapping, 2xH-5), 3.45 (6H, s, 2xOMe), 3.46 (1H, d, *J*10.6, CH*H*N), 3.54-3.75 (12H, m, 2xH-2, 2xH-3, 4xH-6, 2xCH_2O), 7.56 (2H, d, *J*8.0, 2xAr H-2), 7.82 (2H, d, *J*8.0, 2xAr H-3); δ_C (100.6 MHz; D_2O): 21.78 (CH_3), 53.46 and 53.84 (2 × CH_2), 55.19 (2 × CH_3), 69.70 (2 × CH), 70.01 (2 × CH_2), 70.17 (2 × CH_2), 70.39 (2 × CH), 71.23 (2 × CH), 73.03 and 73.11 (2 × CH), 79.29 and 79.40 (2 × C), 99.29 (2 × CH), 127.37 (2 × CH), 130.28 (2 × CH), 131.41 (C), 145.56 (C); MS (FAB) m/z (%): 269 (100%), 713 (98), 692 (67, $M+Na^+$), 61 (51); Accurate mass (FAB): Found 670.2380 ($M+H^+$ $C_{27}H_{44}NO_{16}S$ requires 670.2381).

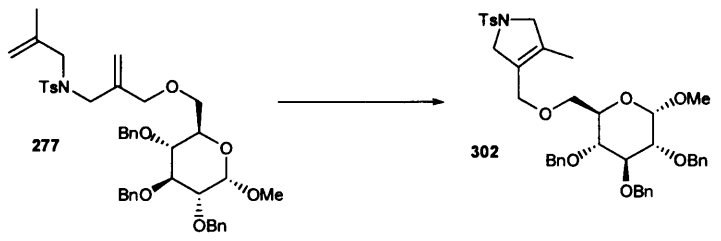
6.3.6 (3*R*, 4*S*)-3,3-*Bis*-(methyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3,4-diol-hydrochloride 271



The octol **295** (0.012 g, 0.017 mmol) was subjected to the general tosyl deprotection procedure (see experiment 6.2.9) using sodium metal (0.0010 g, 0.035 mmol) in liquid ammonia (5-10 ml). After the addition of sodium was complete the reaction was warmed to room temperature and the liquid ammonia was allowed to evaporate. The crude residue was dissolved into water and transferred to a round bottom flask, the water was removed under reduced pressure and the remaining material was purified by flash column chromatography on silica (100% methanol) to give the *amine* **271** (0.004 g, 45%) as an oil. To aid analysis dilute HCl (aq) was added to the amine in water until the solution was approximately pH 2~3 giving the corresponding hydrochloride salt of **271**. $[\alpha]^{20}_{D}$: +6.5 (c= 0.12 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3177br (OH), 1574m, 1403w, 1329m, 1156s, 1058m, 1009s, 955s, 813s, 708m; δ_H (400 MHz; D_2O): 3.47 (6H, s, 2xOMe), 3.49 (2H, d, *J*13.6, 2xC*H*HN), 3.50 (2H, app t, *J*9.6, 2xH-4), 3.60 (2H, dd, *J*9.6, 3.6, 2xH-2), 3.64 (2H, d, *J*13.6, 2xCH*H*N), 3.71 (2H, app t, *J*9.6, 2xH-3), 3.72 (2H, dd, *J*10.4, 2.8, 2xH-6), 3.77 (2H, dd, *J*10.4, 4.0, 2xH-6), 3.80-3.90 (6H, m, 2xH-5, 2xCH_2O), 4.86 (2H, d, *J*3.6, 2xH-1); δ_C (100.6 MHz; D_2O): 51.82, (2 × CH_2), 55.28 (2 × CH_3), 69.59 (2 × CH), 70.27 (2 × CH_2), 70.40 and 70.52 (2 × CH), 71.21 (2 × CH), 71.84 (2 × CH_2), 73.02 and 73.08 (2 × CH), 79.14 (2 × C), 99.41 (2 × CH); (ES) m/z (%): 516 ($M+H^+$, 100%), 538 (22, $M+Na^+$); Accurate mass (FAB): Found 516.2291 ($M+H^+$ $C_{20}H_{38}NO_{14}$ requires 516.2292).

6.4 Alternative route to diols 266 and 267

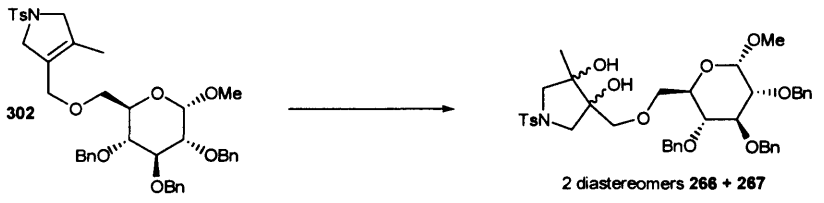
6.4.1 3-Methyl-1-(toluene-4-sulfonyl)-4-(methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside]ylmethyl)-2,5-dihydro-1*H*-pyrrole 302[88]



The diene **277** (0.067 g, 0.082 mmol) dissolved in toluene (2.0 ml) was added to tricyclohexylphosphine-[1,3-*bis*(3,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene]-

[benzylidine] Ruthenium (IV)dichloride (Grubbs 2nd generation catalyst) **301** (0.0056 g, 0.0065 mmol). The reaction mixture was refluxed (80 °C) for 8 h before another portion of catalyst (0.0056 g, 0.0065 mmol) was added. The reaction mixture was stirred a further 18 h before being filtered through a pad of silica and washed through with ethyl acetate (3x10 ml). The solvent was removed under reduced pressure and the resulting crude mixture was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *alkene* **302** as an off-white oil (0.030 g, 52%) together with recovered starting material (0.0278 g, 46%). $[\alpha]^{20}_D$: +43.7 (c=1.00 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3030w, 2914w, 1598w, 1454m, 1343m, 1160s, 1093s, 1043s, 911w, 815w, 736s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.53 (3H, s, CH_3), 2.34 (3H, s, Ar 4-Me), 3.33 (3H, s, OMe), 3.46 (1H, app t, *J*9.9, H-4), 3.49 (1H, dd, *J*9.9, 3.6, H-2), 3.42-3.52 (2H, m overlapping, CH_2N), 3.64-3.68 (1H, m, H-3), 3.74-3.98 (5H, m, CH_2, CH_2O, H-6), 4.05-4.14 (2H, m, H-5, H-6), 4.48 (1H, d, *J*11.1, PhC*H*HO), 4.57 (1H, d, *J*3.6, H-1), 4.64 (1H, d, *J*12.0, PhC*H*HO), 4.75 (1H, d, *J*12.0, PhCH*H*O), 4.77 (1H, d, *J*10.8, PhC*H*HO), 4.84 (1H, d, *J*11.1, PhCH*H*O), 4.96 (1H, d, *J*10.8, PhCH*H*O), 7.20-7.35 (17H, m, 15xPh-H, 2xAr H-3), 7.65 (2H, d, *J*8.1, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 11.29 (CH_3), 21.32 (CH_3), 55.00 (CH_3), 56.22 (CH_2), 58.817 (CH_2), 65.22 (CH_2), 68.89 (CH_2), 69.79 (CH), 73.23 (CH_2), 74.828 (CH_2), 75.58 (CH_2), 79.40 (CH), 79.74 (CH), 81.88 (CH), 97.96 (CH), 127.29 (2 × CH), 127.53 (CH), 127.57 (CH), 127.77 (CH), 127.78 (CH), 127.97 (2 × CH), 128.24 (2 × CH), 128.28 (3 × CH), 128.31 (4 × CH), 129.56 (2 × CH), 130.61 (C), 133.16 (C), 133.95 (C), 137.97 (C), 138.12 (C), 138.58 (C), 143.20 (C); MS (ES) m/z (%): 731 ($M+H^+$, 100%); Accurate mass (FAB): Found 714.3101 ($M+H^+$ $C_{41}H_{48}NO_8S$ requires 714.3101).

6.4.2 (3*S*, 4*S*) and (3*R*, 4*R*)-3-Methyl-1-(toluene-4-sulfonyl)-4-(methyl-[O^2, O^3, O^4-tribenzyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3,4-diol 266 and 267[89]



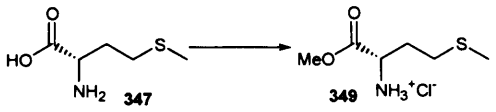
The alkene **302** (0.061 g, 0.082 mmol) was dissolved in acetone (2 ml) and to this solution OsO_4 (2.5% by wt in ^{t}BuOH solution, 0.0033 ml, 0.0033 mmol) was added. This solution was then added to NMO (0.014 g, 0.12 mmol) dissolved in water (1 ml). The reaction mixture was stirred for 24 h before being quenched with 1% aqueous sodium dithionite (10 ml). The mixture was stirred a further 30 min before the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (50:50, ethyl

acetate: petroleum-ether) to give the diol **266** (0.017 g, 26%) as a colourless oil together with diastereoisomer **267** (0.01 g, 16%) as a colourless oil and a mixed fraction (0.021 g, 33%). Data as for 6.2.6.

Studies towards the synthesis of (1→6) linked homoaza-*O*- and homoaza-*N*-disaccharides

6.5 Synthesis of the diene precursors 345 and 346[106]

6.5.1 L-Methionine methyl ester hydrochloride 349



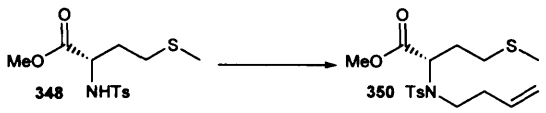
L-Methionine **347** (21.9 g, 150 mmol) was suspended in methanol (175 ml) at 0 °C and thionyl chloride (34.9 g, 21.3 ml, 300 mmol) was slowly added to the stirring solution. The solution was warmed to room temperature and stirred for 24 h before the solvent was removed under reduced pressure to give the *methyl ester* **349** (29.2 g, 99%) as a white solid. Mp: 146-147 °C (from methanol) (Lit.[180], 135 °C (from acetone)); $[\alpha]^{20}_{D}$: +26.3 (c=1.81 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3053s, 2986m, 1751m (C=O), 1421m, 1266s, 896m; δ_H (250 MHz; CD_3OD; Me_4Si): 1.96 (3H, s, CH_3S), 2.14-2.16 (2H, m, CH_2CH_2S), 2.5 (2H, app t, *J*7.4, CH_2CH_2S), 3.7 (3H, s, OMe), 4.06 (1H, app t, *J*6.4, CHN); δ_C (62.9 MHz; CD_3OD; Me_4Si): 15.03 (CH_3), 29.43 (CH_2), 29.48 (CH_2), 51.83 (CH_3), 53.44 (CH), 169.69 (C); (ES) m/z (%): 164 ($M+H^+$, 45%), 147 (30, $M-NH_2$), 327 (22); Accurate mass (FAB): Found 164.0746 ($M+H^+$ $C_6H_{14}NO_2S$ requires 164.0745).

6.5.2 (*S*)-4-Methylsulfanyl-2-(toluene-4-sulfonylamino)-butyric acid methyl ester 348

Methionine methyl ester **349** (13.9 g, 70 mmol) was dissolved in CH_2Cl_2 (150 ml) and cooled to 0 °C. To this solution triethylamine (15.6 g, 21.5 ml, 150.0 mmol) was added and the mixture was stirred for 20 min. *p*-Toluenesulfonyl chloride (14.7 g, 77.0 mmol) and DMAP (0.86 g, 7.0 mmol) were then added to the stirring solution and the mixture was stirred for a further 20 min. The mixture was warmed to room temperature and stirred for 3 hours before being quenched with water (20 ml). The organic product was extracted into dichloromethane (3x30 ml). The organic extracts were combined, dried ($MgSO_4$) and the solvent was removed

under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (50:50, diethyl ether: petroleum-ether) to give the *sulfonamide* **348** (20.2 g, 92%) as a white solid. Mp: 53-54 °C; $[\alpha]^{20}_D$: -8.2 (c=1.75 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3329m (NH), 3054s, 2986m, 1742m (C=O), 1598s, 1494s, 1421m, 1346m, 1270s, 1163s, 1091m, 1010w, 896m, 815m.; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.82-2.02 (2H, m, C*H*$_2$CH$_2$S), 2.06 (3H, s, CH_3S), 2.42 (3H, s, Ar 4-Me), 2.55 (2H, br t, *J*6.8, CH_2CH_2S), 3.53 (3H, s, OMe), 4.07 (1H, dt, *J*7.8, 4.8, CHN), 5.28 (1H, br d, *J*7.8, NH), 7.30 (2H, d, *J*8.0, 2xAr H-3), 7.70 (2H, d, *J*8.0, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 15.34 (CH_3), 21.51 (CH_3), 29.61 (CH_2), 32.60 (CH_2), 52.61 (CH), 54.61 (CH_3), 127.29 (2 × CH), 129.66 (2 × CH), 136.50 (C), 144.77 (C), 169.62 (C); MS (ES) m/z (%): 258 (M-CO_2Me, 100%), 318 (35, $M+H^+$); Accurate mass (FAB): Found 318.0834 ($M+H^+$ $C_{13}H_{20}NO_4S_2$ requires 318.0834); Anal. Found: C, 49.32; H, 5.95; N, 4.47; $C_{13}H_{19}O_4NS_2$ requires: C, 49.19; H, 6.03; N, 4.41.

6.5.3 (*S*)-2-[But-3-enyl-(toluene-4-sulfonyl)amino]-4-methylsulfanyl butyric acid methyl ester 350



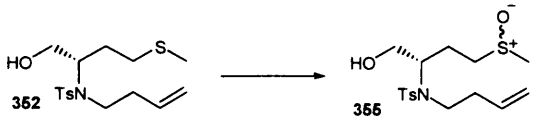
The sulfonamide **348** (1.00 g, 3.15 mmol), was dissolved in DMF (10 ml), and cooled to 0 °C. To this stirring solution K_2CO_3 (0.48 g, 3.47 mmol) was added followed by 4-bromobut-1-ene (0.51 g, 0.38 ml, 3.8 mmol) and the reaction was stirred at room temperature for 48 h. The reaction mixture was poured into water (20 ml) and the organic species extracted into ethyl acetate (3x10 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (20:80, ethyl acetate: petroleum-ether) to give the *alkylated product* **350** (0.24 g, 18%, 87% based on recovered SM) as a yellow oil together with recovered starting material **348** (0.76 g). $[\alpha]^{20}_D$: -35.2 (c=1.38 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3053s, 2986m, 1741w (C=O), 1598s, 1421m, 1266s, 1157s, 1091m, 1010w, 896m, 806m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.82-2.02 (1H, m, C*H*HCH$_2$S), 2.10 (3H, s, CH_3S), 2.13-2.34 (1H, m, CH*H*CH$_2$S), 2.42 (3H, s, Ar 4-Me) 2.44-2.61 (4H, m, CH_2CH_2S, C*H*$_2$CH=CH$_2$), 3.12 (1H, m, NC*H*H), 3.33 (1H, ddd, *J*15.4, 10.5, 5.3, NCH*H*), 3.50 (3H, s, OMe), 4.66 (1H, dd, *J*9.0, 5.2, CHN), 5.03 (1H, dd, *J*8.2, 1.4, CH=C*H*H), 5.07 (1H, dd, *J*14.0, 1.4, CH=CH*H*), 5.62-5.76 (1H, dddd, *J*13.5, 8.2, 6.9, 3.7, C*H*=CH$_2$), 7.28 (2H, d, *J*9.0, 2xAr H-3), 7.72 (2H, d, *J*9.0, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 15.34 (CH_3), 21.50 (CH_3), 29.90 (CH_2), 30.62 (CH_2), 35.10 (CH_2), 45.90 (CH_2), 52.05 (CH_3), 58.88 (CH) 117.11 (CH_2), 127.48 (2 × CH), 128.13 (CH), 129.40 (2 × CH), 134.61 (C), 143.39 (C), 171.07 (C); MS (ES) m/z (%): 389

($M+NH_4^+$, 100%), 372 (35, $M+H^+$), 390 (22); Accurate mass (FAB): Found 372.1304 ($M+H^+$ $C_{17}H_{26}NO_4S_2$ requires 372.1303).

6.5.4 (*S*)-*N*-But-3-enyl-*N*-(1-hydroxymethyl-3-methylsulfanylpropyl)-4-methylbenzenesulfonamide 352

The methyl ester **350** (0.96 g, 2.6 mmol) was dissolved in THF (20 ml) and cooled to 0 °C. To this solution lithium aluminium hydride (0.11 g, 3.01 mmol) was added portion-wise over 5 min. After 1 h the reaction mixture was added carefully to iced water (40 ml) and the resulting mixture was stirred for 10 min before aqueous HCl (1 $moldm^{-3}$, 20 ml) was added. The organic product was extracted into ethyl acetate (3x30 ml), the organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (25:75, ethyl acetate: petroleum-ether) to give the *alcohol* **352** (0.65 g, 73%) as a colourless oil. $[\alpha]^{20}_D$: -3.73 (c=1.00 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3611m (OH), 3053s, 2984m, 2921m, 1641 (C=C), 1598s, 1421m, 1336s, 1261s, 1155s, 1119m, 1010w, 896m, 806m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.54-1.79 (2H, m, CH_2CH_2S), 1.98 (3H, s, CH_3S), 2.17-2.33 (2H, m, CH_2CH_2S), 2.42 (3H, s, Ar 4-Me) 2.36-2.51 (2H, m obscured, $CH_2CH{=}CH_2$), 3.11-3.35 (2H, m, NCH_2), 3.59 (2H, app t, *J*6.2, CH_2OH), 3.88 (1H, app quintet, *J*6.2, CHN), 5.05 (1H, dd, *J*10.6, 1.6, CH=CHH), 5.07 (1H, dd, *J*16.0, 1.6, CH=CHH), 5.73 (1H, ddt, *J*16.0, 10.0, 6.9, $CH{=}CH_2$), 7.30 (2H, d, *J*8.3, 2xAr H-3), 7.76 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 15.38 (CH_3), 21.50 (CH_3), 29.00 (CH_2), 30.88 (CH_2), 35.34 (CH_2), 44.21 (CH_2), 59.44 (CH), 63.43 (CH_2), 117.25 (CH_2), 127.28 (2 × CH), 129.72 (2 × CH), 134.78 (CH), 137.82 (C), 143.51 (C); MS (ES) m/z (%): 344 ($M+H^+$, 70%), 372 (35) 706 (45), 361 (35, $M+NH_4^+$); Accurate mass (FAB): Found 344.1354 ($M+H^+$ $C_{16}H_{26}NO_3S_2$ requires 344.1354); Anal. Found: C, 55.76; H, 7.50; N, 3.97; $C_{16}H_{25}O_3NS_2$ requires: C, 55.95; H, 7.34; N, 4.08.

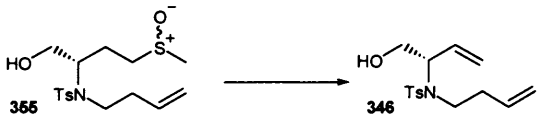
6.5.5 (*S*)-*N*-But-3-enyl-*N*-(1-hydroxymethyl-3-methanesulfonylpropyl)-4-methylbenzenesulfonamide 355



The alcohol **352** (0.72 g, 2.1 mmol) was dissolved in methanol (25 ml) and cooled to 0 °C. Sodium periodate (0.49 g, 2.3 mmol) in water (12 ml) was added to the stirring solution and a

white precipitate formed. After 1.5 h the reaction mixture was filtered through celite and the filtrate was concentrated under vacuum. The resulting crude material was dissolved in ethyl acetate and was washed with water (2x10 ml). The organic product was extracted into ethyl acetate (3x10 ml), the organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum to the give the *sulfoxide* **355** (approximately 1:1 mixture of diastereoisomers) (0.67 g, 89%) as a white solid. Data for isomeric mixture: Mp 94-95 °C; ν_{max} (CH_2Cl_2)/ cm^{-1}: 3692w (OH), 3053w, 2986m, 1598w, 1421m, 1335s, 1267s, 1156s, 1090m, 1047w, 896m, 759m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.78-1.97 (1H, m, C*H*HCH$_2$S), 2.03-2.15 (1H, m, CH*H*CH$_2$S), 2.26-2.50 (2H, m obscured C*H*$_2$CH=CH$_2$), 2.40 (3H, s, Ar 4-Me), 2.52 and 2.53 (3H, s, SCH$_3$), 2.59-2.80 (2H, m, CH$_2$S), 3.12-3.35 (2H, m, NCH$_2$), 3.52 (1H, d, *J*10.0, C*H*HOH), 3.52 (1H, d, *J*10.0, CH*H*OH), 3.84-3.98 (1H, m, CHN), 5.04 (1H, d, *J*10.6, CH=C*H*H), 5.05 (1H, d, *J*16.0, CH=C*H*H), 5.60-5.80 (1H, m, C*H*=CH$_2$), 7.30 (2H, d, *J*8.1, 2xAr H-3), 7.74 (2H, d, *J*8.1, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.48 (CH$_3$), 22.72 and 23.39 (CH$_2$), 35.26 (CH$_2$), 38.44 and 38.72 (CH$_3$), 43.86 and 44.08 (CH$_2$), 51.08 (CH$_2$), 58.72 and 59.66 (CH), 63.25 (CH$_2$), 117.37 (CH$_2$), 127.20 (2 × CH), 129.75 (2 × CH), 134.68 (CH), 137.72 (C), 143.58 (C); MS (ES) m/z (%): 360 ($M+H^+$, 70%), 737 (30), 377 (28, $M+NH_4^+$); Accurate mass (FAB): Found 360.1304 ($M+H^+$ $C_{16}H_{26}NO_4S_2$ requires 360.1303).

6.5.6 (*S*)-*N*-But-3-enyl-*N*-(1-hydroxymethylallyl)-4-methylbenzenesulfonamide 346



The sulfoxide **355** (0.22 g, 0.628 mmol) was dissolved in dry dichlorobenzene (10 ml) and the solution was degassed (3 x cycles freeze-pump-thaw). The reaction mixture was then heated to 185 °C for 1.5 h. Dichlorobenzene was removed under reduced pressure and the resulting brown residue was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *diene* **346** (0.14 g, 75%, 91% based on recovered SM) as a yellow oil together with recovered sulfoxide **355** (0.01 g). $[\alpha]^{20}_D$: +25.6 (c=1.26 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3624w (OH), 3053s, 2986m, 1641 (C=C), 1421m, 1261s, 1155s, 1119m, 1010w, 896m, 806m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.42 (5H, br s, Ar 4-Me, C*H*$_2$CH=CH$_2$), 3.08 (1H, ddd, *J*14.9, 9.0, 6.2, NC*H*H), 3.32 (1H, ddd, *J*14.9, 9.2, 6.7, NCH*H*), 3.68 (1H, dd, *J*11.4, 8.5, C*H*HOH), 3.70 (1H, dd, *J*11.4, 5.9, CH*H*OH), 4.34-4.42 (1H, m, CHN), 5.02 (1H, dd, *J*16.0, 1.2, CHCH=C*H*H), 5.07 (1H, d, *J*10.0, 1.2, CH$_2$CH=C*H*H), 5.13 (1H, d, *J*17.2, 1.2, CH$_2$CH=CH*H*), 5.14 (1H, dd, *J*10.3, 1.2, CHCH=CH*H*), 5.47 (1H, ddd, *J*16.0, 10.3, 6.0,

NCHC*H*=CH_2), 5.75 (1H, ddt, *J*17.2, 10.0, 6.9, CH_2CH=CH_2), 7.30 (2H, d, *J*8.3, 2xAr H-3), 7.74 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.47 (CH_3), 35.37 (CH_2), 44. 49 (CH_2), 61.73 (CH), 62.46 (CH_2), 117.32 (CH_2), 119.49 (CH_2), 127.29 (2 × CH), 129.65 (2 × CH), 132.48 (CH), 134.83 (CH), 137.38 (C), 143.47 (C); MS (ES) m/z (%): 296 ($M+H^+$, 70%), 313 (42, $M+NH_4^+$), 609 (39); Accurate mass (FAB): Found 296.1320 ($M+H^+$ $C_{15}H_{22}NO_3S$ requires 296.1320); Anal. Found: C, 60.87; H, 7.32; N, 4.73; $C_{15}H_{21}O_3NS$ requires: C, 60.99; H, 7.17; N, 4.74.

6.5.7 (*S*)-*N*-(1-Hydroxymethyl-3-methylsulfanylpropyl)-4-methylbenzenesulfonamide 351

The methyl ester **348** (1.0 g, 3.15 mmol) was dissolved in methanol (20 ml) and reduced following the procedure outlined in experiment 6.5.4 using lithium aluminium hydride (0.13 g, 3.47 mmol). The reaction was left 1 h before being worked up. The resulting crude material was purified by flash column chromatography on silica (40:60, ethyl acetate: petroleum-ether) to give the *alcohol* **351** (0.59 g, 65%) as white solid; $[\alpha]^{20}_D$: -15.9 (c=2.38 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3688w (OH), 3368w (NH), 3054s, 2985m, 2924m, 2854w, 1598w, 1421m, 1260s, 1160s, 1092w, 895m, 816m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.70 (2H, ddd, *J*14.2, 9.7, 7.1, C*H*$_2$$CH_2S$), 1.96 (3H, s, CH_3S), 2.32-2.39 (2H, m, CH_2C*H*$_2$S), 2.43 (3H, s, Ar 4-Me), 3.39-2.48 (1H, m, CHN), 3.52 (1H, dd, *J*11.2, 4.1 C*H*HOH), 3.58 (1H, dd, *J*11.2, 4.1 CH*H*OH), 5.24 (1H, br d, *J*8.0, NH), 7.32 (2H, d, *J*8.0, 2xAr H-3), 7.80 (2H, d, *J*8.0, 2xAr H-3); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 15.03 (CH_3), 21.51 (CH_3), 30.21 (CH_2), 30.82 (CH_2), 54.53 (CH), 64.43 (CH_2), 127.11 (2 × CH), 129.75 (2 × CH), 137.55 (C), 143.61 (C); MS (ES) m/z (%): 290 ($M+H^+$, 100%), 307 (45, $M+NH_4^+$), 596 (51); Accurate mass (FAB): Found 290.0884 ($M+H^+$ $C_{12}H_{20}NO_3S_2$ requires 290.0885).

6.5.8 (*S*)-*N*-But-3-enyl-*N*-(1-hydroxymethyl-3-methylsulfanylpropyl)-4-methylbenzenesulfonamide 352

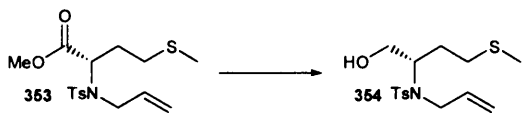
Caesium carbonate (0.12 g, 0.38 mmol) was added to the alcohol **351** (0.1 g, 0.35 mmol) dissolved in acetonitrile (2 ml). The reaction was stirred for 0.5 h before 4-bromobut-1-ene (0.06 g, 0.05 ml, 0.45 mmol) was added and the mixture was then refluxed for a total of 6 h. The mixture was filtered and washed with CH_2Cl_2 (10 ml). The filtrate was washed with water (30 ml) and these washings were extracted with ethyl acetate (3x20 ml). The organic

layers were combined, dried ($MgSO_4$) and the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *alkylated alcohol* **352** (0.032 g, 27%, 46% based on recovered SM) as a colourless oil together with recovered starting material **352** (0.017 g). Data as for experiment 6.5.4.

6.5.9 (*S*)-2-[Allyl-(toluene-4-sulfanyl)-amino]-4-butyric acid methyl ester 353

The methyl ester **348** (2.23 g, 7.0 mmol) was alkylated using potassium carbonate (5.25 g, 38 mmol) and allyl bromide (1.36g, 0.97 ml, 10 mmol) following the procedure outlined in experiment 6.5.3. The reaction was stirred at room temperature for 15 h before being worked up to give the *alkylated product* **353** (2.11 g, 84%) as yellow oil. $[\alpha]^{20}_D$: -33.3 (c=2.41 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3063w, 2953m, 2921m, 1742s, (C=O), 1641w, 1598m, 1495w, 1436s, 1344s, 1306s, 1289s, 1274m, 1272m, 1267m, 1265m, 1260s, 1258s, 1234m, 815m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.97 (1H, ddd, *J*14.2, 8.0, 6.2, C*H*HCH$_2$S), 2.07 (3H, s, SCH_3), 2.17 (1H, d app td, *J*14.2, 7.6, 6.4, CH*H*CH$_2$S), 2.42 (3H, s, CH_3), 2.43-2.66 (2H, m, CH$_2$C*H*$_2$S), 3.51 (3H, s, OMe), 3.76 (1H, dd, *J*16.3, 7.1, NC*H*H), 3.95 (1H, dd, *J*16.3, 5.7, NCH*H*), 4.69 (1H, dd, *J*8.0, 7.6, CH), 5.07 (1H, dd, *J*17.2, 1.4, CH=C*H*H), 5.19 (1H, dd, *J*10.0, 1.4, CH=C*H*H), 5.82 (1H, dddd, *J*17.2, 10.0, 7.1, 5.7, C*H*=CH$_2$), 7.28 (2H, d, *J*8.3, 2xAr H-3), 7.71 (2H, d, *J*8.3, 2xAr H-3); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 15.36 (CH_3), 21.53 (CH_3), 29.60 (CH_2), 30.57 (CH_2), 48.65 (CH_2), 52.05 (CH_3), 58.58 (CH), 118.00 (CH_2), 127.54 (2 × CH), 129.44 (2 × CH), 134.87 (CH), 137.05 (C), 143.44 (C), 171.10 (C); MS (ES) m/z (%): 358 ($M+H^+$, 45%), 359 (15); Accurate mass (FAB): Found 358.1146 ($M+H^+$ $C_{16}H_{24}NO_4S_2$ requires 358.1147); Anal. Found: C, 53.61; H, 6.45; N, 3.83; $C_{16}H_{23}O_4NS_2$ requires: C, 53.76; H, 6.48; N, 3.92.

6.5.10 (*S*)-*N*-Allyl-*N*-(1-hydroxymethyl-3-methylsulfanylpropyl)-4-methylbenzenesulfonamide 354



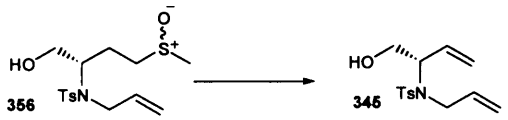
The methyl ester **353** (0.113 g, 5.9 mmol) was dissolved in THF (45 ml) and reduced with lithium aluminium hydride (0.29 g, 7.7 mmol) using the same procedure outlined in experiment 6.5.4. The reaction was worked up after 3 h and the resulting crude material was

purified by flash column chromatography on silica (20:80, ethyl acetate: petroleum-ether) to give the *alcohol* **354** (1.62 g, 83%) as a colourless oil. $[\alpha]^{20}_D$: -7.8 (c=0.98 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3599w (OH), 3057m, 2921m, 1653w, 1599m (aromatic), 1419m, 1334s, 1305s, 1274s, 1272m, 1263w, 1260w, 1257w, 1158m, 1119m, 1091s, 1045s, 992s, 816s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.66-1.79 (2H, m, C*H*$_2$CH$_2$S), 1.99 (3H, s, SCH_3), 2.16-2.37 (2H, m, CH_2C*H*$_2$S), 2.42 (3H, s, Ar 4-Me), 3.58 (1H, dd, *J*12.7, 6.0 C*H*HOH), 3.63 (1H, dd, *J*12.7, 5.7 CH*H*OH), 3.79 (1H, dd, *J*16.3, 6.7, NC*H*H), 3.89-3.99 (2H, m, NCH*H*, CHN), 5.14 (1H, dd, *J*10.1, 1.2, CH=C*H*H), 5.27 (1H, dd, *J*17.2, 1.2, CH=C*H*H), 5.86 (1H, dd app t, *J*17.2, 10.1, 6.7, C*H*=CH_2), 7.30 (2H, d, *J*8.3, 2xAr H-3), 7.75 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 15.32 (CH_3), 21.46 (CH_3), 28.78 (CH_2), 30.74 (CH_2), 47.00 (CH_2), 59.21 (CH), 63.37 (CH_2), 117.99 (CH_2), 127.23 (2 × CH), 129.65 (2 × CH), 135.51 (CH), 137.83 (C), 143.44 (C); MS (ES) m/z (%): 330 ($M+Na^+$, 100%), 313 (60, $M+H^+$); Found 330.1197 ($M+H^+$ $C_{15}H_{24}NO_3S_2$ requires 330.12198); Anal. Found: C, 54.53; H, 6.71; N, 4.17; $C_{15}H_{23}O_3NS_2$ requires: C, 54.68; H, 7.03; N, 4.25.

6.5.11 (*S*)-*N*-Allyl-*N*-(1-hydroxymethyl-3-methanesulfonylpropyl)-4-methylbenzenesulfonamide 356

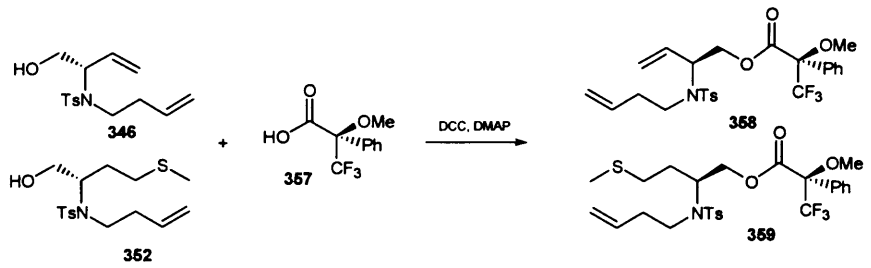
The sulfide **354** (2.7 g, 8.5 mmol) was oxidised using sodium periodate (2.0 g, 9.4 mmol in 5.0 ml water) in methanol (100 ml) using the procedure outlined in experiment 6.5.5. After 4 h the reaction was worked up to give the *sulfoxide* **356** (2.80 g, 99%) as a colourless oil (approx. 1:1 mixture of diastereoisomers). Data for isomeric mixture: ν_{max} (CH_2Cl_2)/ cm^{-1}: 3359s (OH), 3053s, 2973s, 2927s, 1743s (S=O), 1605m, 1430m, 1348s, 1268m, 1265s, 1262s, 874s, 816s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.86-2.13 (2H, m, C*H*$_2$CH$_2$S), 2.43 (3H, s, Ar 4-Me), 2.52 and 2.55 (3H, s, SCH_3), 2.58-2.82 (2H, m, CH_2S), 3.58-3.64 (2H, m, NCH_2), 3.79-3.93 (2H, m, C*H*$_2$OH), 3.95-4.06 (1H, m, CHN), 5.16 (1H, d, *J*10.1, 1.2, CH=C*H*H), 5.26 (1H, dd, *J*17.2, 1.2, CH=CH*H*), 5.86 (1H, m, C*H*=CH_2), 7.31 (2H, d, *J*8.3, 2xAr H-3), 7.75 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.50 (CH_3), 22.32 and 23.02 (CH_2), 38.41 and 38.76 (CH_3), 46.82 and 47.05 (CH_2) 50.84 and 51.02 (CH_2), 58.76 and 59.63 (CH), 63.28 (CH_2), 118.35 (CH_2), 127.27 (2 × CH), 129.75 (2 × CH), 135.23 (CH), 135.36 (C), 143.63 (C); MS (ES) m/z (%): 346 ($M+H^+$, 100%), 347 (20), 348 (15); Accurate mass (FAB): Found 346.1147 ($M+H^+$ $C_{15}H_{24}NO_4S_2$ requires 346.1147).

6.5.12 (*S*)-*N*-Allyl-*N*-(1-hydroxymethylallyl)-4-methylbenzenesulfonamide 345



The sulfoxide **356** (2.7 g, 8.3 mmol) was dissolved in dichlorobenzene (100 ml) and underwent elimination as described in experiment 6.5.6. The reaction was worked up after 1.5 h and the resulting crude material was purified by flash column chromatography on silica (20:80, ethyl acetate: petroleum-ether) to give the *diene* **345** (1.29 g, 56%) as a colourless oil. $[\alpha]^{20}_{D}$: +23.8 (c=1.93 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3598w (OH), 3063w, 2927w, 1598m, 1495m, 1419w, 1335s, 1306m, 1272m, 1267s, 1265m, 1259m, 1161w, 1091w, 1042m, 933m, 872m, 816m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.40 (3H, s, Ar 4-Me), 3.57-3.78 (3H, m, NC*H*H, C*H*$_2$OH), 3.95 (2H, dd, *J*16.3, 5.3, NCH*H*), 4.42 (1H, app td, *J*7.1, 6.4, CHN), 5.02 (1H, dd, *J*17.3, 1.4, CHCH=C*H*H), 5.12 (1H, dd, *J*10.0, 1.2, CH_2CH=C*H*H), 5.14 (1H, dd, *J*10.1, 1.4, CHCH=CH*H*), 5.16 (1H, dd, *J*17.5, 1.2, CH_2CH=CH*H*), 5.86 (1H, ddd, *J*17.3, 10.1, 6.4, CHC*H*=CH_2), 5.77-5.93 (1H, m, C*H*=CH_2), 7.27 (2H, d, *J*8.3, 2xAr H-3), 7.71 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.39 (CH_3), 47.30 (CH_2), 61.72 (CH), 62.50 (CH_2), 117.73 (CH_2), 119.58 (CH_2), 127.20 (2 × CH), 129.55 (2 × CH), 132.42 (2 × CH), 137.49 (C), 143.36 (C); MS (ES) m/z (%): 1212 (100%), 282 (22, $M+H^+$); Accurate mass (FAB): Found 282.1164 ($M+H^+$ $C_{14}H_{20}NO_3S$ requires 282.1164).

6.5.13 Synthesis of the Mosher's esters 358 and 359

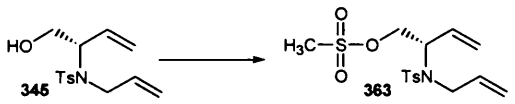


To a stirring solution of the alcohols **352** and **346** and their respective racemic standards (0.02 g, 0.07 mmol) in CH_2Cl_2 (1 ml) was added Mosher's acid (0.019 g, 0.08 mmol), DCC (0.017 g, 0.08 mmol) and DMAP (0.008 g, catalytic). The solutions were allowed to stir for 2 h before being filtered over celite (x3) and washed with ethyl acetate. Solvent was removed under reduced pressure to give the crude Mosher's esters **358** and **359**.

Racemic **346**→**358**: δ_F (400 MHz; $CDCl_3$): -71.94 (s), -71.99 (s); **346**→**358** - δ_F (400 MHz; $CDCl_3$): -71.94 (s). Racemic **352**→**359**: δ_F (400 MHz; $CDCl_3$): -71.72 (s), -71.79 (s); **352**→**359**: δ_F (400 MHz; $CDCl_3$): -71.79 (s).

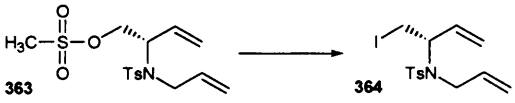
6.6 Synthesis of alkyl halide derivatives - attempts to form the diene ether

6.6.1 (*S*)-Methanesulfonic acid 2-[allyl-(toluene-4-sulfonyl)-amino]-but-3-enyl ester 363



The alcohol **345** (0.30 g, 1.10 mmol) was dissolved in CH_2Cl_2 (6 ml) and the mixture was cooled to 0 °C. To this solution triethylamine (0.12 g, 0.17 ml, 1.2 mmol) was added dropwise. The solution was stirred for 20 min and methanesulfonylchloride (0.14 g, 0.19 ml, 1.2 mmol) was then added and the solution was stirred at 0 °C for 4 h. The reaction mixture was quenched with water (10 ml) and the organic product was extracted into diethyl ether (3x10 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under vacuum to give the *mesylate* **363** (0.33 g, 84%) as a colourless oil. $[\alpha]^{20}_D$: +16.1 (c=2.35 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3688w (OH), 3054w, 2963s, 1653w, 1605w, 1593w, 1506w, 1363m, 1346m, 1277w, 1271s, 1268s, 1266s, 1262m, 1258m, 1178m, 1162w, 1091w, 816m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.40 (3H, s, Ar 4-Me), 2.98 (3H, s, OSO_2CH_3), 3.68 (1H, dd, *J*16.3, 6.7, NC*H*H), 3.92 (1H, dd, *J*16.3, 5.5, NCH*H*), 4.30 (1H, dd, *J*10.6, 7.1, C*H*HOSO$_2$CH$_3$), 4.35 (1H, dd, *J*10.6, 6.9, CH*H*OSO$_2$CH$_3$), 4.62 (1H, app q, *J*7.0, CHN), 5.12 (1H, dd, *J*16.0, 1.2, CH=C*H*H), 5.17 (1H, dd, *J*17.6, 1.2, CH=C*H*H), 5.20 (1H, dd, *J*11.0, 1.2, CH=CH*H*), 5.26 (1H, dd, *J*10.7, 1.2, CH=CH*H*), 5.58-5.82 (2H, m, C*H*=CH$_2$), 7.28 (2H, d, *J*7.8, 2xAr H-3), 7.69 (2H, d, *J*7.8, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.34 (CH_3), 37.26 (CH_3), 47.87 (CH_2), 52.47 (CH), 68.09 (CH_2), 118.29 (CH_2), 120.83 (CH_2), 127.18 (2 × CH), 129.57 (2 × CH), 131.26 (CH), 134.69 (CH), 137.24 (C), 143.53 (C); MS (ES) m/z (%): 359 ($M+NH_4^+$, 100%), 360 (35, $M+H^+$), 378 (22); Accurate mass (FAB): Found 360.0939 ($M+H^+$ $C_{15}H_{22}NO_5S_2$ requires 360.0939).

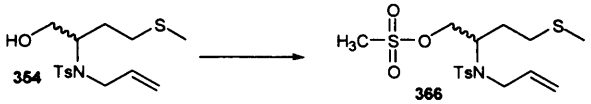
6.6.2 (*S*)-*N*-Allyl-*N*-(1-iodomethylallyl)-4-methylbenzenesulfonamide 364



The mesylate **363** (0.10 g, 0.28 mmol) dissolved in acetone (5 ml) was treated with sodium iodide (0.17 g, 1.1 mmol) as described in experiment 6.2.3. After refluxing for 12 h the reaction mixture was worked up. The resulting crude material was purified by flash column chromatography on silica (20: 80, ethyl acetate: petroleum-ether) to give the *iodide* **364** (0.30 g, 29%, 52% based on recovered SM) as a yellow oil together with recovered starting material **363** (0.048 g). δ_H (300 MHz; $CDCl_3$; Me_4Si): 2.43 (3H, s, Ar 4-Me), 3.30 (1H, dd, *J*8.3, 7.3,

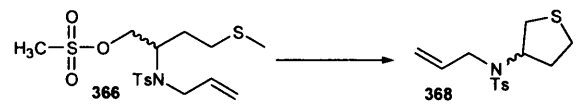
CH*H*I), 3.40 (1H, dd, *J*8.3, 5.3, CH*H*I), 3.66 (1H, ddt, *J*13.4, 6.1, 1.0, NC*H*H), 3.93 (1H, ddt, *J*13.4, 4.6, 1.0, NCH*H*), 4.50 (1H, app q, *J*5.8, CHN), 5.13 (1H, d app t, *J*16.5, 1.0, CH_2CH=C*H*H), 5.18 (1H, dd, *J*14.5, 1.0, CHCH=CH*H*), 5.22 (1H, dd, *J*8.7, 1.0, CHCH=C*H*H), 5.25 (1H, d app t, *J*8.7, 1.0, CH_2CH=CH*H*), 5.66 (1H, ddd, *J*14.5, 8.7, 5.8, C*H*=CH_2), 5.77-5.90 (1H, dddd, *J*16.5, 8.7, 6.1, 4.6, C*H*=CH_2), 7.30 (2H, d, *J*8.3, 2xAr H-3), 7.73 (2H, d, *J*8.3, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 6.25 (CH_2), 21.53 (CH_3), 47.80 (CH_2), 62.00 (CH), 118.10 (CH_2), 120.76 (CH_2), 127.35 (2 × CH), 129.61 (2 × CH), 133.4 (CH), 135.46 (CH), 137.35 (C), 143.48 (C).

6.6.3 (*R*, *S*)-Methanesulfonic acid 2-[allyl-(toluene-4-sulfonyl)-amino]-4-methylsulfanylbutyl ester 366



The alcohol **354** (1.52 g, 4.6 mmol) dissolved in CH_2Cl_2 (30 ml) was treated with triethylamine (0.46 g, 0.65 ml) and methanesulfonylchloride (1.06 g, 0.72 ml) using the procedure outlined in experiment 6.6.1. The reaction was left 4 h before being worked up to give the *mesylate* **366** (1.62 g, 86%) as a yellow oil. ν_{max} (CH_2Cl_2)/ cm^{-1}: 3057w, 2984w, 1598m, 1495m, 1420m, 1344m, 1306m, 1276m, 1271m, 1267m, 1264s, 1254m, 1178s, 1161s, 1091s, 1042s, 1015m, 959m, 878m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.75-1.91 (2H, m, C*H*$_2$CH$_2$S), 2.04 (3H, s, SCH_3), 2.25-2.39 (2H, m CH_2C*H*$_2$S), 2.43 (3H, s, Ar 4-Me), 2.95 (3H, s, OSO_2CH_3), 3.80 (1H, dd, *J*16.2, 6.4, NC*H*H), 3.9 (1H, dd, *J*16.2, 6.4, NCH*H*), 4.15-4.34 (3H, m, CH_2N, CHN), 5.15 (1H, dd, *J*10.0, 1.2, CH=C*H*H), 5.23 (1H, dd, *J*17.0, 1.2, CH=CH*H*), 5.71-5.87 (1H, dd app t, J17.0, 10.0, 6.4, C*H*=CH_2), 7.31 (2H, d, *J*8.3, 2xAr H-3), 7.74 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 15.31 (CH_3), 21.46 (CH_3), 28.83 (CH_2), 30.34 (CH_2), 37.41 (CH_3), 47.73 (CH_2), 55.97 (CH), 69.05 (CH_2), 118.67 (CH_2), 127.29 (2 × CH), 129.72 (2 × CH), 134.83 (CH), 137.61 (C), 143.63 (C); MS (ES) m/z (%): 425 ($M+NH_4^+$, 100%), 408 (40, $M+H^+$); Accurate mass (FAB): Found 430.0792 ($M+Na^+$ $C_{16}H_{25}NO_5S_3Na$ requires 430.0793).

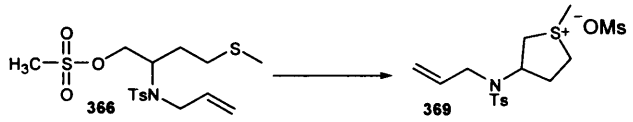
6.6.4 (*R*, *S*)-3-[Allyl-(toluene-4-sulfonyl)-amino]-tetrahydrothiophene 368



The mesylate **366** (0.40 g, 1.23 mmol) dissolved in acetone (8 ml) was treated with sodium iodide (0.55 g, 7.70 mmol) using the procedure described in experiment 6.2.3. The reaction

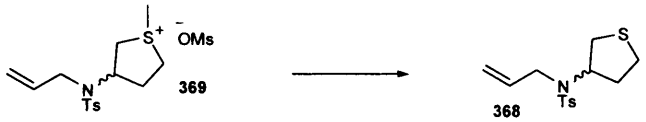
was worked up after 24 h and the resulting crude material was purified by flash column chromatography on silica (20:80, ethyl acetate: petroleum-ether) to give the *cyclic sulfide* **368** (0.21 g, 56%) as a yellow oil. ν_{max} (CH_2Cl_2)/ cm^{-1}: 3054w, 1599m, 1339m, 1281s, 1158m, 1090m, 1012w, 1001w, 813m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.82-1.99 (1H, m, C*H*HCH$_2$S), 2.02-2.18 (1H, m, CH*H*CH$_2$S), 2.43 (3H, s, Ar 4-Me), 2.69-2.78 (4H, m, CHC*H*$_2$S, CHCH$_2$C*H*$_2$S), 3.84-3.89 (2H, m, CH$_2$N), 4.33-4.46 (1H, m, CHN), 5.15 (1H, dd, *J*10.0, 1.2, CH=C*H*H), 5.26 (1H, dd, *J*17.9, 1.2, CH=CH*H*), 5.71-5.87 (1H, dddd, *J*17.9, 10.0, 7.1, 5.7 C*H*=CH$_2$), 7.31 (2H, d, *J*8.1, 2xAr H-3), 7.72 (2H, d, *J*8.1, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.49 (CH_3), 26.83 (CH_2), 30.91 (CH_2), 32.54 (CH_2), 46.49 (CH_2), 60.95 (CH), 117.18 (CH_2), 127.08 (2 × CH), 129.76 (2 × CH), 135.68 (CH), 137.53 (C), 143.43 (C); MS (ES) m/z (%): 315 ($M+NH_4^+$, 100%), 155 (78, $CH_3C_6H_4SO_2^+$), 298 (48, $M+H^+$); Accurate mass (FAB): Found 298.0936 ($M+Na^+$ $C_{14}H_{20}NO_2S_2$ requires 298.0936).

6.6.5 (*R, S*)-3-[Allyl-(toluene-4-sulfonyl)-amino]-1-methyltetrahydrothiophenium; methanesulfonate 369



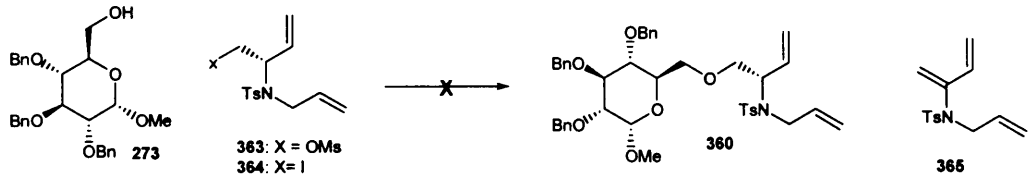
The mesylate **366** (0.06 g, 0.14 mmol) dissolved in acetone (1 ml) was refluxed for 48 h. On cooling a white solid precipitated and solvent was removed under reduced pressure to yield the *salt* **369** (0.072 g, 100%) as a white solid which was taken onto to the next step without further purification. δ_H (250 MHz; D_2O): 2.12-2.25 (1H, m, C*H*HCH$_2$S), 2.31-2.46 (1H, m, CH*H*CH$_2$S), 2.37 (3H, s, Ar 4-Me), 2.73 (3H, s, CH_3), 2.82 (3H, s, CH_3), 3.20 (1H, ddd, *J*12.6, 9.9, 7.6, SC*H*HCH$_2$), 3.26 (1H, dd, *J*13.8, 7.4, CHC*H*HS), 3.44 (1H, dd, *J*13.8, 10.0, CHCH*H*S), 3.72 (1H, ddd, *J*12.6, 7.8, 4.2, SCH*H*CH$_2$), 3.86 (2H, d, *J*5.5, C*H*$_2$CH=), 4.80 (1H, dddd, *J*13.8, 10.0, 7.4, 6.9, CH), 5.18 (1H, dd, *J*10.2, 1.4, CH=C*H*H), 5.25 (1H, dd, *J*17.2, 1.4, CH=CH*H*), 5.78 (1H, dd app t, *J*17.2, 10.2, 5.5, CH=), 7.41 (2H, d, *J*8.0, 2xAr H-3), 7.72 (2H, d, *J*8.0, 2xAr H-2); δ_C (62.9 MHz; D_2O): 21.11 (CH_3), 26.11 (CH_3), 29.78 (CH_2), 38.86 (CH_3), 41.52 (CH_2), 42.33 (CH_2), 47.32 (CH_2), 59.17 (CH), 118.40 (CH_2), 127.41 (2 × CH), 129.80 (2 × CH), 134.56 (CH), 135.07 (C), 146.27 (C); MS (ES) m/z (%): 312 (M^+, 100), 298 (10, $M+H^+$-CH_3).

6.6.6 (*R, S*)-3-[Allyl-(toluene-4-sulfonyl)-amino]-tetrahydrothiophene 368



Sodium iodide (0.042 g, 0.28 mmol) was added to a solution of the salt **369** (0.038 g, 0.23 mmol) dissolved in acetone (1 ml) as described in experiment 6.2.3. The mixture was worked up after heating for 56 h and the resulting crude material was purified by flash column chromatography (20:80, ethyl acetate: petroleum ether) to give the *cyclic sulfide* **368** (0.012 g, 46%). Data as for 6.6.4.

6.6.7 Attempt at the etherification coupling



A solution of the alcohol **273** (0.10 g, 0.22 mmol) in THF (5 ml) was added to a 0 °C solution of sodium hydride (0.009 g of a 60% dispersion in oil, 0.39 mmol) in THF (1 ml). The resulting mixture was stirred for 20 min a solution of the mesylate **363** or iodide **364** (0.32 mmol) in THF (3 ml) was added dropwise. The reaction mixture was heated at reflux for 4 h before being quenched with saturated ammonium chloride (10 ml). Water (10 ml) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3x30 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude mixture was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *recovered alcohol* **273** (0.06 g) as a white solid and the *triene* **365** as a colourless oil (~33%). Data for **365**- δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.43 (3H, s, Ar 4-Me), 3.40 (2H, m, NCH_2), 4.84 (1H, d, *J*1.0, C=C*H*H), 5.01-5.11 (2H, m, $CH_2CH=CH_2$), 5.17 (1H, d, *J*8.8, C(CH=C*H*H)), 5.31 (1H, d, *J*1.0, C=CH*H*), 5.47 (1H, d, *J*14.1, C(CH=CH*H*)), 6.26 (1H, dd, *J*14.1, 8.8, C(C*H*=CH_2)), 5.64-5.78 (1H, m, $CH_2CH=CH_2$), 7.28 (2H, d, *J*8.0, Ar H-3), 7.70 (2H, d, *J*8.0, Ar H-2).

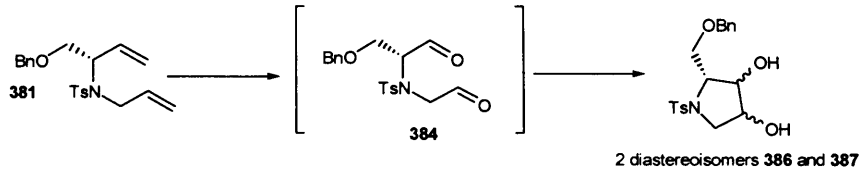
6.7 Investigations into an alternative approach to the diene ether–cyclisation of the diene before attachment of the sugar moiety 273

6.7.1 (*S*)-*N*-Allyl-*N*-(1-benzyloxymethyl-allyl)-4-methyl-benzenesulfonamide 381

The alcohol **345** (0.24 g, 0.84 mmol) was dissolved in DMF (5 ml) and treated with sodium hydride (60%, 0.04 g, 1.5 mmol) and benzyl bromide (0.29 g, 0.2 ml, 1.7 mmol) as described in 6.1.2. After 3 h the reaction was worked up. The resulting crude material was purified by column chromatography (10:90, ethyl acetate: petroleum-ether) to give the *protected diene*

381 (0.21 g, 68%) as a colourless oil. ν_{max} (CH_2Cl_2)/ cm^{-1}: 3053m, 2936m, 2853m, 1609m, 1348s, 1165s, 1082s, 1036m, 944w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.40 (3H, s, Ar 4-Me), 3.59 (1H, dd, *J*9.9, 6.4 CH*H*O), 3.66 (1H, dd, *J*9.9, 4.4, C*H*HO), 3.77 (1H, dd, *J*16.5, 6.4 CH*H*N), 3.94 (1H, dd, *J*16.5, 6.0 C*H*HO), 4.38 (1H, d, *J*12.0, PhC*H*HO), 4.49 (1H, d, *J*12.0, PhCH*H*O), 4.64-4.72 (1H, m, CHN), 5.06 (1H, dd, *J*17.2, 1.4, CH=C*H*H), 5.10 (1H, dd, *J*17.1, 0.9, CH=C*H*H), 5.18 (1H, dd, *J*10.0, 1.4, CH=C*H*H), 5.21 (1H, dd, *J*10.3, 0.9, CH=C*H*H), 5.67-5.89 (2H, m, 2xC*H*=CH_2), 7.18-7.38 (7H, m, 5xPh-H, 2xAr H-3), 7.74 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.44 (CH_3), 47.25 (CH_2), 59.24 (CH), 70.45 (CH_2), 72.89 (CH_2), 117.02 (CH_2), 118.80 (CH_2), 127.60 (2 × CH), 127.66 (CH), 127.70 (2 × CH), 128.28 (2 × CH), 129.28 (2 × CH), 133.87 (CH), 135.77 (CH), 137.79 (C), 138.09 (C), 142.88 (C); MS (ES) m/z (%): 378 ($M+H^+$, 100%), 395 (50, $M+NH_4^+$), 411 (48); Accurate mass (FAB): Found 378.1375 ($M+H^+$ $C_{19}H_{24}NO_5S$ requires 378.1375).

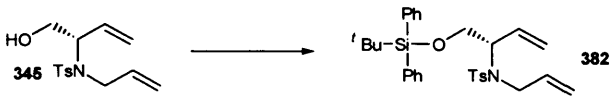
6.7.2 (2*S*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-benzyloxymethyl-pyrrolidine-3,4-diols 386 and 387



The diene **381** (0.18 g, 0.49 mmol) was dissolved in CH_2Cl_2 (10 ml) and treated with ozone using the general procedure (see experiment 6.2.5). The reaction was quenched with DMS (0.12 g, 0.14 ml, 1.94 mmol) and was stirred at room temperature for 18 h before being worked up to give crude material, which was used without further purification. This material (0.26 g, 0.7 mmol, based on **381**) underwent pinacol coupling following the general procedure (see experiment 6.2.6) using samarium metal (0.44 g, 2.92 mmol), diiodomethane (0.65 g, 0.20 ml, 2.43 mmol) and ^{t}BuOH (0.22 g, 0.27 ml, 2.92 mmol) in THF (24.3 ml). The reaction was stirred for 18 h before being worked up and the resulting crude material was purified by column chromatography (50:50, ethyl acetate: petroleum-ether) to give the *diols* **386** and **387** (in an approximate 1:1 ratio from ^{13}C NMR of the crude mixture), (diastereoisomer 1 (eluted 1st), 0.0235 g, diastereoisomer 2 (eluted 2nd), 0.0122 g, mixed fraction, 0.0205 g, 33% total yield from **381**). Data for diastereoisomer 1 **386**: ν_{max} (CH_2Cl_2)/ cm^{-1}: 3624s (OH), 3064m, 2954m, 2853m, 1619w, 1430w, 1343w, 1279s, 1086s, 1086w, 1036s, 746s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.41 (3H, s, Ar 4-Me), 3.19 (1H, dd, *J*14.0, 5.3, C*H*HN), 3.47 (1H, dd, *J*14.0, 6.9, CH*H*N), 3.53-3.68 (2H, m, C*H*HO, C*H*OH), 3.93 (1H, dd, *J*10.0, 3.5, CH*H*O), 4.11-4.12 (1H, m, CHN), 4.20-4.24 (1H, m, C*H*OH), 4.60 (2H, s, $PhCH_2$), 7.18-7.39 (7H, m, 7xPh-H, 2xAr H-3), 7.74 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.55 (CH_3), 52.71 (CH_2), 63.59 (CH), 69.85 (CH), 71.83 (CH_2), 73.76 (CH_2), 75.26 (CH), 127.76 (2 × CH),

127.85 (2 × CH), 127.91 (CH), 128.51 (2 × CH), 129.53 (2 × CH), 133.55 (C), 137.71 (C), 143.69 (C); MS (ES) m/z (%): 400 ($M+Na^+$, 100%), 416 (32), 378 (10, $M+H^+$). Data for diastereoisomer 2 **387**: ν_{max} (CH_2Cl_2)/ cm^{-1}: 3624m (OH), 3053m, 2944m, 1605m, 1336m, 1276s, 1271s, 1265s, 1254s, 1018s, 897s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.44 (3H, s, Ar 4-Me), 3.29 (1H, dd, *J*11.4, 5.1, NC*H*H), 3.46 (1H, dd, *J*11.4, 1.4, NCH*H*), 3.57-3.70 (1H, m, CHN), 3.79-4.12 (4H, m, CH_2O, C*H*OH, C*H*OH), 4.55 (1H, d, *J*11.5, C*H*HPh), 4.64 (1H, d, *J*11.5, CH*H*Ph), 7.25-7.29 (9H, m, 5xPh-H, 2xAr H-3), 7.70 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.51 (CH_3), 53.37 (CH_2), 59.94 (CH), 68.45 (CH_2O, 69.98 (CH), 72.09 (CH), 74.29 (CH_2), 127.41 (2 × CH), 128.11 (2 × CH), 128.42 (CH), 128.73 (2 × CH), 129.87 (2 × CH), 134.91 (C), 137.82 (C), 143.97 (C); (ES) m/z (%): 400 ($M+Na^+$, 100%), 416 (30), 378 (15, $M+H^+$).

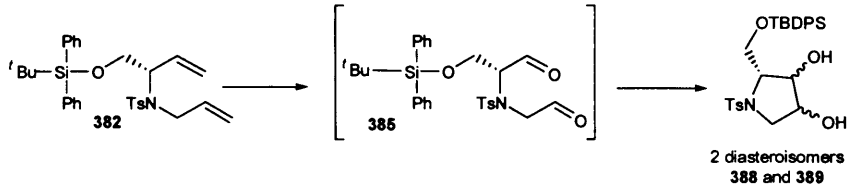
6.7.3 (*S*)-*N*-Allyl-*N*-[1-(*tert*-butyl-diphenyl-silanyloxymethyl)-allyl]-4-methylbezenesulfonamide 382



The alcohol **345** (0.18 g, 0.64 mmol) was dissolved in CH_2Cl_2 (5 ml) and cooled to 0 °C. Triethylamine (0.07 g, 0.1 ml) was added and the solution was stirred for 30 min. After this time $^tBuPh_2SiCl$ (0.18 g, 0.17 ml) followed by DMAP (0.003 g, 0.032 mmol) was added to the mixture. The reaction was stirred for 22 h before being quenched with water (5 ml). The organic product was extracted into ethyl acetate (3x10 ml), the organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (40:60, dichloromethane: petroleum-ether) to give the *protected alcohol* **382** (0.18 g, 55%) as a colourless oil. ν_{max} (CH_2Cl_2)/ cm^{-1}: 3060s, 3048s, 2986s, 2932m, 2305m, 1653w, 1599m, 1550m, 1472m, 1428s, 1280s, 1249s, 1159s, 1091s, 897s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.04 (9H, s, $C(CH_3)_3$), 2.40 (3H, s, Ar 4-Me), 3.66 (1H, dd, *J*16.5, 6.4 CH*H*N), 3.75 (1H, dd, *J*10.6, 6.8 C*H*HO), 3.81 (1H, dd, *J*10.6, 6.7, CH*H*O), 3.93 (1H, dd, *J*16.5, 6.2 C*H*HN), 4.48-4.56 (1H, app q, *J*6.7, CHN), 5.0 (1H, dd, *J*10.2, 1.4, CH=C*H*H), 5.04 (1H, dd, *J*17.5, 1.4, CH=CH*H*), 5.08 (1H, dd, *J*17.2, 1.2, CH=C*H*H), 5.14 (1H, dd, *J*10.5, 1.2, CH=CH*H*), 5.66-5.82 (2H, m, 2 x C*H*=CH_2), 7.21 (2H, d, *J*8.1, 2xAr H-3), 7.35-7.49 (6H, m, 6xPh-H), 7.56-7.64 (4H, m, 4xPh-H), 7.70 (2H, d, *J*8.1, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 19.14 (C), 21.46 (CH_3), 26.78 (3 × CH_3), 47.82 (CH_2), 61.58 (CH), 64.55 (CH_2), 117.13 (CH_2), 118.99 (CH_2), 127.32 (2 × CH), 127.69 (4 × CH), 129.43 (2 × CH), 129.75 (CH), 133.19 (2 × C), 133.88 (CH), 135.61 (4 x CH), 135.82

(CH), 138.23 (C), 142.88 (C); MS (ES) m/z (%): 537 ($M+NH_4^+$, 40%), 520 (20, $M+H^+$); Accurate mass (FAB): Found 520.2342 ($M+H^+$ $C_{30}H_{38}NO_3S$ requires 520.2342).

6.7.4 (2*R*, 3*R*, 4*S*)-2-*tert*-Butyldiphenylsilanyloxymethyl)-1-(toluene-4-sulfonyl)-pyrrolidine-3,4-diols 388 and 389

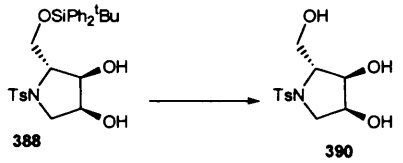


The diene **382** (0.15 g, 0.30 mmol) was dissolved in CH_2Cl_2 (10 ml) and treated with ozone using the general procedure (see experiment 6.2.5). The reaction was quenched with dimethylsulfide (0.07 g, 0.09 ml, 1.2 mmol) and was stirred at room temperature for 18 h before being worked up to give crude material which was used without further purification.

This material (0.15 g, 0.30 mmol, based on **382**) underwent pinacol coupling following the general procedure (see experiment 6.2.6) using samarium metal (0.27 g, 0.18 mmol), diiodomethane (0.40 g, 0.11 ml, 1.5 mmol) and ^{t}BuOH (0.13 g, 0.17 ml, 0.18 mmol) in THF (15 ml). The reaction was stirred for 18 h before being worked up and the resulting crude product was purified by flash column chromatography on silica (50:50, ethyl acetate: petroleum-ether) to give the *diols* **388** and **389** (in an approximate 2:1 ratio crude ^{13}C NMR of the crude mixture) (diastereoisomer 1 (eluted 1st), 0.013 g, diastereoisomer 2 (eluted 2nd), 0.0183 g, mixed fraction, 0.004 g, 22% total yield from **382**). Data for diastereoisomer 1 **388**: ν_{max} (CH_2Cl_2)/ cm^{-1}: 3606m (OH), 3560m (OH), 2074s, 2982s, 2312m, 1605w, 1550m, 1430s, 1348s, 1276s, 1252s, 1164s, 896s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.09 (9H, s, $C(CH_3)_3$), 2.43 (3H, s, Ar 4-Me), 3.21 (1H, dd, *J*10.8, 5.1, C*H*HN), 3.44-3.51 (1H, m, CHN), 3.63 (1H, dd, *J*10.8, 5.3, CH*H*N), 3.85 (1H, dd, *J*10.6, 7.4, C*H*HO), 3.90 (1H, dd, *J*10.6, 3.9, CH*H*O), 4.20-4.28 (2H, m, 2 x C*H*OH), 7.29 (2H, d, *J*7.8, 2xAr H-3), 7.34-7.50 (5H, m, 5xPh-H), 7.61-7.74 (7H, m, 5xPh-H, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 19.22 (C), 21.56 (CH_3), 26.94 (3 × CH_3), 52.69 (CH_2), 65.13 (CH), 65.70 (CH_2), 70.00 (CH), 75.08 (CH), 127.89 (2 × CH), 128.00 (4 × CH), 129.49 (2 × CH), 130.02 (2 × CH), 133.57 (2 × C), 134.72 (C), 135.53 (2 × CH), 135.62 (2 × CH), 143.53 (C); (ES) m/z (%): 526 ($M+H^+$, 80%), 543 (48, $M+NH_4^+$); Accurate mass (FAB): Found 526.2084 ($M+H^+$ $C_{28}H_{36}NO_5S$ requires 526.2084). Data for diastereoisomer 2 **389**: ν_{max} (CH_2Cl_2)/ cm^{-1}: 3691m (OH), 3599m (OH), 3053s, 2987s, 2305m, 1599m, 1550m, 1495w, 1422s, 1346s, 1276s, 1272s, 1253s, 814s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.11 (9H, s, $C(CH_3)_3$), 2.43 (3H, s, Ar 4-Me), 3.30 (1H, dd, *J*11.5, 5.3, C*H*HN), 3.63 (1H, dd, *J*11.5, 3.0, CH*H*N), 3.63-3.69 (1H, m, CHN), 3.84-3.89 (1H, m, C*H*OH), 3.95 (1H, dd, *J*10.1, 8.2, C*H*HO), 4.07 (1H, dd, *J*10.1, 7.1, CH*H*O), 4.13 (1H, dd, *J*10.8, 5.3, C*H*OH),

7.28 (2H, d, *J*8.3, 2xAr H-3), 7.39-7.50 (5H, m, 5xPh-H), 7.60 (2H, d, *J*8.3, 2xAr H-2), 7.67-7.76 (5H, m, 5xPh-H); (ES) m/z (%): 526 (88, $M+H^+$), 448 (80, M-Ph). Accurate mass (FAB): Found 526.2084 ($M+H^+$ $C_{28}H_{36}NO_5S$ requires 526.2084).

6.7.5 (2*R*, 3*R*, 4*S*)-2-Hydroxymethyl-1-(toluene-4-sulfonyl)-pyrrolidine-3,4-diol 390



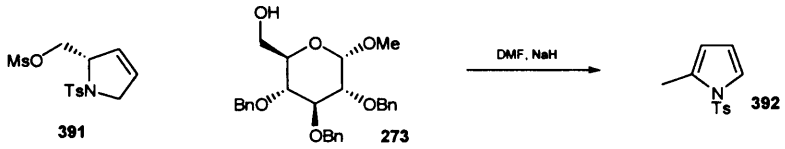
The diol **388** (1st eluted diastereoisomer from reaction 6.8.4) (0.01 g, 0.020 mmol) was dissolved in THF and cooled to 0 °C. Tetra-butylammonium fluoride (0.006 g, 0.007 ml, 0.023 mmol) was added to the solution and the reaction mixture was stirred for 2 h before the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (4:96, methanol: dichloromethane) to give the *triol* **390** (0.004 g, 80%) as colourless oil. $[\alpha]^{20}_D$: -18.7 (c= 1.11, MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3580w (OH), 2899w, 2781w, 2360m, 1652m, 1599m, 1539m, 1269s, 1264s, 1262s, 1258s, 1093m, 808m; δ_H (250 MHz; D_2O): 2.49 (3H, s, Ar-4-Me), 3.26 (1H, dd, *J*10.6, 5.5, NC*H*H), 3.49-3.54 (1H, m, CHN), 3.63 (1H, dd, *J*10.6, 5.3, NCH*H*), 3.79 (1H, dd, *J*12.2, 2.8, C*H*HO), 3.93 (1H, dd, *J*12.2, 4.3, CH*H*O), 4.19 (1H, app t, *J*4.4, C*H*OH), 4.27-4.33 (1H, m, CH_2CHOH), 7.53 (2H, d, *J*8.0, 2xAr H-3), 7.83 (2H, d, *J*8.0, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$): 21.58 (CH_3), 53.34 (CH_2), 63.06 (CH_2), 65.75 (CH), 69.59 (CH), 73.78 (CH), 127.89 (2 × CH), 129.68 (2 × CH), 133.02 (C), 144.05 (C); δ_C (62.9 MHz; $(CD_3)_2O$; Me_4Si): 21.02 (CH_3), 52.67 (CH_2), 63.30 (CH_2), 68.08 (CH), 70.07 (CH), 73.63 (CH), 128.41 (2 × CH), 129.82 (2 × CH), 134.68 (C), 143.70 (C); MS (ES) m/z (%): 242 ($M+H^+$-$2H_2O$, 100%), 288 (88, $M+H^+$); Accurate mass (FAB): Found 288.0906 ($M+H^+$ $C_{12}H_{18}NO_5S$ requires 288.0906).
This agrees with the reported literature ^{13}C NMR data[118]

6.7.6 (*S*)-1-(Toluene-4-sulfonyl)-2-methanesulfonylmethyl-2,5-dihydro-1*H*-pyrrole 391

A solution of the diene **363** (0.43 g, 1.19 mmol) in CH_2Cl_2 (8.0 ml) was added to *bis*(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride **299** (Grubbs catalyst) (0.049 g, 0.059 mmol) as described in experiment 6.9.3. The solution was refluxed for 3 h before being filtered through a pad of silica and washed through with ethyl acetate (3x10 ml). The solvent was removed under vacuum and the resulting crude mixture was purified by flash

column chromatography on silica (30:70, ethyl acetate: petroleum-ether) mixture to give the *alkene* **391** as an orange oil (0.25 g, 63%). α_D: -147.7 (c=1.37 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3051w, 2858m, 2749m, 1357m, 1272s, 1270s, 1267s, 1264s, 1176m, 1096m, 872w, 817w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.44 (3H, s, Ar 4-Me), 3.08 (3H, s, OSO_2CH_3), 4.12 (1H, d app q, *J*14.9, 2.3, NC*H*H), 4.20 (1H, dd app t, *J*14.9, 5.3, 2.3, NCH*H*), 4.38 (1H, dd, *J*10.3, 5.7, C*H*HOSO_2CH_3), 4.50 (1H, dd, *J*10.3, 3.2, CH*H*OSO_2CH_3), 4.62-4.65 (1H, m, CHN), 5.66 (1H, d app q, *J*6.2, 2.3, CHC*H*=CH), 5.77 (1H, ddd, *J*6.2, 5.3, 2.3, CH_2CH=CH), 7.34 (2H, d, *J*8.0, 2xAr H-3), 7.73 (2H, d, *J*8.0, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.60 (CH_3), 37.27 (CH_3), 55.97 (CH_2), 65.46 (CH), 71.46 (CH_2), 125.99 (CH), 127.54 (2 × CH), 127.72 (CH), 130.02 (2 × CH), 133.67 (C), 144.18 (C); MS (FAB) m/z (%): 332 (100%, $M+H^+$), 155 (82), 236 (61, $M-SO_2CH_3$); Accurate mass (FAB): Found 332.0627 ($M+H^+$ $C_{13}H_{18}NO_5S_2$ requires 332.0626).

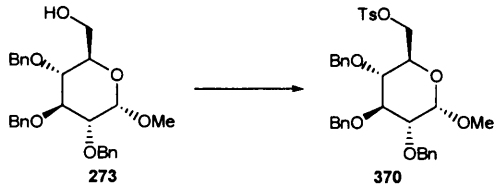
6.7.7 Attempt to attach the sugar moiety 273



A solution of the alcohol **273** (0.10 g, 0.22 mmol) in DMF (5 ml) was added to a 0 °C solution of sodium hydride (0.009 g of a 60% dispersion in oil, 0.39 mmol) in DMF (1 ml). The resulting mixture was stirred for 20 min a solution of the mesylate **391** (0.10 g, 0.32 mmol) in DMF (3 ml) was added dropwise. The reaction mixture was heated at 80 °C for 2 h. The reaction was quenched with saturated ammonium chloride (10 ml). Water (10 ml) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3x30 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude mixture was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *pyrrole* **392** as a colourless oil (0.021 g, 21%). δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.28 (3H, s, CH_3), 2.41 (3H, s, Ar 4-Me), 5.93 (1H, m, CH), 6.16 (1H, m, CH), 7.21 (3H, m, 2xAr H-3, CH), 7.67 (2H, d, *J*8.0, 2xAr H-2).

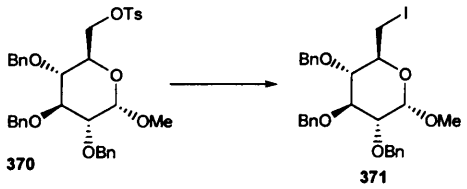
6.8 The synthesis of sugar moiety alkyl halide derivatives

6.8.1 Methyl-[O^2, O^3, O^4-tribenzyl-O^6-(4-methylbenzenesulfonamide)-α-D-glucopyranoside] 370



The protected sugar **273** (1.0 g, 2.15 mmol) was dissolved in CH_2Cl_2 (10 ml) and treated with triethylamine (0.48 g, 0.66 ml, 4.7 mmol), tosyl chloride (0.45 g, 2.37 mmol) and DMAP (0.03 g, 0.21 mmol) as described in experiment 6.7.2. After 1.5 h the reaction was worked up and the resulting crude material was purified by flash column chromatography on silica (20:80, ethyl acetate: petroleum-ether) to give the *tosylate* **370** (1.05 g, 79%) as a colourless oil. $[\alpha]^{20}_D$: +41.7 (c=1.10 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2923w, 1496m, 1454m, 1363m, 1272m, 1265m, 1259m, 1190s, 1178s, 1092s, 1045m, 1028m, 1002m, 934w, 816w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.40 (3H, s, Ar 4-Me), 3.31 (3H, s, OMe), 3.43-3.49 (2H, m, H-2, H-4), 3.72-3.78 (2H, d app t, *J*10.0, 6.2, H-5), 3.95 (1H, app t, *J*9.4, H-3), 4.07-4.24 (2H, m, CH_2OTs), 4.43 (1H, d, *J*11.0, PhC*H*HO), 4.52 (1H, d, *J*3.4, H-1), 4.62 (1H, d, *J*12.1, PhC*H*HO) 4.76 (1H, d, *J*11.0, PhCH*H*O), 4.78 (1H, d, *J*11.0, PhC*H*HO), 4.82 (1H, d, *J*12.1, PhCH*H*O), 4.97 (1H, d, *J*11.0, PhCH*H*O), 7.13-7.15 (2H, d, *J*8.3, 2xAr H-3), 7.25-7.32 (15H, m, 15xPh-H), 7.76 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.59 (CH_3), 55.31 (CH_3), 68.50 (CH), 68.60 (CH_2), 73.41 (CH_2), 74.95 (CH_2), 75.69 (CH_2), 77.51 (CH), 79.71 (CH), 81.82 (CH), 98.02 (CH), 127.63 (CH), 127.81 (CH), 127.86 (2 × CH), 127.89 (2 × CH), 128.07 (3 × CH), 128.11 (2 × CH), 128.39 (4 × CH), 128.47 (2 × CH), 129.78 (2 × CH), 132.92 (C), 137.78 (C), 137.98 (C), 138.56 (C), 144.80 (C); MS (ES) m/z (%): 636 ($M+NH_4^+$, 100%), 619 (20, $M+H^+$); Found 587.2105 (M-OMe $C_{34}H_{35}O_7S$ requires 587.2104). This is in agreement with the reported literature NMR data[181]

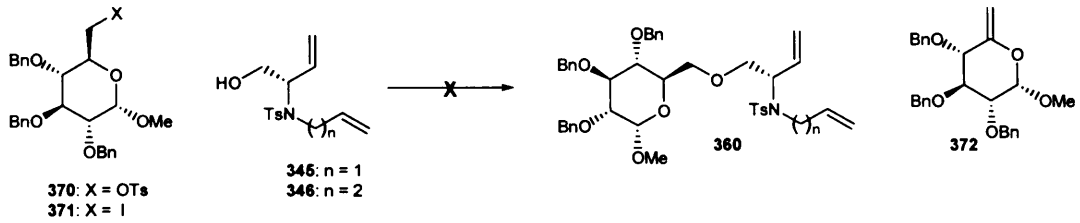
6.8.2 Methyl-[O^2, O^3, O^4-tribenzyl-O^6-(iodo)-α-D-glucopyranoside] 371



The tosylate **370** (0.49 g, 0.79 mmol) was dissolved in acetone (15 ml) and treated with sodium iodide (0.47 g, 3.1 mmol) as described in experiment 6.2.3. The reaction was refluxed for 48 h before being worked up to give the *iodide* **371** (0.39 g, 85%) as a white solid. Mp: 65-67 °C (Lit.[182] 68-69 °C); $[\alpha]^{20}_D$: +65.0 (c=1.20 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3054w, 2927w, 1605w, 1497m, 1454m, 1359m, 1276m, 1271m, 1267m, 1265m, 1260s, 1254m, 1086s, 1048s, 1028m, 909s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.28 (1H, dd, *J*9.1, 4.3, H-6), 3.33 (1H, app t, *J*8.7, H-4), 3.42 (3H, s, OMe), 3.43-3.44 (1H, m, H-5), 3.47 (1H, dd, *J*9.1, 5.7, H-6), 3.53 (1H, dd, *J*8.7, 3.7, H-2), 4.01 (1H, app t, *J*8.7, H-3), 4.61 (1H, d, *J*3.7, H-1), 4.65 (1H, d, *J*12.1, PhC*H*HO), 4.68 (1H, d, *J*11.0, PhC*H*HO) 4.79 (1H, d, *J*11.0, PhC*H*HO), 4.94 (1H, d, *J*11.0, PhCH*H*O), 4.94 (1H, d, *J*12.1, PhCH*H*O), 4.99 (1H, d, *J*11.0, PhCH*H*O), 7.25-7.37

(15H, m, 15xPh-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 7.61 (CH_2), 55.49 (CH_3), 69.28 (CH), 73.42 (CH_2), 75.33 (CH_2) 75.76 (CH_2), 80.08 (CH), 81.48 (CH), 81.55 (CH), 98.10 (CH), 127.68 (CH), 127.82 (CH), 127.91 (2 × CH), 128.07 (2 × CH), 128.24 (CH), 128.24 (2 × CH), 128.42 (2 × CH), 128.49 (2 × CH), 128.63 (2 × CH), 138.00 (C), 138.24 (C), 138.53 (C); MS (ES) m/z (%): 692 ($M+NH_4^+$, 100%), 693 (30, $M+H_3O^+$); Found 543.1031 (M-OMe $C_{27}H_{28}O_4I$ requires 543.1032).

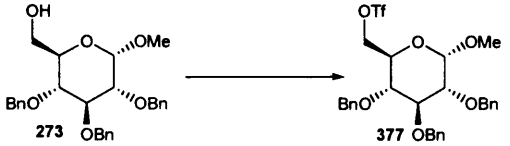
6.8.3 Attempt at the etherification coupling



A solution of the alcohols **345** or **346** (0.23 mmol) in THF (5 ml) were added to a 0 °C solution of sodium hydride (0.01 g of a 60% dispersion in oil, 0.42 mmol) in THF (1 ml). The resulting mixture was stirred for 20 min before a solution of the tosylate **370** or iodide **371** (0.34 mmol) in THF (3 ml) was added dropwise. The reaction mixture was heated at reflux for 4 h before being quenched with saturated ammonium chloride (10 ml). Water (10 ml) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3x30 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude mixture was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *recovered alcohols* **345** or **346** (~60%) as colourless oils and the *alkene* **372** as a colourless oil (30-50%). Data for **372** : δ_H (300 MHz; $CDCl_3$; Me_4Si): 3.35 (3H, s, OMe), 3.50 (1H, dd, *J*9.0, 3.5, H-2), 3.83 (1H, app t, *J*9.0, H-4), 3.90 (1H, app t, *J*9.0, H-3), 4.54 (1H, d, *J*3.5, H-1), 4.58-4.85 (8H, m, 3xCH_2Ph, C=CH_2), 7.17-7.26 (15H, m, 15xPh).

6.9 The synthesis of (1→6) linked homoaza-*O*-disaccharides

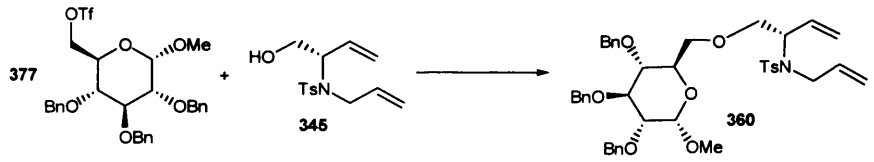
6.9.1 Methyl-[O^2, O^3, O^4, -tribenzyl- O^6-(trifluoromethanesulfonyl)-α-D-glucopyranoside] 377[115]



The alcohol **273** (0.096 g, 0.21 mmol) was dissolved in dry toluene (2.1 ml) and the mixture was cooled to 0 °C. To this solution triethylamine (0.022 g, 0.032 ml, 0.23 mmol) was added

and the solution was stirred 20 mins before the addition of trifluoromethanesulfonic anhydride (0.088 g, 0.051 ml, 0.31 mmol). The reaction mixture was stirred a further 30 min at 0 °C before being quenched with ice water (5 ml). The aqueous layer was extracted with ethyl acetate (3x10 ml) and the organic layers were combined and washed with a 5% aqueous $NaHCO_3$ solution (10 ml). The organic layer was dried ($MgSO_4$) and solvent was removed under vacuum to yield the *triflate* **377** as a colourless oil (0.11 g, 93%) which was taken onto the next step without further purification. δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.37 (3H, s, OMe), 3.41 (1H, dd, *J*10.1, 9.4, H-4), 3.52 (1H, dd, *J*9.4, 3.4, H-2), 3.84 (1H, ddd, *J*10.1, 7.4, 5.1, H-5), 4.01 (1H, app t, *J*9.4, H-3), 4.45 (1H, dd, *J*10.8, 5.1, H-6), 4.53 (1H, d, *J*3.4, H-1), 4.55 (1H, d, *J*12.6, PhC*H*HO), 4.60 (1H, dd, *J*10.8, 7.4, H-6), 4.74 (1H, d, *J*11.3, PhC*H*HO), 4.80 (1H, d, *J*11.0, PhC*H*HO), 4.83 (1H, d, *J*12.6, PhCH*H*O), 4.85 (1H, d, *J*11.3, PhCH*H*O), 5.01 (1H, d, *J*11.0, PhCH*H*O), 7.19-7.37 (15H, m, 15xPh-H). This is in agreement with the reported literature NMR data.[115]

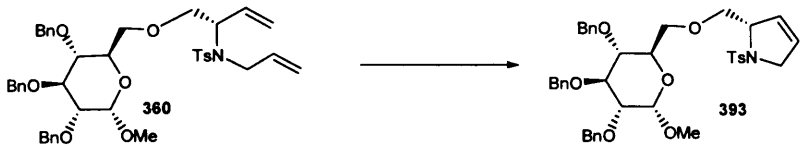
6.9.2 (*S*)-*N*-Allyl-*N*-[(Methyl-[O^2, O^3, O^4,-tribenzyl-α-D-glucopyranoside]ylmethyl)allyl]-4-methylbenzenesulfonamide 360



A solution of the alcohol **273** (0.12 g, 0.41 mmol) in THF (5 ml) was added to a 0 °C solution of sodium hydride (0.034 g of a 60% dispersion in oil, 1.37 mmol) in THF (1 ml). The resulting mixture was stirred for 20 min a solution of the triflate **377** (0.29 g, 0.49 mmol) in THF (3 ml) was added dropwise. The reaction mixture was stirred at room temperature for 4 h before being quenched with saturated ammonium chloride (10 ml). Water (10 ml) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3x30 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude mixture was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *diene* **360** as a colourless oil (0.21 g, 68%). $[\alpha]^{20}_D$: +49.8 (c=6.64 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3070w, 3019s, 2398m, 1521m, 1476m, 1332m, 1214s, 1162m, 929s, 802s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.37 (3H, s, CH_3, Ar), 3.38 (3H, s, OMe), 3.44 (3H, app t, *J*9.4, H-4), 3.50 (1H, dd, *J*9.4, 3.5, H-2), 3.54-3.75 (6H, m, H-5, 2xH-6, C*H*HN, CH_2O), 3.93 (1H, dd, *J*13.1, 6.0, CH*H*N), 3.98 (1H, app t, *J*9.4, H-3), 4.49-4.54 (1H, m, CHN), 4.55 (1H, d, *J*11.0, PhC*H*HO), 4.60 (1H, d, *J*3.5, H-1), 4.67 (1H, d, *J*12.0, PhC*H*HO), 4.80 (1H, d, *J*12.0, PhCH*H*O), 4.82 (1H, d, *J*11.0, PhCH*H*O), 4.86 (1H, d, *J*11.0, PhCH*H*O), 4.99 (1H, d, *J*11.0, PhCH*H*O), 5.03 (1H, dd, *J*10.8, 1.4, C*H*H=CH), 5.06 (1H, dd,

*J*10.8, 1.4, C*H*H=CH), 5.09 (1H, dd, J17.2, 1.4, CH=CH*H*), 5.14 (1H. dd, *J*17.9, 1.4, CH=CH*H*), 5.63-5.86 (2H, m, 2xC*H*=CH_2), 7.22-7.40 (17H, m, 15xPh-H, 2xAr H-3), 7.72 (1H, d, *J*8.3, 2xAr H-2) δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.41 (CH_3), 47.89 (CH_2), 55.12 (CH_3), 59.35 (CH), 69.94 (CH_2), 70.12 (CH), 72.30 (CH_2), 73.32 (CH_2), 74.97 (CH_2), 75.76 (CH_2), 77.84 (CH), 79.95 (CH), 82.02 (CH), 97.98 (CH), 117.18 (CH_2), 119.05 (CH_2), 127.31 (2 × CH), 127.57 (CH), 127.67 (CH), 127.73 (2 × CH), 127.87 (CH), 128.00 (2 × CH), 128.05 (2 × CH), 128.34 (2 × CH), 128.38 (2 × CH), 128.42 (2 × CH), 129.40 (2 × CH), 133.78 (CH), 135.75 (CH), 138.07 (C), 138.11 (C), 138.31 (C), 138.71 (C), 142.99 (C); MS (ES) m/z (%): 750 ($M+Na^+$, 100%), 751 (50), 585 (48), 745 (22, $M+NH_4^+$); Accurate mass (FAB): Found 750.3074 ($M+Na^+$ $C_{42}H_{49}NO_8SNa$ requires 750.3077).

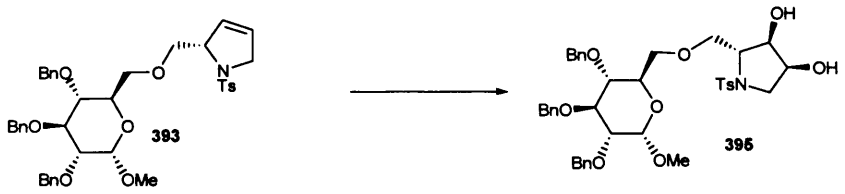
6.9.3 (*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2,O^3,O^4,-tribenzyl-α-D-glucopyranoside]ylmethyl)-2,5-dihydro-1*H*-pyrrole 393



A solution of the diene **360** (0.13 g, 0.172 mmol) dissolved in CH_2Cl_2 (1.5 ml) was added to *bis*(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride **299** (Grubbs catalyst) (0.0076 g, 0.0092 mmol). The reaction mixture was refluxed (45 °C) for 3.5 h before being filtered through a pad of silica and washed through with ethyl acetate (3x10 ml). The solvent was removed under reduced pressure and the resulting crude mixture was purified by flash column chromatography on silica (25:75, ethyl acetate: petroleum-ether) to give the *alkene* **393** as an off-white oil (0.097 g, 81%). $[\alpha]^{20}_D$: -17.7 (c=2.61 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2918m, 2869m, 2577w, 1700m, 1600m, 1496m, 1345m, 1273s, 1270s, 1268s, 1264s, 1256s, 1134m, 1028m, 826w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.42 (3H, s, Ar 4-Me), 3.40 (3H, s, OMe), 3.48 (1H, app t, *J*8.7, C*H*HO), 3.55 (1H, dd, *J*9.4, 3.5, H-2), 3.57 (1H, overlapping app t, *J*9.4, H-4), 3.68-3.78 (3H, m, H-5, 2xH-6), 4.01 (1H, dd, *J*8.7, 4.15, CH*H*O), 4.01 (1H, overlapping app t, *J*9.4, H-3), 4.09-4.21 (2H, m, CH_2N), 4.54-4.56 (1H, m, CHN), 4.60 (1H, d, *J*11.0, PhC*H*HO), 4.66 (1H, d, *J*3.5, H-1), 4.69 (1H, d, *J*12.0, PhC*H*HO), 4.82 (1H, d, *J*12.0, PhCH*H*O), 4.85 (1H, d, *J*11.0, PhC*H*HO), 4.89 (1H, d, *J*11.0, PhCH*H*O), 5.00 (1H, d, *J*11.0, PhCH*H*O), 5.62 (1H, dd, *J*6.2, 4.4, CHC*H*=CH), 5.77 (1H, ddd, *J*6.2, 4.1, 2.1, CH_2C*H*=CH), 7.27-7.41 (17H, m, 15xPh-H, 2xAr H-3), 7.71 (1H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.42 (CH_3), 55.09 (CH_3), 55.75 (CH_2), 59.35 (CH), 66.02 (CH), 69.94 (CH_2), 73.27 (CH_2), 74.97 (2 × CH_2), 75.75 (CH_2), 77.67 (CH), 79.83 (CH), 81.97

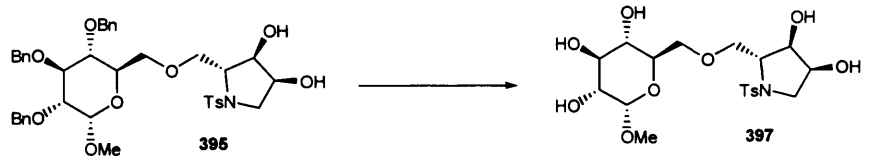
(CH), 98.07 (CH), 125.49 (CH), 127.43 (2 × CH), 127.56 (CH), 127.65 (CH), 127.72 (2 × CH), 127.81 (CH), 128.01 (4 × CH), 128.37 (2 × CH), 128.37 (2 × CH), 128.51 (CH), 128.42 (2 × CH), 129.40 (2 × CH), 134.10 (C), 138.10 (C), 138.24 (C), 138.65 (C), 143.50 (C); MS (ES) m/z (%): 717 (100, $M+NH_4^+$); Accurate mass (FAB): Found 669.2765 (M-OMe $C_{39}H_{43}NO_7S$ requires 669.2760).

6.9.4 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4,-tribenzyl-α-D-glucopyranoside]ylmethyl)-pyrrolidone-3,4-diol 395



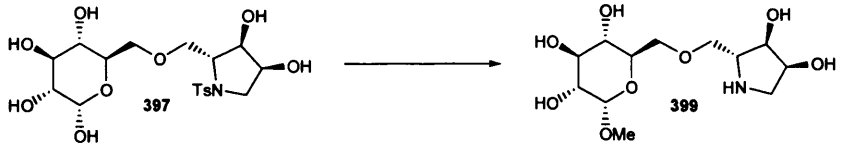
The alkene **393** (0.056 g, 0.080 mmol) was dissolved in acetone (2 ml) and to this solution OsO_4 (2.5% by wt in ^{t}BuOH solution, 0.0032 ml, 0.0032 mmol) was added. This solution was then added to NMO (0.019 g, 0.16 mmol) dissolved in water (1 ml). The reaction mixture was stirred for 24 h before being quenched with a 1% aqueous sodium dithionite solution (10 ml). The mixture was stirred a further 30 min before the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (50:50, ethyl acetate: petroleum-ether) to give the *diol* **395** as a colourless oil (0.042 g, 73%). $[\alpha]^{20}_D$: +24.5 (c=0.98 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3580bw, 2899m, 2747m, 2558m, 2461m, 2339w, 1733m, 1652m, 1616m, 1521w, 1496w, 1345m, 1273s, 1270s, 1264s, 1262s, 1163m, 1070m, 808w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.40 (3H, s, Ar 4-Me), 3.21 (1H, dd, *J*11.2, 4.1, C*H*HN), 3.36 (3H, s, OMe), 3.44-3.60 (6H, m, H-2, H-4, CH_2O, CHN, CH*H*N), 3.64-3.74 (3H, m, H-5, 2xH-6), 3.88-4.07 (2H, m, H-3, C*H*OH), 4.10-4.15 (1H, m, C*H*OH), 4.59 (1H, d, *J*3.2, H-1) 4.62 (1H, d, *J*11.0, PhC*H*HO), 4.64 (1H, d, *J*12.2, PhC*H*HO), 4.77 (1H, d, *J*12.2, PhCH*H*O), 4.82 (1H, d, *J*10.8, PhC*H*HO), 4.89 (1H, d, *J*11.0, PhCH*H*O), 4.92 (1H, d, *J*10.8, PhCH*H*O), 7.25-7.37 (17H, m, 15xPh-H, 2xAr H-3), 7.69 (1H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.51 (CH_3), 52.29 (CH_2), 55.29 (CH_3), 62.40 (CH), 69.76 (CH), 69.89 (CH), 69.96 (CH_2) 73.06 (CH_2), 73.34 (CH_2), 75.01 (CH_2), 75.52 (CH), 75.81 (CH_2), 77.60 (CH), 79.98 (CH), 81.87 (CH), 98.08 (CH), 127.65 (2 × CH), 127.72 (2 × CH), 127.86 (4 × CH), 128.00 (4 × CH), 128.39 (2 × CH), 128.43 (3 × CH), 129.48 (2 × CH), 133.44 (C), 138.03 (C), 138.08 (C), 138.54 (C), 143.62 (C); MS (ES) m/z (%): 801 (20%), 722 (10), 756 (54, $M+Na^+$); Accurate mass (FAB): Found 734.2999 ($M+H^+$ $C_{40}H_{48}NO_{10}S$ requires 734.2999).

6.9.5 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-([methyl-α-D-glucopyranoside]ylmethyl)-pyrrolidine-3,4-diol 397



The diol **395** (0.10g, 0.14 mmol) was dissolved in a mixture of ethanol (8.5 ml) and acetic acid (1.05 ml) and was subjected to the general hydrogenolysis procedure (outlined in 6.2.7) using $Pd(OAc)_2$ (0.029 g, 0.13 mmol). The reaction mixture was stirred for 3 h before being filtered. The solvent was removed under vacuum and the resulting crude reaction mixture was purified by flash column chromatography on silica (10:90, methanol: ethyl acetate) to give the *pentol* **397** as a white foam (0.061 g, 92%). α_D: +15.1 (c=0.95 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3406br (OH), 2924m, 1598w, 1342s, 1272s, 1262s, 1256m, 1162s, 1092s, 1049s, 815m, 757s; δ_H (400 MHz; D_2O): 2.40 (3H, s, Ar 4-Me), 3.15 (1H, dd, *J*10.3, 6.0, C*H*HN), 3.36 (1H, app t, *J*9.4, H-4), 3.37 (3H, s, OMe), 3.50 (1H, dd, *J*9.4, 3.7 H-2), 3.55-3.58 (2H, m (including dd *J*10.3, 6.0, CH*H*N and 1H, dd, *J*8.6, 4.6, H-6)), 3.61 (1H, app t, *J*9.4, H-3), 3.66-3.75 (5H, m, H-5, H-6, CH_2O, CHN), 4.13 (1H, app t, *J*3.7, CHC*H*OH), 4.27 (1H, app td, *J*6.0, 3.7, CH_2CHOH), 4.75 (1H, d, *J*3.7, H-1) 7.42 (2H, d, *J*8.3, 2xAr H-3), 7.73 (1H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; D_2O; Me_4Si): 21.11 (CH_3), 52.16 (CH_2), 55.54 (CH_3), 65.26 (CH), 69.76 (CH), 70.13 (CH), 70.55 (CH_2) 77.80 (CH), 71.24 (CH_2), 71.60 (CH), 73.29 (CH), 73.51 (CH), 99.70 (CH), 128.01 (2 x CH), 130.33 (2 x CH), 132.27 (C), 145.92 (C); MS (ES) m/z (%): 154 (100%), 136 (90), 486 (80, $M+Na^+$); Accurate mass (FAB): Found 486.1410 ($M+Na^+$ $C_{19}H_{29}NO_{10}SNa$ requires 486.1410).

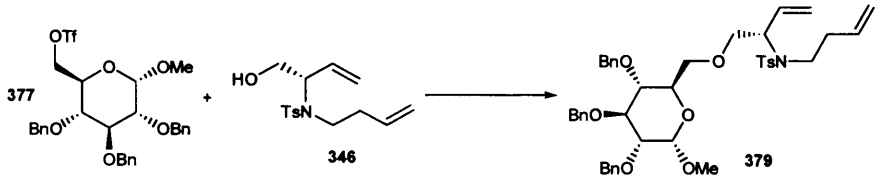
6.9.6 (2*R*, 3*R*, 4*S*)-2-([methyl-α-D-glucopyranoside]ylmethyl)-pyrrolidone-3,4-diol 399



The pentol **397** (0.036 g, 0.078 mmol) was subjected to the general tosyl deprotection procedure (see experiment 6.2.9) using sodium metal (0.0045 g, 0.20 mmol) and liquid ammonia (5-10 ml). After the addition of sodium was complete the reaction was warmed to room temperature and the liquid ammonia was allowed to evaporate. The crude residue was dissolved into water and transferred to a round bottom flask, the water was removed under reduced pressure and the resulting crude material was purified by flash column chromatography on silica (100% methanol) to give the *amine* **399** as a clear oil (0.022 g,

92%). $[\alpha]^{20}_D$: + 49.5 (c= 0.14 MeOH); ν_{max} (solid state)/ cm^{-1}: 3303 br (OH, NH), 2921m, 1548w, 1417m, 1342w, 1106m, 1035s, 1009s, 901w; δ_H (400 MHz; D_2O): 3.02 (1H, br d, *J*12.4, C*H*HN), 3.28 (1H, dd, *J*12.4, 5.2, CH*H*N), 3.39 (1H br m, CHN), 3.47 (3H, s, OMe), 3.50 (1H, app t, *J*9.6, H-4), 3.60 (1H, dd, *J*9.6, 3.6, H-2), 3.71 (1H, app t, *J*9.6, H-3), 3.73-3.83 (5H, m, H-5, 2xH-6, CH_2O), 4.06 (1H, app t, *J*6.0, CHC*H*OH), 4.25 (1H, dd, *J*6.0, 5.2, CH_2C*H*OH), 4.84 (1H, d, *J*3.6, H-1); δ_C (100.6 MHz; D_2O): 49.91 (CH_2), 55.17 (CH_3), 60.50 (CH), 69.51 (CH), 69.52 (CH_2), 70.46 (CH), 70.55 (CH_2), 70.75 (CH), 71.19 (CH), 72.91 (CH), 73.01 (CH), 99.36 (CH); MS (ES) m/z (%): 323 (100%), 310 (65, $M+H^+$); Accurate Mass (FAB): Found 310.1501 ($M+H^+$ $C_{12}H_{24}NO_8$ requires 310.1502).

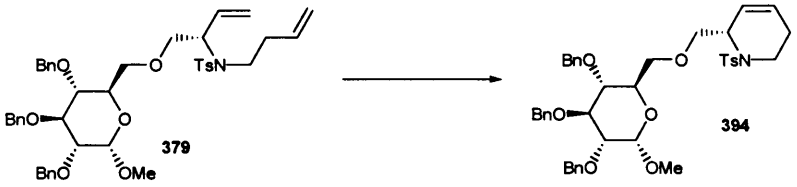
6.9.7 (*S*)-*N*-But-3-enyl-*N*-[(methyl-[O^2, O^3, O^4,-tribenzyl-α-D-glucopyranoside]ylmethyl)allyl]- 4-methylbenzenesulfonamide 379



A solution of the alcohol **346** (0.36 g, 1.23 mmol) in THF (10 ml) was added to a 0 °C solution of sodium hydride (0.074 g of a 60% dispersion in oil, 3.06 mmol) in THF (1 ml). The resulting mixture was stirred for 20 min before a solution of the triflate **377** (0.877 g, 1.47 mmol) in THF (5 ml) was added dropwise. The reaction mixture was stirred for 4 h before being quenched with saturated aqueous ammonium chloride (25 ml). Water (20 ml) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3x50 ml). The organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum. The resulting crude mixture was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *diene* **379** as a colourless oil (0.52 g, 57%). $[\alpha]^{20}_D$: +34.4 (c=1.0 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3045w, 2927w, 1495m, 1455m, 1338m, 1264s, 1261s, 1258s, 1250m, 1073s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.41 (3H, s, Ar 4-Me), 2.39-2.42 (2H, m, CH_2N), 3.07-3.19 (1H, m, C*H*HCH=CH_2), 3.26-3.36 (1H, m, CH*H*CH=CH_2), 3.42 (3H, s, OMe), 3.45-3.74 (7H, m, H-2, H-4, H-5, 2xH-6, CH_2O), 4.03 (1H, app t, *J*9.4, H-3), 4.52-4.56 (1H, m, CHN), 4.62 (1H, d, *J*11.0, PhC*H*HO), 4.65 (1H, d, *J*3.7, H-1), 4.72 (1H, d, *J*12.0, PhC*H*HO), 4.85 (1H, d, *J*12.0, PhCH*H*O), 4.87 (1H, d, *J*10.8, PhCH*H*O), 4.87 (1H, d, *J*11.0, PhCH*H*O), 4.91 (1H, d, *J*10.8, PhCH*H*O), 5.06 (1H, dd, *J*18.0, 1.2, C*H*H=CH), 5.12 (1H, dd, *J*9.4, 1.2, CH*H*=CH), 5.14 (1H, dd, *J*17.2, 1.2, C*H*H=CH), 5.23 (1H, dd, *J*10.6, 1.2, CH*H*=CH), 5.65-5.82 (2H, m, 2xCH_2=C*H*), 7.27-7.41 (17H, m, 15xPh-H, 2xAr H-3), 7.77 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.40 (CH_3), 35.19 (CH_2), 44.92 (CH_2), 55.12 (CH_3), 59.26 (CH), 70.03 (CH_2), 70.11 (CH), 72.56 (CH_2), 73.32 (CH_2), 74.97

(CH_2), 75.74 (CH_2), 77.83 (CH), 79.97 (CH), 82.02 (CH), 97.96 (CH), 116.67 (CH_2), 118.86 (CH_2), 127.15 (2 × CH), 127.57 (CH), 127.70 (CH), 127.75 (2 × CH), 127.87 (CH), 127.99 (2 × CH), 128.04 (2 × CH), 128.34 (2 × CH), 128.39 (2 × CH), 128.42 (2 × CH), 129.43 (2 × CH), 133.83 (CH), 134.91 (CH), 137.99 (C), 138.12 (C), 138.30 (C), 138.72 (C), 142.99 (C); MS (ES) m/z (%): 764 (100%, $M+Na^+$), 759 (88, $M+NH_4^+$), 765 (80, MH^++Na^+); Accurate mass: Found 742.3415 ($M+H^+$ $C_{43}H_{52}NO_8S$ requires 742.3414).

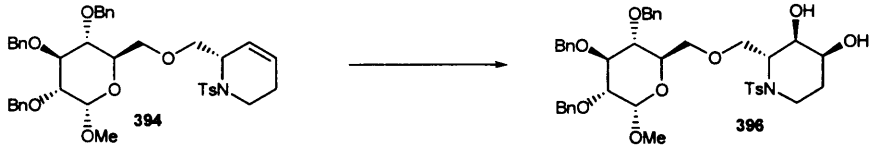
6.9.8 (*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4,-tribenzyl-α-D-glucopyranoside]ylmethyl)-1,2,5,6-tetrahydropyridine 394



A solution of the diene **379** (0.49 g, 0.66 mmol) in CH_2Cl_2 (10 ml) was added to *bis*(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride **299** (Grubbs catalyst) (0.028 g, 0.033 mmol) as described in experiment 6.9.3. The solution was refluxed (45 °C) for 3.5 h before being filtered through a pad of silica and washed through with ethyl acetate (3x10 ml). The solvent was removed under vacuum and the resulting crude mixture was purified by flash column chromatography on silica (25:75, ethyl acetate: petroleum-ether) to give the *alkene* **394** as an orange oil (0.37 g, 77%). $[\alpha]^{20}_D$: -38.3 (c=1.95 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2918m, 1598m, 1454s, 1272s, 1160m, 1123w, 1098s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.71-1.91 (2H, m, C*H*$_2$CH=CH), 2.39 (3H, s, Ar 4-Me), 3.17 (1H, ddd, *J*14.0, 10.3, 5.5, C*H*HN), 3.39 (3H, s, OMe), 3.50 (1H, app t, *J*9.4, H-4), 3.45-3.54 (1H, m, overlapping, C*H*HO), 3.54 (1H, dd, *J*9.4, 3.6, H-2), 3.67-3.75 (4H, m, H-5, 2xH-6, CH*H*O), 3.84 (1H, app dd, *J*14.0, 3.9, CH*H*N), 4.00 (1H, app t, *J*9.4, H-3), 4.45-4.47 (1H, m, CHN), 4.55 (1H, d, *J*11.2, PhC*H*HO), 4.63 (1H, d, *J*3.6, H-1), 4.68 (1H, d, *J*12.2, PhC*H*HO), 4.82 (1H, d, *J*12.2, PhCH*H*O), 4.84 (1H, d, *J*10.8, PhC*H*HO), 4.89 (1H, d, *J*11.2, PhCH*H*O), 4.99 (1H, d, *J*10.8, PhCH*H*O), 5.71-5.78 (2H, m, 2xCH=), 7.23 (2H, d, *J*8.3, 2xAr H-3), 7.25-7.37 (15H, m, 15xPh-H), 7.70 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.42 (CH_3), 23.07 (CH_2), 39.56 (CH_2), 52.43 (CH), 55.08 (CH_3), 69.97 (CH_2), 70.07 (CH), 73.31 (CH_2), 74.05 (CH_2), 75.00 (CH_2), 75.76 (CH_2), 77.90 (CH), 79.92 (CH), 82.03 (CH), 98.03 (CH), 125.44 (CH), 126.28 (CH), 126.87 (2 × CH), 127.57 (CH), 127.61 (CH), 127.69 (2 × CH), 127.84 (CH), 128.01 (2 × CH), 128.04 (2 × CH), 128.35 (4 × CH), 128.39 (2 × CH), 129.52 (2 × CH), 138.13 (C), 138.25 (C), 138.36 (C), 138.68 (C), 142.98 (C); MS (ES) m/z (%): 861

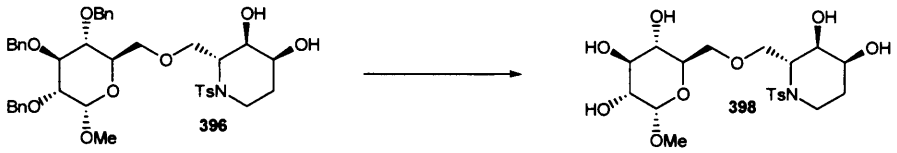
(100%), 731 (63, M+NH_4^+), 862 (55), 732 (29, M+H^++NH_4^+); Accurate mass (FAB): Found 736.2920 (M+Na^+ $C_{41}H_{47}NO_8SNa$ requires 736.2920).

6.9.9 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4,-tribenzyl-α-D-glucopyranoside]ylmethyl)-piperidine-3,4-diol 396



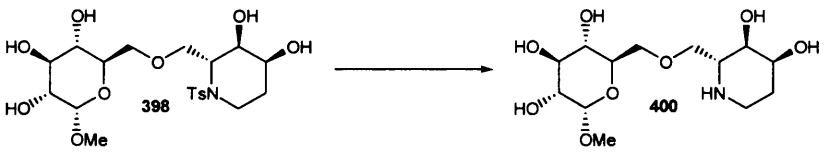
The alkene **394** (0.29 g, 0.40 mmol) dissolved in acetone (2.0 ml), underwent dihydroxylation using the procedure outlined in experiment 6.9.4 using OsO_4 (2.5% by wt in ^tBuOH solution, 0.16 ml, 0.016 mmol) and NMO (0.071 g, 0.61 mmol) dissolved in water (2.0 ml). The reaction mixture was stirred for 24 h before being quenched with a 1% aqueous sodium dithionite solution (10 ml). The mixture was stirred a further 30 min before the solvent was removed under vacuum. The resulting crude reaction mixture was purified by flash column chromatography on silica (50:50, ethyl acetate: petroleum-ether) to give the *diol* **396** as colourless oil (0.20 g, 66%). $[\alpha]^{20}_D$: +18.9 (c=1.61 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3056w, 2922m, 1599m, 1558w, 1496m, 1453m, 1333s, 1273s, 1266s, 1158m, 1072m, 871w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.56-1.72 (2H, m, C*H*$_2$CHOH), 2.37 (3H, s, Ar 4-Me), 3.10 (1H, ddd, *J*11.7, 6.6, 3.7, C*H*HN), 3.40 (3H, s, OMe), 3.42 (1H, app t, *J*9.6, H-4), 3.50 (1H, dd, *J*9.6, 3.7, H-2), 3.53-3.56 (4H, m, CH_2O, 2xH-6), 3.66-3.71 (2H, m, H-5, CH*H*N), 3.77-3.87 (1H, m, CH_2C*H*OH), 3.97 (1H, dd, *J*5.7, 3.2, CHC*H*OH), 3.99 (1H, app t, *J*9.6, H-3), 4.30 (1H, app t br, *J*5.7, CHN), 4.54 (1H, d, *J*11.0, PhC*H*HO), 4.60 (1H, d, *J*3.7, H-1), 4.67 (1H, d, *J*12.2, PhC*H*HO), 4.80 (1H, d, *J*12.2, PhCH*H*O), 4.81 (1H, d, *J*10.8, PhCH*H*O), 4.89 (1H, d, *J*11.0, PhCH*H*O), 4.98 (1H, d, *J*10.8, PhCH*H*O), 7.23 (2H, d, *J*8.3, 2xAr H-3), 7.27-7.37 (15H, m, 15xPh-H), 7.76 (2H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.37 (CH_3), 27.46 (CH_2), 41.02 (CH_2), 55.22 (CH_3), 58.40 (CH), 62.64 (CH), 68.25 (CH), 69.92 (CH), 70.29 (CH_2), 70.55 (CH_2), 73.24 (CH_2), 74.83 (CH_2), 75.68 (CH_2), 77.82 (CH), 79.89 (CH), 81.84 (CH), 97.88 (CH), 127.36 (2 × CH), 127.58 (3 × CH), 127.61 (CH), 127.80 (CH), 127.90 (2 × CH), 127.97 (2 × CH), 128.27 (2 × CH), 128.33 (4 × CH), 129.38 (2 × CH), 137.48 (C), 137.99 (C), 138.97 (C), 138.51 (C), 143.14 (C); MS (ES) m/z (%): 765 (100%, M+NH_4^+), 766 (48, M+H^++NH_4^+); Accurate mass (FAB): Found 770.2975 (M+Na^+ $C_{41}H_{49}NO_{10}SNa$ requires 770.2975).

6.9.10 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-([methyl-α-D-glucopyranoside]ylmethyl)-piperidine 3,4-diol 398



The diol **396** (0.12g, 0.17 mmol) was dissolved in a mixture of ethanol (12 ml) and acetic acid (1.3 ml) and was subjected to the general hydrogenolysis procedure (outlined in experiment 6.2.7) using $Pd(OAc)_2$ (0.034 g, 0.15 mmol). The reaction mixture was stirred 3 h before being filtered and the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (10:90, methanol: ethyl acetate) to give the *pentol* **398** as a white foam (0.06 g, 76%). $[\alpha]^{20}_D$: +30.3 (c=1.16 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3408br (OH), 2924m, 1339w, 1272s, 1156s, 1053m, 1049s, 729s, 692s; δ_H (400 MHz; D_2O): 1.39-1.43 (1H, m, C*H*HCHOH), 1.63 (1H, app qd, *J*12.6, 4.9, CH*H*CHOH), 2.37 (3H, s, Ar 4-Me), 3.17 (1H, app td, *J*12.6, 2.8, C*H*HN), 3.27 (1H, app t, *J*9.0, H-4), 3.30 (1H, dd, *J*9.4, 3.7 H-2), 3.35 (3H, s, OMe), 3.48-3.63 (7H, m, CH*H*N, CH_2O, 2xH-6, H-3, H-5), 3.84 (1H, ddd, *J*12.6, 4.5, 3.2, CH_2CHOH), 3.90 (1H, app t, *J*3.2, CHC*H*OH), 4.28 (1H, app t br, *J*3.2, CHN), 4.62 (1H, d, *J*3.7, H-1) 7.29 (2H, d, *J*8.0, 2xAr H-3), 7.78 (1H, d, *J*8.3, 2xAr H-2); δ_C (100.6 MHz; D_2O): 20.50 (CH_3), 26.79 (CH_2), 41.42 (CH_2), 48.88 (CH), 54.72 (CH_3), 59.92 (CH), 66.82 (CH), 68.49 (CH), 70.67 (CH_2), 70.80 (CH_2), 71.47 (CH), 72.50 (CH), 74.11 (CH), 100.28 (CH), 127.69 (2 × CH), 129.51 (2 × CH), 138.52 (C), 143.57 (C); MS (ES) m/z (%): 191 (100%), 500 (86, $M+Na^+$); Accurate mass (FAB): Found 478.1746 ($M+H^+$ $C_{20}H_{32}NO_{10}S$ requires 478.1747).

6.9.11 (2*R*, 3*R*, 4*S*)-2-([Methyl-α-D-glucopyranoside]ylmethyl)-piperidine 3,4-diol 400

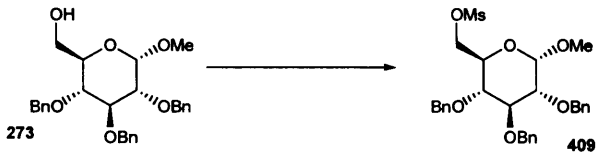


The pentol **398** (0.029 g, 0.062 mmol) was subjected to the general tosyl deprotection procedure (6.2.9) using sodium metal (0.0035 g, 0.15 mmol) and liquid ammonia (5-10 ml). After the addition of sodium was complete the reaction was warmed to room temperature and the liquid ammonia was allowed to evaporate. The crude residue was dissolved into water and transferred to a round bottom flask, the water was removed under reduced pressure and the resulting crude material was purified by flash column chromatography on (100% methanol) to give the *amine* **400** as a clear oil (0.0098 g, 49%). $[\alpha]^{20}_D$: +56.3 (c=0.27 MeOH); ν_{max} (solid

state)/ cm^{-1}: 3307br (OH, NH), 2922m, 1634m, 1544w, 1416m, 1337w, 1107m, 1076m, 1040s, 900m, 831w, 755w; δ_H (400 MHz; D_2O): 1.77 (1H, dddd, *J*14.8, 7.6, 5.2, 2.8, C*H*HCHOH), 1.88 (1H, d app q, *J*14.8, 2.8, CH*H*CHOH), 2.80-2.91 (2H, m, CH_2N), 3.08 (1H, ddd, *J*10.0, 6.8, 3.2, CHN), 3.47 (3H, s, OMe), 3.49 (1H, app t, *J*9.6, H-4), 3.55 (1H, dd, *J*10.0, 2.8, CHC*H*OH), 3.59 (1H, dd, *J*9.6, 3.6, H-2), 3.69 (1H, dd, *J*10.4, 6.8, C*H*HO) 3.70 (1H, app t overlapping, *J*9.6, H-3), 3.77 (4H, m, H-5, 2xH-6, CH*H*O), 4.12 (1H, app q, *J*2.8, C*H*OH), 4.84 (1H, d, *J*3.6, H-1); δ_C (100.6 MHz; D_2O): 30.66 (CH_2), 38.28 (CH_2), 54.08 (CH), 55.20 (CH_3), 67.65 (CH), 69.39 (CH), 69.52 (CH), 69.66 (CH_2), 70.50 (CH), 71.19 (CH), 71.51 (CH_2), 73.03 (CH), 99.38 (CH); MS (ES) m/z (%): 324 (M+H+, 100%); Accurate mass (FAB): Found 324.1658 ($M+H^+$ $C_{13}H_{26}NO_8$ requires 324.1658).

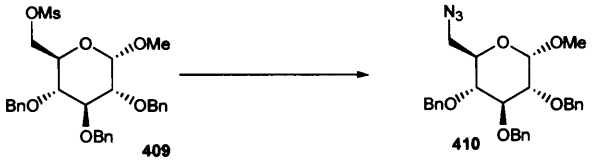
6.10 The synthesis of the (1→6) linked homoaza-*N*-disaccharides

6.10.1 Methyl-[O^2, O^3, O^4,-tribenzyl-O^6-(methanesulfonyl)-α-D-glucopyranoside] 409[182]



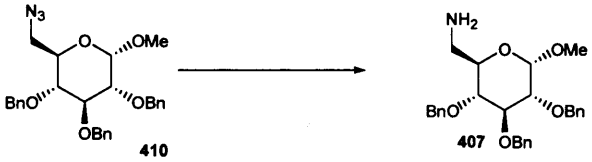
Triethylamine (0.2 g, 0.29 ml, 2.1 mmol) was added to a solution of alcohol **273** (0.087 g, 1.87 mmol) in CH_2Cl_2 (5 ml) at 0 °C. After 20 min methanesulfonyl chloride (0.43 g, 0.29 ml, 3.75 mmol) was added dropwise to the solution and the mixture was stirred for 1.5 h before being quenched with saturated aqueous ammonium chloride (5 ml) and water (5 ml). The aqueous extract was extracted with ethyl acetate (3x 20 ml). The organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum to give the *mesylate* **409** as a yellow oil (0.88 g, 86%). $[\alpha]^{20}_D$: + 60.0 (c=1.23 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2912s, 1726s, 1602m, 1496m, 1454s, 1360s, 1280m, 1272s, 1178s, 1092s, 1028s, 810m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.97 (3H, OSO_2CH_3), 3.38 (3H, s, OMe), 3.48 (1H, dd, *J*10.0, 9.4, H-4), 3.51 (1H, dd, *J*9.4, 3.7, H-2), 3.84 (1H, ddd, *J*10.0, 4.0, 2.0, H-5), 4.01 (1H, app t, *J*9.4, H-3), 4.33 (1H, dd, *J*11.0, 2.0, H-6), 4.38 (1H, dd, *J*11.0, 4.0, H-6), 4.59 (1H, d, *J*3.7, H-1), 4.62 (1H, d, *J*10.8, PhC*H*HO), 4.65 (1H, d, *J*12.2, PhC*H*HO), 4.79 (1H, d, *J*12.2, PhCH*H*O), 4.82 (1H, d, *J*11.2, PhC*H*HO), 4.91 (1H, d, *J*10.8, PhCH*H*O), 5.00 (1H, d, *J*11.2, PhCH*H*O), 7.27-7.37 (15H, m, 15xPh-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 37.51 (CH_3), 55.45 (CH_3), 68.40 (CH_2), 68.63 (CH), 73.4 (CH_2), 75.11 (CH_2), 75.75 (CH_2), 77.51 (CH), 79.79 (CH), 81.80 (CH), 98.17 (CH), 127.68 (CH), 127.90 (2 × CH), 127.97 (CH), 128.07 (CH), 128.07 (4 × CH), 128.43 (2 × CH), 128.51 (4 × CH), 137.75 (C), 137.95 (C), 138.50 (C). This is in agreement with the reported literature NMR data.[182]

6.10.2 Methyl-[O^2, O^3, O^4,-tribenzyl-O^6-(azido)-α-D-glucopyranoside] 410[183]



Sodium azide (0.084 g, 1.29 mmol) was added to the mesylate **409** (0.18 g, 0.32 mmol) in DMF (10 ml). The reaction mixture was heated (80 °C) for 3 h before being quenched with saturated aqueous ammonium chloride solution (10 ml) followed by water (10 ml). The aqueous layer was extracted with ethyl acetate (3x20 ml), the organic layers were combined, dried ($MgSO_4$) and solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *azide* **410** as a colourless oil (0.11 g, 72%). ν_{max} (CH_2Cl_2)/ cm^{-1}: 2982s, 2304s, 1684m, 1668m, 1652m, 1646m, 1634m, 1576m, 1558m, 1540m, 1418m, 1360m, 1253s, 1050s. 893s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.33 (1H, dd, *J*13.0, 5.5, H-6), 3.41 (3H, s, OMe), 3.44 (1H, overlapping app t, *J*9.6, H-4), 3.46 (1H, dd, *J*13.0, 2.5, H-6), 3.55 (1H, dd, *J*9.6, 3.7, H-2), 3.80 (1H, ddd, *J*9.6, 5.5, 2.5, H-5), 4.01 (1H, app t, *J*9.6, H-3), 4.59 (1H, d, *J*11.0, PhC*H*HO), 4.63 (1H, d, *J*3.7, H-1), 4.68 (1H, d, *J*12.0, PhC*H*HO), 4.81 (1H, d, *J*12.0, PhCH*H*O), 4.82 (1H, d, *J*11.0, PhC*H*HO), 4.91 (1H, d, *J*11.0, PhCH*H*O), 5.00 (1H, d, *J*11.0, PhCH*H*O), 7.23-7.40 (15H, m, 15xPh-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 51.35 (CH_2), 55.31 (CH_3), 69.90 (CH), 73.37 (CH_2), 75.08 (CH_2), 75.70 (CH_2), 78.32 (CH), 79.96 (CH), 81.79 (CH), 98.00 (CH), 127.62 (2 × CH), 127.90 (5 × CH), 128.03 (2 × CH), 128.38 (2 × CH), 128.44 (4 × CH), 137.92 (C), 138.00 (C), 138.58 (C); MS (ES):430 (100%), 436 (72), 507 (51, $M+NH_4^+$), 512 (42, $M+Na^+$), 490 (12, $M+H^+$). This is in agreement with the reported literature NMR data.[183]

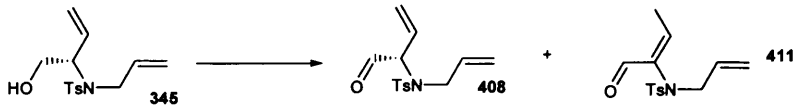
6.10.3 Methyl-[O^2, O^3, O^4,-tribenzyl-O^6-amino-α-D-glucopyranoside] 407[122]



A solution of the azide **410** (0.46 g, 0.93 mmol) in diethyl ether (12 ml) was added to a suspension of $LiAlH_4$ (0.19 g, 5.13 mmol) in diethyl ether (21 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1 h before being diluted with diethyl ether (10 ml). Saturated aqueous $NaHCO_3$ solution (10 ml) was carefully added and the mixture was stirred for a further 30 min at room temperature. The mixture was extracted with diethyl ether (3x20 ml), the organic extracts were combined, dried ($MgSO_4$) and solvent was removed

under vacuum. The resulting crude material was purified by flash column chromatography on silica (1:15, methanol: CH_2Cl_2) to give the *amine* **407** as a white solid (0.22 g, 50%). Mp: 85-86 °C (Lit.[183], 86-89 °C); $[\alpha]^{20}_D$: + 63.4 (c=1.17 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2580br (NH), 2910s, 1728w, 1496m, 1454w, 1358m, 1270s, 1266m, 1258s, 1084s, 1070s, 1050s, 914w, 870w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.73 (1H, dd, *J*13.5, 6.0, H-6), 2.99 (1H, dd, *J*13.5, 2.5, H-6), 3.36 (1H, app t, *J*9.4, H-4), 3.39 (3H, s, OMe), 3.51 (1H, dd, *J*9.4, 3.5, H-2), 3.58 (1H, ddd, *J*9.4, 6.0, 2.5, H-5), 4.01 (1H, app t, *J*9.4, H-3), 4.58 (1H, d, *J*3.5, H-1), 4.62 (1H, d, *J*11.2, PhC*H*HO), 4.67 (1H, d, *J*12.2, PhC*H*HO), 4.81 (1H, d, *J*12.2, PhCH*H*O), 4.84 (1H, d, *J*11.0, PhC*H*HO), 4.89 (1H, d, *J*11.2, PhCH*H*O), 5.00 (1H, d, *J*11.0, PhCH*H*O), 7.29-7.36 (15H, m, 15xPh-H); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 42.72 (CH_2), 55.00 (CH_3), 71.56 (CH), 73.26 (CH_2), 74.85 (CH_2), 75.63 (CH_2), 78.53 (CH), 80.11 (CH), 82.07 (CH), 97.85 (CH), 127.53 (CH), 127.69 (CH), 127.79 (CH), 127.83 (CH), 127.89 (2 × CH), 128.02 (2 × CH), 128.33 (3 × CH) 128.39 (4 × CH), 138.10 (2 × C), 138.72 (C); MS (ES):432 (100%), 464 (75, $M+H^+$), 324 (59); Accurate mass (FAB): Found 464.2436 ($M+H^+$ $C_{28}H_{34}NO_5$ requires 464.2437).

6.10.4 *N*-Allyl-*N*-(1-formyl-allyl)-4-methyl-benzenesulfonamide 408

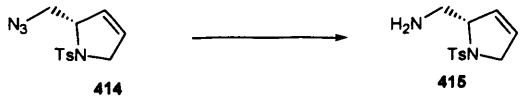


Dess-Martin periodinane (0.093 g, 0.22 mmol) was added to a solution of the alcohol **345** (0.056 g, 0.2 mmol) in dichloromethane (1.5 ml). The reaction was stirred at room temperature for 2 h. Diethyl ether (5 ml) was added to the reaction mixture and the resulting solution was sonnicated and filtered. The filtrate was washed with a 5% aqueous solution of sodium thiosulfate. The organic layers were combined, dried ($MgSO_4$) and reduced under vacuum to yield the crude aldehyde **408** (0.076 g, 84%). δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.51-3.93 (2H, m, NCH_2), 4.64 (1H, d, *J*4.6, CH), 5.03-5.43 (4H, m, 2xCH=C*H*$_2$), 5.53-5.85 (2H, m, 2xC*H*=CH_2), 7.21 (2H, d, *J*8.1, 2xAr H-3), 7.74 (2H, d, *J*8.1, 2xAr H-2), 9.62 (1H, s, CH). The following significant peaks were observed in the crude ^{1}H-NMR to suggest formation of **411**: disappearance of the CH signal at 4.64 and the CHO signal at 9.62, appearance of - δ_H (250 MHz; $CDCl_3$; Me_4Si): 3.85 (2H, d, *J*10.4, NCH_2), 4.91-5.30 (4H, m, 2xCH=C*H*$_2$), 5.52-5.87 (2H, m, 2xC*H*=CH_2), 7.27 (2H, d, *J*8.1, 2xAr H-3), 7.71 (2H, d, *J*8.1, 2xAr H-2), 9.11 (1H, s, CH).

6.10.5 (*S*)-1-(Toluene-4-sulfonyl)-2-azidomethyl-2,5-dihydro-1*H*-pyrrole 414

Sodium azide (0.19 g, 2.94 mmol) was added to a solution of the mesylate **391** (0.24 g, 0.74 mmol) in DMF (10 ml) as described in experiment 6.10.2. The reaction mixture was heated (80 °C) for 4 h before being quenched with saturated aqueous ammonium chloride (10 ml) followed by water (10 ml). The aqueous layer was washed with ethyl acetate (3x20 ml), the organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum. The resulting crude material was purified using flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *azide* **414** as a white solid (0.16 g, 77%). Mp: 98-99 °C; $[\alpha]^{20}_D$: -165.4 (c=1.46 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2922m, 2858m, 2739m, 2109s (N_3), 1684m, 1598m, 1348s, 1270s, 1265s, 1164s, 1017m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.43 (3H, s, Ar 4-Me), 3.53 (1H, dd, *J*12.4, 3.0, C*H*HN$_3$), 3.69 (1H, dd, *J*12.4, 5.7, CH*H*N$_3$), 4.07-4.26 (2H, m, NCH_2), 4.56-4.60 (1H, m, CHN), 5.61 (1H, d app q, *J*6.4, 2.0, CHC*H*=CH), 5.77 (1H, ddd, *J*6.4, 4.4, 2.0, CH_2C*H*=CH), 7.33 (2H, d, *J*8.5, 2xAr H-3), 7.72 (2H, d, *J*8.5, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.50 (CH_3), 55.28 (CH_2), 55.77 (CH_2), 65.37 (CH), 127.16 (CH), 127.18 (CH), 127.44 (2 × CH), 129.87 (2 × CH), 134.12 (C), 143.90 (C); MS (ES) m/z (%): 167 (100%), 128 (50), 279 (42, M+H$^+$), 301 (29, M+Na$^+$); Anal. Found: C, 51.93; H, 5.24; N, 20.14; $C_{12}H_{14}O_2N_4S$ requires: C, 51.78; H, 5.07; N, 20.13.

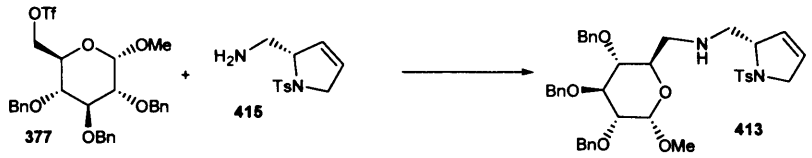
6.10.6 (*S*)-1-(Toluene-4-sulfonyl)- 2-methylamine-2,5-dihydro-1*H*-pyrrole 415



Triphenylphosphine (0.05 g, 0.2 mmol) was added to a solution of the azide **414** (0.057 g, 0.20 mmol) in THF (0.6 ml) and water (0.1 ml). The reaction mixture was stirred for 20 h before the solvent was removed under vacuum. The resulting crude product was purified by flash column chromatography on silica (5: 95, methanol: dichloromethane) to give the *amine* **415** as colourless oil (0.046 g, 90%). α_D: -170.6 (c=1.00 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3382br (NH), 29923m, 2868m, 2741w, 1734w, 1597m, 1523w, 1493m, 1466m, 1338s, 1305m, 1272m, 1266s, 1263vs, 1163s, 1094s, 841m, 808m; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.42 (3H, s, Ar 4-Me), 2.85 (1H, dd, *J*13.5, 3.9, C*H*HNH$_2$), 3.12 (1H, dd, *J*13.5, 4.4, CH*H*NH$_2$), 4.09-4.22 (2H, m, NCH_2), 4.42-4.52 (1H, m, CHN), 5.54 (1H, d app q, *J*6.2, 2.3, CHC*H*=CH), 5.72 (1H, ddd, *J*6.2, 4.1, 2.0, CH_2C*H*=CH), 7.31 (2H, d, *J*8.0, 2xAr H-3), 7.72 (2H, d, *J*8.5, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.48 (CH_3), 46.13 (CH_2), 56.20 (CH_2), 69.31 (CH),

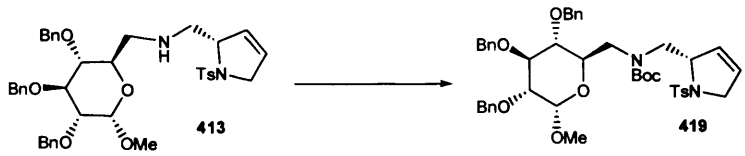
126.57 (CH), 127.45 (2 × CH), 127.89 (CH), 129.78 (2 × CH), 134.30 (C), 143.64 (C); MS (ES) m/z (%): 253 (100%, $M+H^+$), 236 (79, $M-NH_2$), 155 (58); Accurate mass (FAB): Found 253.1012 ($M+H^+$ $C_{12}H_{17}N_2O_2S$ requires 253.1011).

6.10.7 (*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4 tribenzyl-α-D-glucopyranoside-O^6-amino]ylmethyl)-2,5-dihydro-1*H*-pyrrole 413



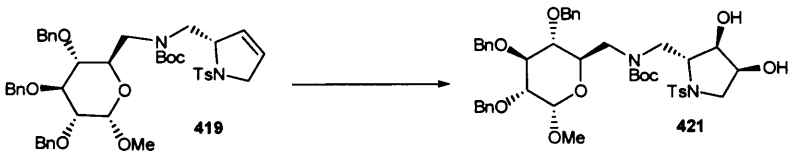
Diisopropylethylamine (0.02 g, 0.028 ml, 0.20 mmol) was added dropwise to a solution of the amine **415** (0.046 g, 0.18 mmol) in THF (0.5 ml) at 0 °C. The resulting mixture was stirred for 20 min before a solution of the triflate **377** (0.13 g, 0.22 mmol) in THF (1 ml) was added. The reaction mixture was stirred for 20 h before being quenched with saturated aqueous ammonium chloride (5 ml). Water (5 ml) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3x10 ml). The organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (100% ethyl acetate) to give the *secondary amine* **413** as a colourless oil (0.076 g, 60%). $[\alpha]^{20}_D$: -26.4 (c=1.45 CH_2Cl_2); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3031w, 2923s, 1599m, 1495m, 1453s, 1343s, 1269s, 1169s, 1091s, 895w; δ_H (250 MHz; $CDCl_3$; Me_4Si): 2.41 (3H, s, Ar 4-Me), 2.76 (1H, dd, *J*12.1, 7.1, H-6), 2.80 (1H, dd, *J*12.4, 5.5, C*H*HNH), 2.96 (1H, dd, *J*12.4, 3.0, CH*H*NH), 2.98 (1H, dd, *J*12.1, 3.0, H-6), 3.41 (3H, s, OMe), 3.43 (1H, app t, *J*9.7, H-4), 3.52 (1H, dd, *J*9.7, 3.5, H-2), 3.78 (1H, ddd, *J*9.7, 7.1, 3.0, H-5), 4.99 (1H, app t, *J*9.4, H-3), 4.11-4.18 (2H, m, CH_2NTs), 4.49-4.50 (1H, m, CHN), 4.60 (1H, d, *J*3.5, H-1), 4.63 (1H, d, *J*10.8, PhC*H*HO), 4.68 (1H, d, *J*12.0, PhC*H*HO), 4.80 (1H, d, *J*12.0, PhCH*H*O), 4.83 (1H, d, *J*11.0, PhC*H*HO), 4.90 (1H, d, *J*10.8, PhCH*H*O), 4.99 (1H, d, *J*11.0, PhCH*H*O), 5.58 (1H, d app q, *J*6.3, 1.4, CHC*H*=CH), 5.61 (1H, ddd, *J*6.3, 4.2, 1.6, CH_2CH=CH), 7.19-7.36 (17H, m, 15xPh-H, 2xAr H-3), 7.70 (1H, d, *J*8.3, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.44 (CH_3), 50.28 (CH_2), 54.48 (CH_2), 55.21 (CH_3), 55.90 (CH_2), 67.03 (CH), 69.02 (CH), 73.26 (CH_2), 74.92 (CH_2), 75.67 (CH_2), 79.34 (CH), 80.03 (CH), 81.92 (CH), 97.88 (CH), 125.71 (CH), 127.45 (2 × CH), 127.53 (CH), 127.67 (CH), 127.83 (CH), 127.89 (2 × CH), 127.92 (CH), 128.01 (2 × CH), 128.32 (2 × CH), 128.36 (2 × CH), 128.38 (2 × CH), 128.49 (2 × CH), 129.73 (2 × CH), 133.99 (C), 138.10 (C), 138.22 (C), 138.69 (C), 143.62 (C); MS (ES): 130 (100%), 699 (92, $M+H^+$), 700 (48, $M+2H^+$); Accurate mass (FAB): Found 699.3104 ($M+H^+$ $C_{40}H_{47}N_2O_7S$ requires 699.3104).

6.10.8 (*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4, -tribenzyl-α-D-glucopyranoside-O^6-carbamic acid *tert*-butyl ester]ylmethyl)-2,5-dihydro-1*H*-pyrrole 419



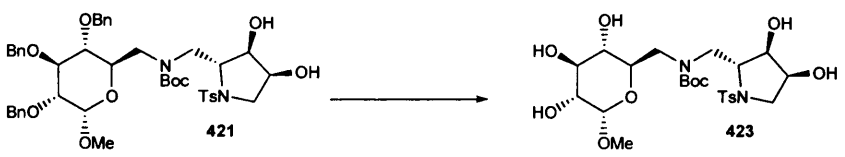
Triethylamine (0.046 g, 0.064 ml, 0.46 mmol) followed by a solution of di-*tert* butyl dicarbonate (0.136 g, 0.62 mmol) in dichloromethane (2 ml) were added to a solution of the secondary amine **413** (0.29 g, 0.41 mmol) in dichloromethane (10 ml) at 0 °C. The resulting mixture was stirred for 18 h before being diluted with dichloromethane (10 ml). Water (20 ml) was added to the mixture and the aqueous layer was extracted with dichloromethane (3x10 ml). The organic layers were combined, dried ($MgSO_4$) and solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (40:60, ethyl acetate: petroleum-ether) to give the *protected amine* **419** (0.33 g, 96%). $[\alpha]^{20}_D$: -29.2 (c= 0.77 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2930s, 1765s, 1689s, 1598m, 1454m, 1412m, 1366s, 1268s, 1163s, 1163s, 1092s, 909s, 815m; δ_H (400 MHz; 323K, $CDCl_3$; Me_4Si,): (signals broad due to restricted rotation) 1.46 (9H, s, $C(CH_3)_3$), 2.41 (3H, s, Ar 4-Me), 3.23 (1H, app t, *J*9.2, H-4), 3.32 (3H, s, OMe), 3.45-3.49 (1H, br m, C*H*HNTs), 3.54 (1H, dd, *J*9.2, 3.7, H-2), 3.61-3.66 (1H, br m, CH*H*NTs), 3.81 (1H, m, H-6), 3.88 (1H, ddd, *J*11.52, 9.2, 2.4, H-5), 3.98 (1H, app t, *J*9.2, H-3), 4.10-4.11 (2H, m, CH_2N), 4.39 (1H, m, H-6), 4.61-4.62 (2H, m, H-1, CHN), 4.65 (1H, d, *J*11.3, PhC*H*HO), 4.66 (1H, d, *J*11.9, PhC*H*HO), 4.76 (1H, d, *J*11.9, PhCH*H*O), 4.80 (1H, d, *J*11.2, PhC*H*HO), 4.90 (1H, d, *J*11.3, PhCH*H*O), 4.97 (1H, d, *J*11.2, PhCH*H*O), 5.58-5.61 (2H, m, 2xCH=), 7.24-7.37 (17H, m, 15xPh-H, 2xAr H-3), 7.68 (1H, d, *J*8.2, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.30 (CH_3), 28.45 (3 × CH_3), 54.76 (CH), 55.48 (CH_2), 66.50 (CH), 73.23 (2 × CH_2), 74.75 (CH_2), 75.62 (2 × CH_2), 76.86 (C), 80.34 (CH), 82.30 (CH), 85.08 (CH), 97.63 (CH), 127.48 (2 × CH), 127.52 (CH), 127.67 (2 × CH), 127.77 (2 × CH), 127.97 (2 × CH), 128.18 (2 × CH), 128.28 (4 × CH), 128.36 (4 × CH), 129.60 (2 × CH), 134.92 (C), 138.38 (C), 138.54 (C), 139.00 (C), 143.42 (C), 155.73 (C); MS (ES) m/z (%): 816 (100%, $M+NH_4^+$), 799 (72, $M+H^+$), 699 (52, M-$COOC(CH_3)_3$); Accurate mass (FAB): Found 799.3629 ($M+H^+$ $C_{45}H_{55}N_2O_9S$ requires 799.3628).

6.10.9 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4,-tribenzyl-α-D-glucopyranoside-O^6-carbamic acid *tert*-butyl ester]ylmethyl)-pyrrolidine-3,4-diol 421



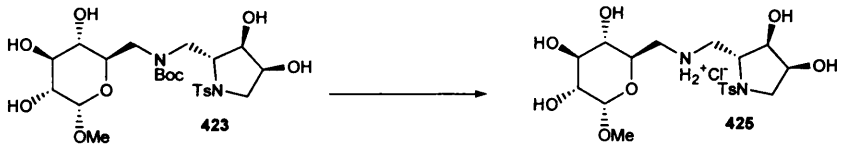
A solution of the alkene **419** (0.21 g, 0.26 mmol) in acetone (2.0 ml) underwent dihydroxylation using the procedure outlined in experiment 6.9.4 using OsO_4 (2.5% by wt in ^{t}BuOH solution, 0.11 ml, 0.011 mmol) and NMO (0.046 g, 0.39 mmol) dissolved in water (2.0 ml). The reaction mixture was stirred for 24 h before being quenched with 1% aqueous sodium dithionite (10 ml). The resulting mixture was stirred a further 30 min before the solvent was removed under vacuum. The resulting crude reaction mixture was purified by flash column chromatography on silica (50:50, ethyl acetate: petroleum-ether) to give the *diol* **421** as a colourless oil (0.14 g, 75%). $[\alpha]^{20}_D$: -2.7 (c=1.15 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3300bw (OH), 2928s, 1673s (C=O), 1659s (C=O), 1616m, 1558w, 1344m, 1271s, 1204s, 1136s, 1123w, 1091s; δ_H (400 MHz, 323K; $CDCl_3$; Me_4Si): 1.43 (9H, s, $C(CH_3)_3$), 2.41 (3H, s, Ar 4-Me), 3.18-3.28 (3H, m, H-4, H-6, C*H*HNTos), 3.32 (3H, s, OMe), 3.38-3.46 (1H, br m, C*H*HBoc), 3.52 (1H, dd, *J*16.7, 5.0, CH*H*NTos), 3.56 (1H, dd, *J*9.5, 3.6, H-2), 3.68-3.76 (2H, br m, CH*H*NBoc), 3.89-3.94 (2H, m, H-5, H-6), 3.98 (1H, app t, *J*9.5, H-3), 4.02 (1H, app t, *J*4.4, CHC*H*OH), 4.17 (1H, app q, *J*4.4, CH_2C*H*OH), 4.61 (1H, d, *J*3.6, H-1), 4.64 (1H, d, *J*11.3, PhC*H*HO), 4.67 (1H, d, *J*12.1, PhC*H*HO), 4.77 (1H, d, *J*12.1, PhCH*H*O), 4.80 (1H, d, *J*11.2, PhC*H*HO), 4.93 (1H, d, *J*11.3, PhCH*H*O), 4.99 (1H, d, *J*11.2, PhCH*H*O), 7.28-7.39 (17H, m, 15xPh-H, 2xAr H-3), 7.72 (1H, d, *J*8.2, 2xAr H-2); δ_C (62.9 MHz, 323K; $CDCl_3$; Me_4Si): 21.47 (CH_3), 28.36 (3 × CH_3), 50.13 (CH_2), 55.62 (CH_2), 54.86 (CH_3), 60.38 (CH_2), 65.04 (CH), 70.02 (CH), 73.26 (CH_2), 74.74 (CH_2), 75.56 (CH_2), 76.30 (CH), 80.03 (2 × CH), 80.30 (CH), 80.86 (C), 82.20 (CH), 97.67 (CH), 127.49 (2 × CH), 127.60 (CH), 127.80 (2 × CH), 127.86 (CH), 128.19 (2 × CH), 128.08 (3 × CH), 128.20 (2 × CH), 128.29 (2 × CH), 128.42 (2 × CH), 129.37 (2 × CH), 133.42 (C), 138.07 (C), 138.38 (C), 138.73 (C), 143.61 (C), 157.40 (C); MS (ES) m/z (%): 559 (100%), 816 (72, M+H^+-OH), 593 (66), 799 (52), 699 (48), 850 (42, M+NH_4^+).

6.10.10 (2*R*, 3*R*, 4*S*)-1-(toluene-4-sulfonyl)-2-(methyl-α-D-glucopyranoside-O^6-carbamic acid *tert*-butyl ester]ylmethyl)-pyrrolidine-3,4-diol 423



The diol **421** (0.10g, 0.13 mmol) was dissolved in a mixture of ethanol (7.9 ml) and acetic acid (0.97 ml) and was subjected to the general hydrogenolysis procedure (outlined in experiment 6.2.7) using $Pd(OAc)_2$ (0.026 g, 0.12 mmol). The reaction mixture was stirred for 4 h before being was filtered and the solvent was removed under vacuum. The resulting crude reaction mixture was purified by flash column chromatography on silica (1:99, methanol: ethyl acetate) to give the *pentol* **423** as an oil (0.028 g, 63%). $[\alpha_D]^{20}{}_D$: +18.8 (c= 1.16 MeOH); ν_{max} (solid state)/ cm^{-1}: 3406 br (OH), 2931w, 1662 (C=O), 1599w, 1478w, 1415m, 1367m, 1263m, 1158s, 1090s, 966w, 893w, 813w, 734s; δ_H (400 MHz, 300K; D_2O) (signals broad due to restricted rotation): 1.44 (9H, s, $C(CH_3)_3$), 2.38 (3H, s, Ar 4-Me), 3.08 (1H, dd, *J*9.2, 8.3, C*H*HNTs), 3.20 (1H, app t, *J*9.2, H-4), 3.29 (3H, s, OMe), 3.30-3.94 (9H, m, 2xH-6, CH_2NBoc, CH*H*NTs, H-3, H-5, CH, CHC*H*OH), 4.32 (1H, app td, *J*7.1, 3.9, CH_2CHOH), 4.79 (1H signal obscured by HOD peak, H-1), 7.40 (2H, d, *J*8.0, 2xAr H-3), 7.69 (2H, d, *J*8.0, 2xAr H-2); δ_C (100.6 MHz, 300K; D_2O): 21.14 (CH_3), 28.14 (3 × CH_3), 49.29 (CH_2), 50.34 and 50.74 (CH_2), 51.16 (CH_2), 54.98 (CH_3), 64.68 and 64.90 (CH), 70.11 and 69.98 (CH), 71.21 (CH), 71.74 (CH), 72.22 and 72.31 (CH), 73.04 (CH), 73.34 and 73.50 (CH), 82.31 and 82.47 (C), 99.25 (CH), 128.06 and 128.15 (2 × CH), 130.26 (2 × CH), 132.62 (C), 143.29 (C), 157.58 (C); MS (ES) m/z (%): 463 (100%, $M+2H^+$-$COOC(CH_3)_3$), 563 (22, $M+H^+$); Accurate mass (FAB): Found 563.2274 ($M+H^+$ $C_{24}H_{39}N_2O_{11}S$ requires 563.2275).

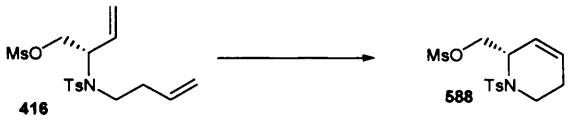
6.10.11 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl) 2-(methyl-α-D-glucopyranoside-O^6-ammonium chloride]ylmethyl)- pyrrolidine-3,4-diol 425



Acetyl chloride (4.26 ml, 60 mmol) was added to a solution of ethyl acetate (14.3 ml, 150 mmol) and ethanol (5.16 ml, 90 mmol) at 0 °C and the mixture was stirred for 30 min. A small portion (1 ml) of the resulting solution was added to the pentol **423** (0.0091 g, 0.016 mmol) and the reaction mixture was stirred for 1 h. The solvent was removed under vacuum to yield the *ammonium chloride salt* **425** (0.0074 g, 95%) as an oil. $[\alpha]^{20}{}_D$: -3.0 (c=0.95 MeOH); ν_{max} / cm^{-1} (solid state): 3334br (OH/NH), 2960m, 1727m, 1652w, 1448m, 1260s, 1155m, 1088s, 801m, 735m, 703m, 665m; δ_H (400 MHz; D_2O): 2.39 (3H, s, Ar 4-Me), 3.23 (1H, dd, *J*10.9, 5.5, C*H*HNTs), 3.28-3.36 (3H, m, H-6, C*H*HNBoc, H-4), 3.39 (1H, dd, *J*13.0, 3.7, CH*H*NBoc), 3.45 (3H, s, OMe), 3.52 (1H, dd, *J*12.7, 2.8, H-6), 3.59 (1H, dd, *J*9.8, 3.7, H-2), 3.64 (1H, dd, *J*10.9, 5.4, CH*H*NTs), 3.66 (1H, app t, *J*9.8, H-3), 3.81 (1H, d app t, *J*8.6, 3.7, CHN), 3.94-3.97 (1H, m, H-5), 3.97 (1H, app t *J*3.7, C*H*OH), 4.21 (1H, app q, *J*3.7,

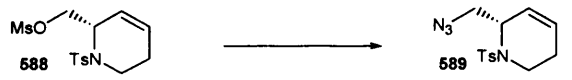
C*H*OH), 7.45 (2H, d, *J*8.0, 2xAr H-3), 7.75 (2H, d, *J*8.0, 2xAr H-2); δ_C (100.6 MHz; D_2O): 21.09 (CH_3), 49.64 (CH_2), 50.33 (CH_2), 52.74 (CH_2), 56.04 (CH_3), 61.63 (CH), 66.95 (CH), 69.51 (CH), 71.34 (CH), 72.07 (CH), 72.99 (CH), 74.12 (CH), 99.86 (CH), 128.44 (2 × CH), 130.32 (2 × CH), 131.08 (C), 146.42 (C); MS (ES) m/z (%): 463 (100%, $M+H^+$); Accurate mass (FAB): Found 463.1750 ($M+H^+$ $C_{19}H_{31}N_2O_9S$ requires 463.1750).

6.10.12 (*S*)-1-(Toluene-4-sulfonyl)-2-methanesulfonylmethyl-1,2,5,6-tetrahydropyridine 588



A solution of the diene **416** (0.67 g, 1.72 mmol) in CH_2Cl_2 (10 ml) was added to *bis*(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride **299** (Grubbs catalyst) (0.071 g, 0.086 mmol) as described in experiment 6.9.3. The solution was refluxed (45 °C) for 3 h before being filtered through a pad of silica and washed through with ethyl acetate (3x10 ml). The solvent was removed under vacuum and the resulting crude mixture was purified by flash column chromatography on silica (20:80 ethyl acetate: petroleum-ether) to give the *alkene* **588** as an orange oil (0.36 g, 60%, 83% based on recovered SM) together with recovered starting material **416** (0.20 g). $[\alpha]^{20}_D$: -90.9 (c=1.00 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3062s, 2937s, 1597s, 1461m, 1356s, 1273m, 1266s, 1212m, 1161s, 1101s, 1061m, 951s, 908m, 816s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.78-1.84 (2H, m, C*H*$_2$CH=CH), 2.42 (3H, s, Ar 4-Me), 3.07 (3H, s, OSO_2CH_3), 3.19 (1H, ddd, *J*14.9, 9.0, 6.4, NC*H*H), 3.86 (1H, d app t, *J*14.9, 3.9, NCH*H*), 4.26 (1H, dd, *J*10.6, 5.3, C*H*HOSO_2CH_3), 4.32 (1H, dd, *J*10.6, 5.7, CH*H*OSO_2CH_3), 4.61-4.68 (1H, m, CHN), 5.62 (1H, dd app t, *J*10.5, 3.8, 2.0, CHC*H*=CH), 5.86-5.90 (1H, m, CH_2CH=CH), 7.29 (2H, d, *J*8.0, 2xAr H-3), 7.71 (2H, d, *J*8.0, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.44 (CH_3), 22.70 (CH_2), 37.67 (CH_3), 39.02 (CH_2), 52.00 (CH), 69.71 (CH_2), 122.35 (CH), 126.91 (2 × CH), 129.00 (CH), 129.72 (2 × CH), 137.59 (C), 143.58 (C); MS (ES) m/z (%): 346 (100%, $M+H^+$), 250 (99, M-SO_2CH_3), 363 (52, $M+NH_4^+$); Accurate mass (FAB): Found 346.0783 ($M+H^+$ $C_{14}H_{20}NO_5S_2$ requires 346.0783); Anal. Found: C, 48.63; H, 5.66; N, 3.88; $C_{14}H_{19}O_5NS_2$ requires: C, 48.68; H, 5.54; N, 4.05.

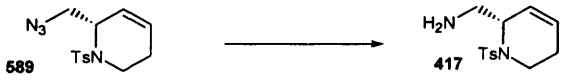
6.10.13 (*S*)-1-(Toluene-4-sulfonyl)-2-azidomethyl-1,2,5,6-tetrahydropyridine 589



Sodium azide (0.25 g, 3.87 mmol) was added to a solution of the mesylate **588** (0.33 g, 0.97 mmol) in DMF (25 ml) as described in experiment 6.10.2. The reaction mixture was heated (80 °C) for 5 h before being quenched with saturated aqueous ammonium chloride solution

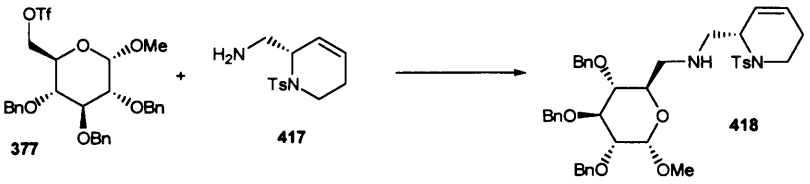
(10 ml) followed by water (10 ml). The aqueous layer was extracted with ethyl acetate (3x20 ml), the organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (80:20 ethyl acetate: petroleum-ether) to give the *azide* **589** (0.20 g, 70%) as a white solid. Mp: 70-72 °C; $[\alpha]^{20}_D$: -84.6 (c= 1.38 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2140m, 1343w, 1263s, 1160s, 1098w, 762m; δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.81-1.88 (2H, m, C*H*$_2$CH=CH), 2.42 (3H, s, Ar 4-Me), 3.22 (1H, d app t, *J*15.8, 7.0, C*H*HN), 3.43 (1H, dd, *J*12.6, 5.9, C*H*HN$_3$), 3.49 (1H, dd, *J*12.6, 5.6, CH*H*N$_3$), 3.88 (1H, d appt, *J*15.8, 4.1, CH*H*N), 4.43-4.46 (1H, m, CHN), 5.63 (1H, dd app t *J*10.5, 3.8, 2.0, CHC*H*=CH), 5.84-5.88 (1H, m, CH$_2$C*H*=CH), 7.28 (2H, d, *J*7.9, 2xAr H-3), 7.71 (2H, d, *J*7.9, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.37 (CH_3), 22.85 (CH_2), 39.00 (CH_2), 52.57 (CH), 54.59 (CH_2), 124.15 (CH), 126.80 (2 × CH), 127.96 (CH), 129.59 (2 × CH), 137.73 (C), 143.34 (C); MS (FAB) m/z (%): 236 (100%, M-CH_2N_3), 155 (32, $CH_3C_6H_4SO_2^+$), 293 (5, $M+H^+$); Accurate mass (FAB): Found 293.1073 ($M+H^+$ $C_{13}H_{17}N_4O_2S$ requires 293.1072); Anal. Found: C, 53.72; H, 5.78; N, 19.25; $C_{13}H_{16}O_2N_4S$ requires: C, 53.41; H, 5.52; N, 19.16.

6.10.14 (*S*)-1-(Toluene-4-sulfonyl)-2-methylamine-1,2,5,6-tetrahydropyridine 417



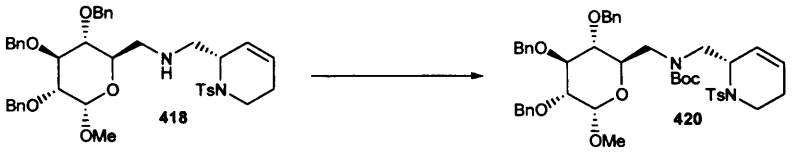
Triphenylphosphine (0.21 g, 0.81 mmol) was added to a solution of the azide **589** (0.24 g, 0.81 mmol) in THF (2.5 ml) and water (0.4 ml). The reaction mixture was stirred for 20 h before the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (5:95, methanol: dichloromethane) to give the *amine* **417** as a colourless oil (0.20 g, 94%). $[\alpha]^{20}_D$: -165.1 (c=1.31 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3640m (NH), 2936m, 1598m, 1341s, 1298m, 1264s, 1212m, 1159s, 1096s, 939s; δ_H (250 MHz; $CDCl_3$; Me_4Si): 1.52-1.63 (2H, br m, C*H*$_2$CH=CH), 2.43 (3H, s, Ar 4-Me), 2.62 (1H, dd, *J*11.2, 7.3, C*H*HNH$_2$), 2.78 (1H, dd, *J*11.2, 3.4, CH*H*NH$_2$), 3.09 (1H, ddd, *J*14.4, 6.8, 2.5, NC*H*H), 3.76 (1H, d app t, *J*14.4, 3.0, CH*H*N), 4.13-4.14 (1H, m, CHN), 5.48-5.59 (2H, m, 2xCH=), 7.18 (2H, d, *J*8.2, 2xAr H-3), 7.63 (2H, d, *J*8.2, 2xAr H-2); δ_C (62.9 MHz; $CDCl_3$; Me_4Si): 21.28 (CH_3), 22.29 (CH_2), 38.31 (CH_2), 45.04 (CH_2), 56.76 (CH), 125.06 (CH), 126.19 (CH), 126.68 (2 × CH), 129.40 (2 × CH), 137.88 (C), 143.05 (C); MS (ES) m/z (%): 267 (100%, $M+H^+$), 279 (97), 545 (85), 224 (49), 250 (22, M-NH_2); Accurate mass (FAB): Found 267.1168 ($M+H^+$ $C_{13}H_{19}N_2O_2S$ requires 267.1167).

6.10.15 (*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4 tribenzyl-α-D-glucopyranoside-O^6-amino]ylmethyl)-1,2,5,6-tetrahydropyridine 418



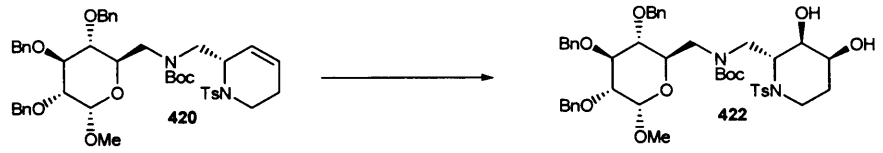
Diisopropylethylamine (0.082 g, 0.11 ml, 0.81 mmol) was added dropwise to a solution of the amine **417** (0.19 g, 0.74 mmol) in THF (10 ml) at 0 °C. The solution was stirred for 20 min before a solution of the triflate **377** (0.48 g, 0.81 mmol) in THF (1 ml) was added. The resulting mixture was stirred for 20 h before being quenched with saturated ammonium chloride (5 ml). Water (5 ml) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3x 10ml). The organic layers were combined, dried ($MgSO_4$) and solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (70:30, ethyl acetate: petroleum-ether) to give the *secondary amine* **418** (0.25 g, 47%) as a colourless oil. $[\alpha]^{20}_D$: -24.2 (c=1.82 CH_2Cl_2); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3033m, 2926s, 1598m, 1495m, 1454m, 1329m, 1268s, 1258s, 1211m, 1159s, 1071m; δ_H (400 MHz; $CDCl_3$; Me_4Si): 1.70-1.81 (2H, m, C*H*$_2$CH=CH), 2.39 (3H, s, Ar 4-Me), 2.69 (1H, dd, *J*12.4, 5.0, C*H*HNH), 2.80 (1H, dd, *J*12.6, 6.8, H-6), 2.86 (1H, dd, *J*12.4, 7.3, CH*H*NH), 2.92 (1H, dd, *J*12.6, 2.6, H-6), 3.20 (1H, ddd, *J*14.7, 11.5, 4.5, C*H*HNTos), 3.42 (3H, s, OMe), 3.46 (1H, app t, *J*9.4, H-4), 3.54 (1H, dd, *J*9.4, 3.5, H-2), 3.77 (1H, ddd, *J*9.4, 6.8, 2.6, H-5), 3.86 (1H, dd, *J*14.7, 5.7, CH*H*NTos), 4.01 (1H, app t, *J*9.4, H-3), 4.35-4.42 (1H, br m, CHN), 4.62 (1H, d, *J*3.5, H-1), 4.65 (1H, d, *J*11.1, PhC*H*HO), 4.70 (1H, d, *J*12.1, PhC*H*HO), 4.81 (1H, d, *J*12.1, PhCH*H*O), 4.85 (1H, d, *J*10.9, PhC*H*HO), 4.91 (1H, d, *J*11.1, PhCH*H*O), 5.00 (1H, d, *J*10.9, PhCH*H*O), 5.60-5.67 (2H, m, 2xCH=), 7.22 (2H, d, *J*8.0, 2xAr H-3), 7.28-7.40 (15H, m, 15xPh), 7.71 (2H, d, *J*8.0, 2xAr H-2); δ_C (100.6 MHz; $CDCl_3$; Me_4Si): 21.39 (CH_3), 21.60 (CH_2), 38.75 (CH_2), 49.77 (CH_2), 53.10 (CH_2), 53.26 (CH), 55.05 (CH_3), 69.42 (CH), 73.21 (CH_2), 74.94 (CH_2), 75.65 (CH_2), 79.28 (CH), 79.96 (CH), 81.96 (CH), 97.73 (CH), 125.76 (CH), 126.87 (CH), 126.87 (2 × CH), 127.48 (CH), 127.57 (CH), 127.77 (CH), 127.83 (2 × CH), 127.89 (2 × CH), 127.97 (2 × CH), 128.28 (4 × CH), 128.33 (2 × CH), 129.43 (2 × CH), 138.06 (2 × C), 138.27 (C), 138.65 (C), 143.00 (C); MS (ES) m/z (%): 713 (100%, $M+H^+$), 714 (47, $M+2H^+$); Accurate mass (FAB): Found 713.3261 ($M+H^+$ $C_{41}H_{49}N_2O_7S$ requires 713.3261).

6.10.16 (*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4,-tribenzyl -α-D-glucopyranoside-O^6-carbamic acid *tert*-butyl ester]ylmethyl) 1,2,5,6-tetrahydropyridine 420



The secondary amine **418** (0.24 g, 0.33 mmol) was subjected to the Boc protection procedure (outlined in experiment 6.10.8) using triethylamine (0.037 g, 0.051 ml, 0.36 mmol) and di-*tert* butyl dicarbonate (0.11 g, 0.50 mmol) in dichloromethane (2 ml). The reaction mixture was stirred for 18 h before being worked up. The resulting crude material was purified by flash column chromatography on silica (30:70 ethyl acetate: petroleum-ether) to give the *protected amine* **420** (0.24 g, 88%) as a colourless oil. $[\alpha]^{20}_D$: - 9.8 (c=1.82 CH_2Cl_2); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2927s, 1688s (C=O), 1454m, 1411m, 1366m, 1261s, 1160s, 1094s, 882w, 815w; δ_H (400 MHz; 323K; $CDCl_3$; Me_4Si,) (signals broad due to restricted rotation): 1.45 (9H, s, $C(CH_3)_3$), 2.37 (3H, s, Ar 4-Me), 1.71-1.73 (2H, br m, CH_2CH=CH), 2.97-3.13 (1H, br m, CHHNTs), 3.19 (1H, app t, *J*9.0, H-4), 2.16-3.21 (1H, br m overlapping, CHHNBoc), 3.31 (3H, s, OMe), 3.52 (1H, dd, *J*9.0, 3.7, H-2), 3.61-3.62 (1H, br m, CHHNTs), 3.85 (1H, app td, *J*9.0, 1.9, H-5), 3.73-3.87 (1H, br m overlapping, CHHNBoc), 3.97 (1H, app t, *J*9.0, H-3), 4.04-4.11 (2H, br m, 2xH-6), 4.59 (1H, d, *J*3.7, H-1), 4.57-4.60 (1H, br m overlapping, CHN), 4.60 (1H, d, *J*11.0, PhCHHO), 4.66 (1H, d, *J*12.2, PhCHHO), 4.77 (1H, d, *J*12.2, PhCHHO), 4.79 (1H, d, *J*11.2, PhCHHO), 4.87 (1H, d, *J*11.0, PhCHHO), 4.97 (1H, d, *J*11.2, PhCHHO), 5.56 (1H, d app t, *J*10.4, 1.8, CHCH=CH), 5.67 (1H, br m, CH_2CH=CH), 7.20 (2H, d, *J*8.0, 2xAr H-3), 7.24-7.37 (15H, m, 15xPh-H), 7.67 (2H, d, *J*8.0, 2xAr H-2); δ_C (100.6 MHz; $CDCl_3$; Me_4Si): 20.98 and 21.39 (CH_3), 22.53 and 23.02 (CH_2), 28.28 (3 × CH_3), 38.00 and 38.26 (CH_2), 47.77 and 49.64 (CH_2), 50.06 and 50.62 (CH_2), 51.48 and 52. 47 (CH), 54.62 (CH_3), 69.31 (CH), 70.61 (CH), 73.13 (CH_2), 74.85 (CH_2), 75.69 (CH_2), 77.2 (C), 79.64 and 79.81 (CH), 80.16 and 82.12 (CH), 97.51 and 98.31 (CH), 126.29 (CH), 126.87 (2 × CH), 127.51 (CH), 127.64 (CH), 127.85 (2 × CH), 127.96 (3 × CH), 128.21 (CH), 128.30 (3 × CH), 128.36 (4 × CH), 129.38 (2 × CH), 129.49 (CH), 138.09 (C), 38.18 (C), 138.46 (C), 138.61 (C), 142.83 and 143.04 (C), 155.17 and 155.47 (C); MS (ES) m/z (%): 573 (100%, M-$C_{12}H_{18}O_2NS$), 681 (52), 713 ($M+2H^+$- $OCOC(CH_3)_3$), 813 (39, $M+H^+$); Accurate mass (FAB): Found 813.3784 (M+H+ $C_{46}H_{57}N_2O_9S$ requires 813.3785).

6.10.17 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-[O^2, O^3, O^4,-tribenzyl -α-D-glucopyranoside-O^6-carbamic acid *tert*-butyl ester]ylmethyl)-piperidine-3,4-diol 422

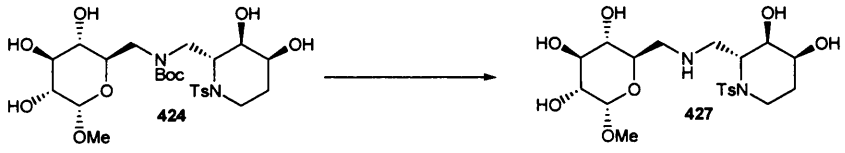


The alkene **420** (0.21 g, 0.25 mmol) in acetone (2.0 ml) underwent dihydroxylation using the procedure outlined in experiment 6.9.4 using OsO_4 (2.5% by wt in ^{t}BuOH solution, 0.10 ml, 0.010 mmol) and NMO (0.040 g, 0.37 mmol) dissolved in water (2.0 ml). The reaction mixture was stirred for 50 h before being quenched with 1% aqueous sodium dithionite (10 ml) and the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *diol* **422** (0.15 g, 71%) as a colourless oil. $[\alpha]^{20}_D$: +30.0 (c=0.94 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3500w (OH), 2931s, 1688s (C=O), 1453m, 1413m, 1262s, 1244w, 1157m, 1090m, 1051m, 998w; δ_H (400 MHz; 323K; $CDCl_3$; Me_4Si,) (signals broad due to restricted rotation): 1.42 (9H, s, $C(CH_3)_3$), 1.60-1.61 (2H, br m, CH_2CH=CH), 2.35 (3H, s, Ar 4-Me), 2.85-2.87 (2H, br m, CH_2NTos), 3.13 (1H, app t, *J*9.6, H-4), 3.28 (3H, s, OMe), 3.30-3.31 (1H, m, CHN), 3.51 (1H, dd, *J*9.6, 3.3, H-2), 3.74 (1H, app t, *J*2.5, CH_2CHOH), 3.78-3.88 (5H, m, 2xH-6, CH_2NBoc, H-5), 3.95 (1H, app t, *J*9.6, H-3), 4.31 (1H, app t, *J*2.5, CHCH=CH), 4.57 (1H, d, *J*3.3, H-1), 4.58 (1H, d, *J*11.0, PhCHHO), 4.67 (1H, d, *J*12.1, PhCHHO), 4.77 (1H, d, *J*12.1, PhCHHO), 4.79 (1H, d, *J*11.1, PhCHHO), 4.88 (1H, d, *J*11.1, PhCHHO), 4.97 (1H, d, *J*11.0, PhCHHO), 7.20 (2H, d, *J*8.0, 2xAr H-3), 7.25-7.38 (15H, m, 15xPh-H), 7.74 (2H, br m, 2xAr H-2); δ_C (100.6 MHz; $CDCl_3$; Me_4Si): 21.43 (CH_3), 27.22 (CH_2), 28.24 (3 × CH_3), 39.36 (CH_2), 46.36 (CH_2), 48.85 (CH_2), 54.79 (CH), 57.78 (CH_3), 66.42 (CH), 67.84 (CH), 70.21 (CH), 73.20 (CH_2), 74.82 (CH_2), 75.68 (CH_2), 77.20 (C), 79.75 (CH), 80.14 and 80.36 (CH), 82.07 (CH), 97.37 (CH), 127.47 (3 × CH), 127.57 (2 × CH), 127.83 (2 × CH), 127.90 (CH), 128.00 (2 × CH), 128.27 (3 × CH), 128.33 (2 × CH), 128.43 (2 × CH), 129.34 (2 × CH), 138.01 (2 × C), 138.17 (C), 138.57 (C), 143.06 (C), 155.89 (C); MS (ES) m/z (%): 607 (100%, $M+2H^+$-($CH_3C_6H_4SO_2$+OCOC$(CH_3)_3$)), 715 (72, $M+2H^+$-(OCOC$(CH_3)_3$+2OH)), 864 (62 $M+NH_4^+$), 747 (33, $M+2H^+$-OCOC$(CH_3)_3$), 847 (31, $M+H^+$); Accurate mass (FAB): Found 847.3837 (M+H+ $C_{46}H_{59}N_2O_{11}S$ requires 847.3840).

6.10.18 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-α-D-glucopyranoside-O^6-carbamic acid *tert*-butyl ester]ylmethyl)-piperidine-3,4-diol 424

The diol **422** (0.11 g, 0.12 mmol) dissolved in a mixture of ethanol (8.6 ml) and acetic acid (0.93 ml) was subjected to the general hydrogenolysis procedure (outlined in experiment 6.2.7) using $Pd(OAc)_2$ (0.025 g, 0.11 mmol). The reaction mixture was stirred for 3 h before being filtered and the solvent was removed under vacuum. The resulting crude reaction mixture was purified by flash column chromatography on silica (7:93, methanol: ethyl acetate) to give the *pentol* **424** (0.066 g, 88%) as an oil. $[\alpha]^{20}_D$: +49.5 (c= 1.35 MeOH); ν_{max} (solid state)/ cm^{-1}: 3405br (OH), 2930w, 1668 (C=O), 1598w, 1463w, 1416m, 1367m, 1247m, 1153s, 1073s, 1006w, 734s; δ_H (400 MHz, 333K; D_2O) (signals broad due to restricted rotation): 1.45 (9H, s, $C(CH_3)_3$), 1.61-1.74 (2H, m, CH_2CHOH), 2.43 (3H, s, Ar 4-Me), 3.12-3.23 (2H, m, *CH*HNTos, H-4), 3.30 (3H, s, OMe), 3.34-3.42 (1H, m, *CH*HNBoc), 3.52 (1H, dd, *J*9.8, 3.5, H-2), 3.57 (1H, app t, *J*9.8, H-3), 3.47-3.67 (4H, m overlapping 2xH-6, H-5, CH*H*NBoc), 3.80-3.99 (3H, m, CH*H*NTos, 2x*CH*OH), 4.69-4.71 (1H, m, CHN), 5.07 (1H, d, *J*3.5 H-1), 7.44 (2H, d, *J*8.3, 2xAr H-3), 7.76 (2H, d, *J*8.3, 2xAr H-2); δ_C (100.6 MHz, 343K; D_2O): 21.56 (CH_3), 27.36 (CH_2), 28.69 (3 × CH_3), 41.09 (2 × CH_2), 46.68 (CH_2), 51.61 (CH_3), 59.47 (CH), 67.10 (CH), 68.36 (CH), 72.44 (2 × CH), 72.94 (CH), 74.29 (CH), 82.88 (C), 99.89 (CH), 127.79 (2 × CH), 130.86 (2 × CH), 132.22 (C), 145.54 (C), 157.42 (C); MS (ES) m/z (%): 477 (100%, M+H$^+$-$COOC(CH_3)_3$), 599 (18, M+Na$^+$), 577 (8, M+H$^+$); Accurate mass (FAB): Found 577.2432 (M+H$^+$ $C_{25}H_{41}N_2O_{11}S$ requires 577.2431).

6.10.19 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-(methyl-α-D-glucopyranoside-O^6-amino]ylmethyl)-piperidine-3,4-diol 427



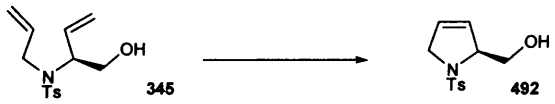
The pentol **424** (0.017 g, 0.029 mmol) underwent deprotection as described in experiment 6.10.11 using a 3M HCl solution in ethyl acetate (~1 ml). The reaction mixture was stirred for 1 h before the solvent was removed under reduced pressure. The resulting crude ammonium chloride salt was basified using a dilute solution of aqueous sodium hydroxide. The resulting crude amine was purified by flash column chromatography on silica (50:50, ethyl acetate:

methanol) to give the *amine* **427** (0.0065 g, 48%) as an oil. $[\alpha]^{20}_D$: +7.7 (c= 1.00 MeOH); ν_{max} (solid state) / cm^{-1}: 3331nr (OH, NH), 2924m, 1597w, 1447w, 1305.2m, 1192w, 1151s, 1001s, 924w, 814m, 749m; δ_H (400 MHz; D_2O): 1.62-1.71 (2H, m, C*H*$_2$CHOH), 2.47 (3H, s, Ar 4-Me), 2.71 (1H, dd, *J*13.0, 4.9, C*H*HNH), 2.81 (1H, dd, *J*12.8, 8.4, H-6), 2.89 (1H, dd, *J*12.8, 9.7, H-6), 2.93 (1H, dd, *J*13.0, 2.7, CH*H*NH), 3.19 (1H, app td, *J*11.5, 3.3, C*H*HNTs), 3.28 (1H, app t, *J*9.2, H-4), 3.41 (3H, s, OMe), 3.57 (1H, dd, *J*9.2, 3.8, H-2), 3.65 (1H, app t, *J*9.2, H-3), 3.64-3.71 (1H, overlapping m, H-5), 3.80 (1H, app br d, *J*11.5, CH*H*NTs), 3.88-3.92 (2H, m, 2xC*H*OH), 4.30-4.34 (1H, m, CHN), 4.67 (1H, d, *J*3.8, H-1), 7.38 (2H, d, *J*8.2, 2xAr H-3), 7.76 (2H, d, *J*8.2, 2xAr H-2); δ_C (62.9 MHz; D_2O; Me_4Si): 21.06 (CH_3), 26.27 (CH_2), 40.15 (CH_2), 46.13 (CH_2), 49.58 (CH_2), 55.62 (CH_3), 60.16 (CH), 66.40 (CH), 68.53 (CH), 69.92 (CH), 71.65 (CH), 72.23 (CH), 73.38 (CH), 99.63 (CH), 127.50 (2 × CH), 130.28 (2 × CH), 136.66 (C), 145.30 (C); MS (ES) m/z (%): 477 (100%, $M+H^+$), 519 (73); Accurate mass (FAB): Found 477.1906 ($M+H^+$ $C_{20}H_{33}N_2O_9S$ requires 477.1907).

The synthesis of all stereoisomers of 2-hydroxymethyl-3-amino-4-hydroxyaminopyrrolidine

6.11 Synthesis of the homoallylic carbamate 502 using RCM

6.11.1 (*S*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-2,5-dihydro-1*H*-pyrrole 492



A solution of the diene **345** (0.49 g, 1.76 mmol) in CH_2Cl_2 (10 ml) was added to *bis*(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride (Grubbs catalyst) **299** (0.073 g, 0.088 mmol) as described in experiment 6.9.3. The reaction mixture was heated for 5 h before being filtered through a pad of silica and washed through with ethyl acetate (3x10 ml). The filtrate was concentrated under reduced pressure and the resulting crude mixture was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether-ether) to give the *alkene* **492** as an off-white solid (0.36 g, 82%). Mp: 99-100 °C; $[\alpha]^{20}_D$: -162.5 (c=1.28 MeOH); ν_{max} (solid state)/ cm^{-1}: 3524 (OH), 2941w, 1595, 1492w, 1377m, 1334s, 1203w, 1155s, 1089m, 1036s, 844w, 816 s; δ_H (400 MHz; $CDCl_3$; Me_4Si): 2.42 (3H, s, Ar 4-Me), 3.69 (1H, dd, *J*11.7, 5.5, C*H*HO), 3.79 (1H, dd, *J*11.7, 3.5, CH*H*O), 4.12 (1H, d app q, *J*15.0, 2.2, *H*H-5), 4.21 (1H, dd app t, *J*15.0, 5.5, 2.2, H*H*-5), 4.43-4.48 (1H, m, CH), 5.54 (1H, dq, *J*6.3, 2.2, H-3), 5.71 (1H, ddd, *J*6.3, 5.5, 2.2, H-4), 7.32 (2H, d, *J*8.4, Ar H-3), 7.71 (2H, d, *J*8.4, Ar H-2); δ_C (100.6 MHz; $CDCl_3$; Me_4Si): 21.55 (CH_3), 56.36 (CH_2), 65.84

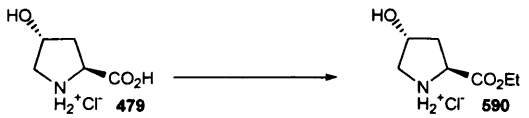
(CH_2), 69.44 (CH), 126.60 (CH), 126.82 (CH), 127.56 (2 × CH), 129.92 (2 × CH), 133.87 (C), 143.97 (C); (ES) m/z (%): 236 (M-OH, 100%), 254 (98, $M+H^+$), 155 (92, $CH_3C_6H_4SO_2^+$); Accurate mass (FAB): Found 254.0851 ($M+H^+$ $C_{12}H_{16}O_3NS$ requires 254.0851).

6.11.2 (*S*)-1-(Toluene-4-sulfonyl)-2-(carbamoyloxymethyl)-2,5-dihydro-1*H*-pyrrole 502

Trichloroacetylisocyanate (0.39 g, 0.25 ml, 2.09 mmol) was added to a 0 °C solution of the alcohol **492** (0.44 g, 1.75 mmol) in dichloromethane (2.6 ml). After 2 h TLC analysis confirmed all starting material had been consumed. Dichloromethane was removed under reduced pressure and the crude mixture was dissolved in methanol (3.5 ml) and cooled to 0 °C. Potassium carbonate (0.72 g, 1.5 moldm^{-3}, 5.2 mmol) was added to the resulting solution and the reaction mixture was stirred for 4 h before the solvent was removed under reduced pressure. Water (10 ml) was added and the resulting solution extracted with ethyl acetate (3x20 ml), the organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield the *carbamate* **502** as a white solid (0.50 g, 99%). Mp: 149-152 °C; $[\alpha]^{20}_D$: -208.8 (c=1.02 MeOH); ν_{max} (solid state)/ cm^{-1}: 3461br (NH), 2921s, 1730s (C=O), 1706s, 1600m, 1402m, 1311m, 1160s, 1049s, 817s; δ_H (300 MHz; $CDCl_3$; Me_4Si): 2.41 (3H, s, Ar 4-Me), 4.06-4.14 (2H, m, 2xH-5), 4.15 (1H, dd, *J*11.1, 5.4, C*H*HO), 4.40 (1H, dd, *J*11.1, 3.9, CH*H*O), 4.61-4.65 (1H, m, H-2), 4.92 (2H, br s, NH_2), 5.50 (1H, d app q, *J*6.3, 2.1, H-3), 5.70 (1H, ddd, *J*6.3, 5.4, 2.1, H-4), 7.30 (2H, d, *J*8.1, Ar H-3), 7.70 (2H, d, *J*8.1, Ar H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.47 (CH_3), 55.67 (CH_2), 66.06 (CH), 66.65 (CH_2), 126.73 (CH), 126.77 (CH), 127.40 (2 × CH), 129.77 (2 × CH), 134.21 (C), 143.70 (C), 156.62 (C); (ES) m/z (%): 236 ($M-OCONH_2$, 100%), 254 (28, $M-CONH_2$), 297 (22, $M+H^+$); Accurate mass (FAB): Found 297.0908 ($M+H^+$ $C_{13}H_{17}O_4N_2S$ requires 297.0909).

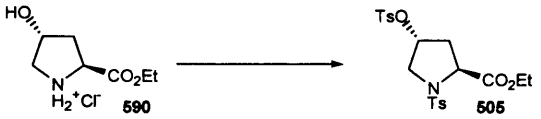
6.12 Synthesis of the homoallylic carbamate 502 from *trans*-hydroxy-L-proline 479

6.12.1 (2*S*, 4*R*)-2-Ethoxycarbonyl-4-hydroxypyrrolidinium; chloride 590



Thionyl chloride (11.18 g, 6.82 ml, 94.0 mmol) was added to a 0 °C solution of *trans*-hydroxy-L-proline **479** (11.2 g, 85 mmol) dissolved in ethanol (100 ml). The solution was refluxed for 5 h before being cooled and the solvent was removed under reduced pressure. The resulting crude material was purified by recrystallisation (from ethanol) to give *the ester* **590** (15.0 g, 90%). Mp: 150-153 °C (from ethanol) (Lit.[184], 153-153.5 °C); $[\alpha]^{20}_D$: -26.4 (c=2.5 MeOH); ν_{max} (solid state)/ cm^{-1}: 3311br (OH), 2954m, 2699s, 1698 (C=O), 1593m, 1401m, 1273s, 1236s, 1076s, 956s; δ_H (300 MHz; D_2O): 1.41 (3H, t, *J*7.1, CH_3CH_2O), 2.40 (1H, ddd, *J*14.2, 10.4, 4.4, *H*H-3), 2.61 (1H, ddt, *J*14.2, 7.8, 1.8, H*H*-3), 3.52 (1H, dt, *J*12.6, 1.8, *H*H-5), 3.64 (1H, dd, *J*12.6, 3.7, H*H*-5), 4.42 (2H, q, *J*7.1, CH_3CH_2O), 4.74-4.84 (2H, m, H-2, H-4); δ_C (75 MHz; D_2O; Me_4Si): 13.12 (CH_3), 36.73 (CH_2), 53.43 (CH_2), 58.21 (CH), 63.87 (CH_2), 69.40 (CH), 169.81 (C); (ES) m/z (%): 160 ($M+H^+$, 100%); Accurate mass (FAB): Found 160.0974 ($M+H^+$ $C_7H_{14}NO_3$ requires 160.0974); Anal. Found: C, 43.18; H, 7.13; N, 7.24; $C_7H_{13}O_3N$ requires: C, 42.97; H, 7.21; N, 7.13.

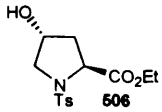
6.12.2 (2*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-ethoxycarbonyl-4-(toluene-4-sulfonyloxy)-pyrrolidine 505



Triethylamine (16.3 g, 22.5 ml, 161 mmol) and DMAP (0.05 g, 0.06 mmol) were added to a stirring solution of the ester **590** (6.3 g, 32.2 mmol) in dichloromethane (41 ml). The reaction mixture was stirred for 1h before a solution of *p*-toluenesulfonyl chloride (15.4 g, 80. 7 mmol) dissolved in dichloromethane (80 ml) was added dropwise. The reaction mixture was stirred for a further 48 h before being diluted with dichloromethane (50 ml). The organic layer was washed with water (3x50 ml) and the organic extract was dried ($MgSO_4$) and the solvent removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (30:70, ethyl acetate: petroleum-ether) to give the *ditosylated pyrrolidine* **505** (14.47 g, 96%) as a white solid together with the *monotosylated product* **506** (0.28 g, 3%) as an oil. Further purification of **505** was achieved by recrystallisation from ethanol. Data for **505** Mp: 76-78 °C (from ethanol) (Lit.[185], 75-76 °C); $[\alpha]^{20}_D$: -74.2 (c=2.43 MeOH); ν_{max} (solid state)/ cm^{-1}: 2878s, 1737s (C=O), 1596m, 1358s, 1346s, 1196m, 1166s, 1159s, 953s; δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.20 (3H, t, *J*7.2, CH_3CH_2O), 2.16 (1H, ddd, *J*14.0, 7.5, 5.1, *H*H-3), 2.32 (1H, dddd, *J*14.0, 7.5, 3.0, 1.5, H*H*-3), 2.39 (3H, s, Ar 4-Me), 2.41 (3H, s, Ar 4-Me), 3.54 (1H, d app t, *J*12.3, 1.5, *H*H-5), 3.66 (1H, dd, *J*12.3, 4.2, H*H*-5), 4.07-4.21 (3H, m, CH_3CH_2O, H-2), 4.92-4.97 (1H, m, H-4), 7.27 (2H, d, *J*8.1, 2xAr H-3), 7.29 (2H, d, *J*8.1, 2xAr H-3), 7.58 (2H, d, *J*8.1, 2xAr H-2), 7.67 (2H, d, *J*8.1, 2xAr H-2); δ_C

(75 MHz; $CDCl_3$; Me_4Si): 13.96 (CH_3), 21.20 (CH_3), 21.63 (CH_3), 37.24 (CH_2), 53.82 (CH_2), 59.22 (CH), 61.75 (CH_2), 78.15 (CH), 127.60 (2 × CH), 127.64 (2 × CH), 129.67 (2 × CH), 129.95 (2 × CH), 132.99 (C), 134.17 (C), 144.05 (C), 145.31 (C), 171.07 (C); (ES) m/z (%): 468 ($M+H^+$, 100%), 485 (58, $M+NH_4^+$); Accurate mass (FAB): Found 468.1150 ($M+H^+$ $C_{21}H_{26}O_7NS_2$ requires 468.1151); Anal. Found: C, 54.17; H, 5.55; N, 2.94; $C_{21}H_{25}O_7NS_2$ requires: C, 53.95; H, 5.39; N, 2.97.

Data for side product: (2*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-ethoxycarbonyl-4-hydroxypyrrolidine **506**

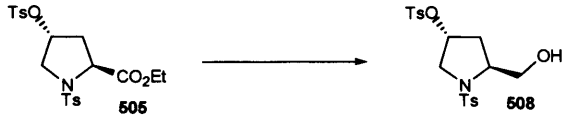


δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.28 (3H, t, *J*6.9, CH_3CH_2O), 1.81 (1H, br s, OH), 2.10 (1H, ddd, *J*13.2, 8.0, 4.8, H-3), 2.22 (1H, dddd, *J*13.2, 8.0, 3.3, 1.8, H-3), 2.43 (3H, s, Ar 4-Me), 3.39 (1H, d app t, *J*11.4, 1.8, H-5), 3.60 (1H, dd, *J*11.4, 3.9, H-5), 4.16-4.24 (2H, m, CH_3CH_2O), 4.39 (1H, app t, *J*8.0, H-2), 4.44-4.47 (1H, m, H-4), 7.31 (2H, d, *J*8.1, 2xAr H-3), 7.78 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 14.03 (CH_3), 21.54 (CH_3), 39.47 (CH_2), 56.34 (CH_2), 59.44 (CH), 61.51 (CH_2), 70.11 (CH), 127.71 (2 × CH), 129.58 (2 × CH), 134.87 (C), 143.78 (C), 172.04 (C).

6.12.3 Attempt at the nucleophilic displacement of 505

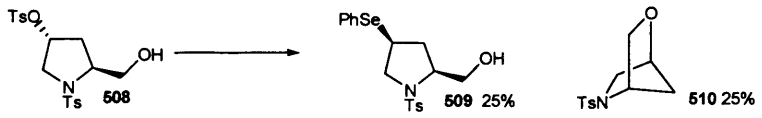
Sodium borohydride (0.04 g, 1.33 mmol) was added to a solution of diphenyldiselenide (0.174 g, 0.69 mmol) in ethanol (1.3 ml). The solution was degassed (3 cycles freeze-pump-thaw) and to this mixture a degassed (3 cycles freeze-pump-thaw) solution of the tosylate **505** (0.41 g, 0.88 mmol) in THF (1.3 ml) was added. The reaction mixture was refluxed for 3 h before solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (10:90, ethyl acetate: petroleum-ether) to give the recovered **505** (racemised) (0.048 g, 12%) and the *pyrrole* **507** (0.01 g, 10%) as an oil. δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.33 (3H, t, *J*7.2, CH_3CH_2O), 4.32 (2H, m, CH_3CH_2O), 6.27 (1H, m, CH), 6.92 (2H, m, 2xCH), 9.31 (1H, br s, NH); δ_C (75 MHz; $CDCl_3$; Me_4Si): 14.46 (CH_3), 60.32 (CH_2), 110.39 (CH), 115.10 (CH), 122.72 (CH), 123.01 (C), 161.26 (C).

6.12.4 (2*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-(hydroxymethyl)-4-(toluene-4-sulfonyloxy)-pyrrolidine 508



LiCl (2.07 g, 42.0 mmol) and $NaBH_4$ (1.86 g, 42.0 mmol) were added to a 0 °C solution of the ester **505** (6.53 g, 14.0 mmol) dissolved in a 1:1 mixture of ethanol (80 ml) and THF (80 ml). The solution was stirred for 3.5 h before being quenched with water (50 ml). Ethanol was removed under reduced pressure and the aqueous layer was extracted with ethyl acetate (3x50 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield the *alcohol* **508** as a white solid (5.41 g, 91%). Mp: 82-84 °C (Lit.[186]; 86-87 °C); $[\alpha]^{20}_D$: -21.5 (c=1.03 MeOH); ν_{max} (solid state)/ cm^{-1}: 3532br (OH), 2963m, 1597m, 1335s, 1173s, 1090m, 889s, 812s; δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.88-1.94 (1H, br dd, *J*14.1, 7.8, *H*H-3), 2.03 (1H, ddd, *J*14.1, 8.4, 4.8, H*H*-3), 2.33 (3H, s, Ar 4-Me), 2.34 (3H, s, Ar 4-Me), 3.28 (1H, br s, OH), 3.51-3.67 (4H, m, C*H*HO, 2xH-5, H-2), 3.81 (1H, dd, *J*11.4, 3.3, CH*H*O), 4.86-4.92 (1H, m, H-4), 7.22 (4H, d, *J*8.1, 4xAr H-3), 7.48 (2H, d, *J*8.1, 2xAr H-2), 7.62 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.16 (CH_3), 21.20 (CH_3), 34.78 (CH_2), 54.97 (CH_2), 59.92 (CH), 63.72 (CH_2), 78.57 (CH), 127.16 (2 ×CH), 127.35 (2 × CH), 129.42 (2 × CH), 129.59 (2 × CH), 132.75 (C), 133.01 (C), 143.77 (C), 144.80 (C); (ES) m/z (%): 254 (M-$HOSO_2C_6H_4CH_3$, 100%), 443 (97, M+NH_4^+), 426 (72, M+H^+); Accurate mass (FAB): Found 426.1045 (M+H^+ $C_{19}H_{24}O_6NS_2$ requires 426.1045); Anal. Found: C, 53.64; H, 5.54; N, 3.06; $C_{21}H_{25}O_7NS_2$ requires: C, 53.63; H, 5.45; N, 3.29.

6.12.5 Attempt at the nucleophilic displacement of 508



Sodium borohydride (0.012 g, 0.32 mmol) was added to a solution of diphenyldiselenide (0.054 g, 0.17 mmol) in ethanol (0.5 ml). The solution was degassed (3 cycles freeze-pump-thaw) and to this mixture a degassed (3 cycles freeze-pump-thaw) solution of the alcohol **508** (0.11 g, 0.27 mmol) in THF (0.5 ml) was added. The reaction mixture was refluxed for 3 h before solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (40:60, ethyl acetate: petroleum-ether) to give the *selenide* **509** (0.028 g, 25%) as a white solid and the bicyclic ether **510** (0.29 g, 25%) as an oil. Data for **509** - δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.72 (1H, m, CH), 2.23 (1H, m, CH), 2.44 (3H, s, Ar 4-Me), 3.77 (1H, m, CH), 3.31 (1H, dd, *J*11.9, 10.3, CH), 3.63-3.82 (4H, m,

4xCH), 7.24-7.42 (7H, m, 2xAr H-3, 5xPh-H), 7.65 (2H, d, *J*8.3, 2xAr H-2); Data for **510** - δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.32 (1H, d, *J*9.8, CH), 1.70 (1H, d, *J*11.4, CH), 2.44 (3H, s, Ar 4-Me), 3.21(1H, dd, *J*9.8, 1.6, CH), 3.40 (1H, d, *J*9.8, CH), 3.67 (1H, d, *J*7.6, 1.6, CH), 3.88 (1H, d, *J*7.8, CH), 4.47 (2H, d, *J*11.4, CH), 7.32 (2H, d, *J*8.3, 2xAr H-3), 7.72 (2H, d, *J*8.3, 2xAr H-2); (ES) m/z (%): 254 ($M+H^+$, 100%),

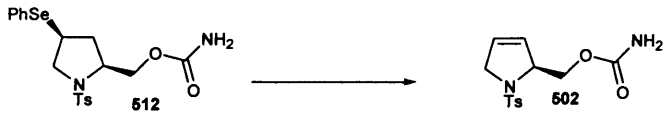
6.12.6 (2*S*, 4*R*)-1-(toluene-4-sulfonyl)-2-(carbamoyloxymethyl)- 4-(toluene-4-sulfonyloxy)-pyrrolidine 511

The alcohol **508** (1.38 g, 3.2 mmol) was dissolved in dichloromethane (5 ml) and formation of the carbamate was achieved as described in 6.12.2 using trichloroacetylisocyanate (0.38 g, 0.24 ml, 3.8 mmol). After 2 h TLC analysis confirmed all starting material had been consumed. Dichloromethane was removed under reduced pressure and the resulting crude material was dissolved in methanol (6.5 ml). The solution was cooled to 0 °C and potassium carbonate (1.34 g, 0.5 $moldm^{-3}$, 9.7 mmol) was added. The solution was stirred for 4 h before being worked up as described previously, to yield the *carbamate* **511** (1.37 g, 91%) as a white solid. Mp: 94-97 °C; $[\alpha]^{20}_D$: -36.1 (c=1.95 MeOH); ν_{max} (solid state)/ cm^{-1}: 2957s, 1723s (C=O), 1597s, 1401m, 1331s, 1346s, 1174s, 1089m, 956s, 889s; δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.95-2.05 (2H, m, 2xH-3), 2.44 (3H, s, Ar 4-Me), 2.46 (3H, s, Ar 4-Me), 3.55 (1H, ddd, *J*12.6, 3.3, 0.9, *H*H-5), 3.61 (1H, dd, *J*12.6, 4.2, H*H*-5), 3.84-3.91 (1H, m, H-2), 4.19 (1H, dd *J*11.4, 5.7, C*H*HO), 4.38 (1H, dd, *J*11.4, 3.3, CH*H*O), 4.81-4.88 (3H, m, H-4, NH_2), 7.30 (2H, d, *J*8.1, 2xAr H-3), 7.32 (2H, d, *J*8.1, 2xAr H-3), 7.59 (2H, d, *J*8.1, 2xAr H-2), 7.70 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.57 (CH_3), 21.64 (CH_3), 35.68 (CH_2), 54.53 (CH_2), 57.12 (CH), 66.40 (CH_2), 78.18 (CH), 127.64 (2 × CH), 127.71 (2 × CH), 129.78 (2 × CH), 129.94 (2 × CH), 133.08 (C), 133.57 (C), 144.13 (C), 145.24 (C), 156.28 (C); (ES) m/z (%): 469 ($M+H^+$, 100%), 486 (51, $M+NH_4^+$); Accurate mass (FAB): Found 469.1103 ($M+H^+$ $C_{20}H_{25}O_7N_2S_2$ requires 469.1103).

6.12.7 (2*S*, 4*S*)-1-(toluene-4-sulfonyl)-2-(carbamoyloxymethyl)-4-phenylselanylpyrrolidine 512

Sodium borohydride (0.19 g, 5.13 mmol) was added to a solution of diphenyldiselenide (0.84 g, 2.70 mmol) in ethanol (10 ml). The solution was degassed (3 cycles freeze-pump-thaw) and to this mixture a degassed (3 cycles freeze-pump-thaw) solution of the carbamate **511** (1.93 g, 4.28 mmol) in THF (10 ml) was added. The reaction mixture was refluxed for 2 h before solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (40:60, ethyl acetate: petroleum-ether) to give the *selenide* **512** (1.76 g, 94%) as a white solid. Mp: 125-128 °C; $[\alpha]^{20}_{D}$: +27.0 (c=1.15 MeOH); ν_{max} (solid state)/ cm^{-1}: 3460br (NH), 2920s, 1698s (C=O), 1613s, 1406m, 1336s, 1152s, 1084m, 733s; δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.71 (1H, ddd, *J*13.2, 10.2, 7.5, *H*H-3), 2.29 (1H, d app t, *J*13.2, 7.5, H*H*-3), 2.43 (3H, s, Ar 4-Me), 2.80 (1H, app tdd, *J*10.2, 7.5, 6.6, H-4), 3.26 (1H, dd, *J*12.3, 10.2, *H*H-5), 3.78 (1H, dd *J*12.3, 6.6, H*H*-5), 3.90-3.98 (1H, m, H-2), 4.17 (1H, dd *J*11.1, 5.7, C*H*HO), 4.26 (1H, dd, *J*11.1, 5.1, CH*H*O), 4.76 (2H, s br, NH_2), 7.25-7.37 (5H, m, 3x Ph-H, 2xAr H-3), 7.40-7.44 (2H, m, 2xPh-H), 7.67 (2H, d, *J*8.4, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.52 (CH_3), 33.36 (CH_2), 36.29 (CH), 55.71 (CH_2), 58.72 (CH), 66.74 (CH_2), 127.34 (2 × CH), 127.29 (C), 128.15 (CH), 129.18 (2 × CH), 129.87 (2 × CH), 134.37 (C), 134.84 (2 × CH), 143.88 (2 × C), 156.38 (C); (ES) m/z (%): 394 (M-$OCONH_2$, 100%), 455 (67, $M+H^+$); Accurate mass (FAB): Found 455.0543 ($M+H^+$ $C_{19}H_{23}O_4N_2SSe$ requires 455.0544); Anal. Found: C, 49.99; H, 4.89; N, 6.32; $C_{19}H_{22}O_4N_2SSe$ requires: C, 50.33; H, 4.89; N, 6.18.

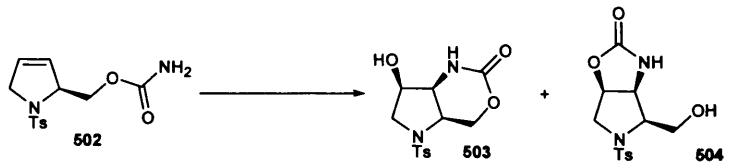
6.12.8 (*S*)-1-(Toluene-4-sulfonyl)-2-(carbamoyloxymethyl)-2,5-dihydro-1*H*-pyrrole 502



Pyridine (0.32 ml, 3.9 mmol) and hydrogen peroxide (0.75 ml, 30% solution) were added to a 0 °C solution of the selenide **512** (1.33 g, 2.9 mmol) dissolved in dichloromethane (16 ml). The reaction mixture was stirred for 2 h before being diluted with dichloromethane (20 ml). The organic layer was washed with 5% aqueous $KHSO_4$ (2x10 ml), saturated aqueous $NaHCO_3$ (2x10 ml) and water (2x10 ml) before being dried ($MgSO_4$). Solvent was removed under reduced pressure and the resulting crude material was purified by flash column chromatography on silica (2:98, methanol: dichloromethane) to give the *alkene* **502** (0.45 g, 52%) as an off-white solid. Data as for 6.11.2

6.13 Synthesis of the (2*S*, 3*S*, 4*R*)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 488

6.13.1 (2*S*, 3*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2,3-(oxazin-2-one)- 4-hydroxypyrrolidine 503 and (2*S*, 3*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-3,4-(oxazol-2-one)-pyrrolidine 504[91]

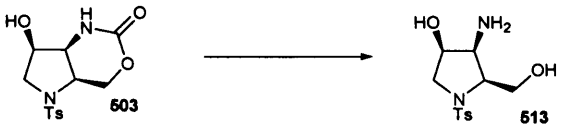


All but a few drops of a freshly prepared solution of aqueous NaOH (11.4 ml, 0.08 moldm^{-3}, 0.91 mmol) was added to a solution of the alkene **502** (0.30 g, 1.0 mmol) in *n*PrOH (12.1 ml). After 5 min ^{t}BuOCl (0.13 g, 1.0 mmol) was added to the reaction followed by $^{i}Pr_2NEt$ (0.005 g, 0.007 ml, 0.05 mmol) after a further 5 min stirring. After another 5 min period $K_2Os(OH)_4O_2$ (0.015 g, 0.04 mmol) dissolved in the remaining aqueous NaOH solution was also added and a green colour was observed, which slowly changed to a dark brown. After 2 h sodium sulphite (0.5 g) was added and the solution was stirred for 30 min. The reaction mixture then extracted with ethyl acetate (3x20 ml), the organic layers were combined and washed with water (20 ml) and brine (20 ml). The organic layer was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (1:99, methanol: ethyl acetate) to give the *5:5 bicyclic carbamate* **504** (0.091 g, 28%) as an off-white solid, together with the *5:6 bicyclic carbamate* **503** (0.066 g, 21%) as a white solid together with recovered starting material **502** (0.11 g, 38%). Data for **504**: Mp: 100-102 °C; $[\alpha]^{20}_D$: -49.0 (c=1.14 MeOH); ν_{max} (solid state)/ cm^{-1}: 3289br (NH/OH), 1730s (C=O), 1599m, 1404w, 1349m, 1242m, 1160s, 970w; δ_H (400 MHz; MeOD): 2.45 (3H, s, Ar 4-Me), 2.91 (1H, app td, *J*9.0, 5.2, H-2). 3.06, (1H, dd, *J*12.0, 5.2, *H*H-5), 3.66 (1H, dd, *J*11.2, 9.0, C*H*HO), 3.72 (1H, dd, *J*12.0, 0.8, H*H*-5), 4.25 (1H, dd, *J*11.2, 5.2, CH*H*O), 4.35 (1H, dd, *J*9.0, 6.0, H-3), 4.91 (1H, ddd, *J*6.0, 5.2, 0.8, H-4), 7.47 (2H, d, *J*8.4, 2xAr H-3), 7.72 (2H, d, *J*8.4, 2xAr H-3); δ_C (100.6 MHz; MeOD): 20.10 (CH_3), 55.82 (CH_2), 58.42 (CH), 59.46 (CH_2), 63.44 (CH), 76.33 (CH), 127.91 (2 × CH), 129.69 (2 × CH), 131.22 (C), 144.76 (C), 160.04 (C); (ES) m/z (%): 313 ($M+H^+$, 100%), 330 (68, $M+NH_4^+$), 252 (25, M-OCONH$_2$); Accurate mass (FAB): Found 313.0858 ($M+H^+$ $C_{13}H_{17}O_5N_2S$ requires 313.0858); Anal. Found: C, 49.75; H, 5.16; N, 8.81; $C_{13}H_{16}O_5N_2S$ requires: C, 49.99; H, 5.16; N, 8.97.

Data for **503**: Mp: 104-108 °C; $[\alpha]^{20}_D$: -24.7 (c=0.29 MeOH); ν_{max} (solid state)/ cm^{-1}: 3260br (NH/OH), 1701s (C=O), 1480w, 1424m, 1340m, 1285w, 1157s, 1065m, 993m, 769m, 666s;

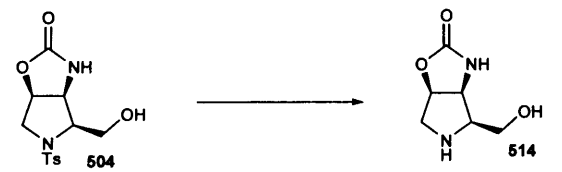
δ_H (300 MHz; MeOD): 2.45 (3H, s, Ar 4-Me), 3.23 (1H, dd, *J*11.1, 6.0, *H*H-5). 3.45, (1H, dd, *J*11.1, 6.0, H*H*-5), 3.73 (1H, dd, *J*6.9, 4.2, H-3), 3.80 (1H, app td, *J*6.0, 4.2 H-4), 4.00 (1H, ddd, *J*6.9, 6.0, 3.0, H-2), 4.33 (1H, *J*11.4, 3.0, C*H*HO), 4.54 (1H, dd, *J*11.4, 6.0, CH*H*O), 7.45 (2H, d, *J*8.1, 2xAr H-3), 7.78 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; MeOD): 22.36 (CH_3), 54.20 (CH_2), 54.94 (CH), 57.63 (CH), 69.96 (CH_2), 71.83 (CH), 129.59 (2 × CH), 131.20 (2 × CH), 136.08 (C), 146.721 (C), 157.80 (C); (ES) m/z (%): 313 (M+H+, 100%), 330 (65, $M+NH_4^+$); Accurate mass (FAB): Found 313.0858 ($M+H^+$ $C_{13}H_{17}O_5N_2S$ requires 313.0858)

6.13.2 (2*S*, 3*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 513



LiOH (0.036 g, 1.5 mmol) was added to a solution of the bicyclic carbamate **503** (0.055 g, 0.18 mmol) dissolved in MeOH (0.4 ml) and water (1.46 ml) and the mixture was heated at reflux (90 °C) for 1.5 h. The solution was cooled and the solvent was removed under reduced pressure. The resulting crude mixture was purified by flash column chromatography on silica (20:80, methanol: ethyl acetate) to give the *aminopyrrolidine* **513** (0.029 g, 57%) as a white solid. Mp: 132-135 °C; $[\alpha]^{20}_D$: -57.6 (c=2.6 MeOH); ν_{max} (solid state)/ cm^{-1}: 3330w (OH/NH), 2888w, 1599w, 1467w, 1341m, 1236w, 1152s, 1030m, 809m, 675s; δ_H (400 MHz, D_2O): 2.39 (3H, s, Ar 4-Me), 3.06 (1H, dd, *J*8.8, 4.4, H-3), 3.28 (1H, dd, *J*11.6, 3.6, *H*H-5), 3.38 (1H, dd, *J*11.6, 1.2, H*H*-5), 3.69 (1H, app td, *J*4.4, 2.8, H-2), 3.72 (1H, dd, *J*12.4, 2.8, C*H*HO), 3.85 (1H, dd, *J*12.4, 4.4, CH*H*O), 3.93 (1H, ddd, *J*8.8, 3.6, 1.2, H-4), 7.44 (2H, d, *J*8.0, 2xAr H-3), 7.72 (2H, d, *J*8.0, 2xAr H-2); δ_C (100.6 MHz; D_2O): 20.67 (CH_3), 54.39 (CH_2), 58.85 (CH), 59.79 (CH_2), 61.67 (CH), 70.30 (CH), 127.501 (2 × CH), 130.17 (2 × CH), 131.30 (C), 145.70 (C); (ES) m/z (%): 287 ($M+H^+$, 100%), 573 (228, $2M+H^+$); Accurate mass (FAB): Found 287.1066 ($M+H^+$ $C_{12}H_{19}O_4N_2S$ requires 287.1066).

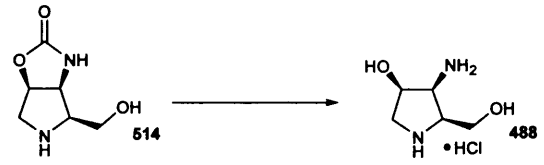
6.13.3 (2*S*, 3*S*, 4*R*)-2-Hydroxymethyl-3,4-(oxazol-2-one)-pyrrolidine 514



The bicyclic carbamate **504** (0.011 g, 0.036 mmol) was subjected to the general tosyl deprotection procedure (see experiment 6.2.9) using sodium metal (0.0021 g, 0.09 mmol) in liquid ammonia (~5 ml). After the addition of sodium was complete the reaction was warmed

to room temperature and the liquid ammonia was allowed to evaporate. The crude residue was dissolved into water and transferred to a round bottom flask. The water was removed under reduced pressure and the resulting crude material was purified by flash column chromatography on silica (25:75, methanol: ethyl acetate) to give the *amine* **514** (0.0054 g, 95%) as a yellow oil. $[\alpha]^{20}_{D}$: -18.50 (c=0.8 MeOH); ν_{max} (solid state)/ cm^{-1}: 3255w (OH/NH), 1736s (C=O), 1558s, 1406s, 1228w, 1097w, 1036m, 956w; δ_H (400 MHz, MeOD): 2.87 (1H, dd, *J*13.2, 4.8, *H*H-5), 2.95 (1H, ddd, *J*7.2, 6.4, 4.8, H-2), 3.23 (1H, d, *J*13.2, H*H*-5), 3.64 (1H, dd, *J*10.8, 7.2, C*H*HO), 3.73 (1H, dd, *J*10.8, 6.4, CH*H*O), 4.31 (1H, dd, *J*7.2, 4.8, H-3), 5.12 (1H, dd, *J*7.2, 4.8, H-4); δ_C (100.6 MHz; MeOD): 52.47 (CH_2), 57.88 (CH), 59.63 (CH_2), 61.27 (CH), 81.77 (CH), 160.78 (C); (ES) m/z (%): 159 ($M+H^+$, 100%); Accurate mass (FAB): Found 159.0770 ($M+H^+$ $C_6H_{11}O_3N_2$ requires 159.0770).

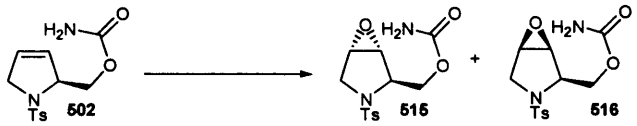
6.13.4 (2*S*, 3*S*, 4*R*)-2-Hydroxymethyl-3-amino-4-hydroxypyrrolidine 488



The bicyclic carbamate **514** (0.011 g, 0.06 mmol) was dissolved in MeOH (0.14 ml) and water (0.54 ml) and underwent hydrolysis as described in experiment 6.13.2 using LiOH (0.025 g, 1.04 mmol). The mixture was heated at reflux (90 °C) for 1.5 h. The solution was cooled and the solvent was removed under reduced pressure. The resulting crude mixture was purified by flash column chromatography on silica (100% methanol basified with NH_3) to give the *aminopyrrolidine* **488** (0.0028 g, 35%) as an orange oil. To aid analysis dilute HCl (aq) was added to the amine in water until the solution was approximately pH 2~3 yielding the corresponding hydrochloride salt of **488**. δ_H (400 MHz, D_2O): 3.49 (1H, dd, *J*12.8, 3.6, *H*H-5), 3.54 (1H, dd, *J*12.8, 2.0, H*H*-5), 4.02 (1H, dd, *J*12.4, 6.0, C*H*HO), 4.06 (1H, dd, *J*12.4, 4.8, CH*H*O), 4.16 (1H, ddd, *J*9.2, 6.0, 4.8, H-2), 4.24 (1H, dd, *J*9.2, 4.8, H-3), 4.79-4.81 (1H, m obscured by HOD peak, H-4); δ_C (100.6 MHz; D_2O): 50.69 (CH_2), 52.18 (CH), 57.10 (CH_2), 58.10 (CH), 67.79 (CH).

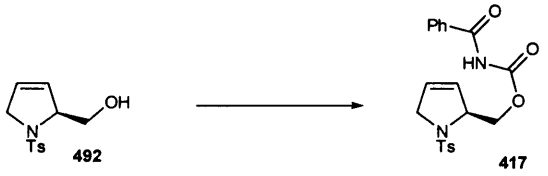
6.14 Synthesis of the (2*S*, 3*S*, 4*S*)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 489

6.14.1 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2- (carbamoyloxymethyl)-3,4-epoxypyrrolidine 515 and (2*R*, 3*S*, 4*R*)-1-(toluene-4-sulfonyl)-2-(carbamoyloxymethyl)-3,4-epoxypyrrolidine 516



Na_2EDTA (3.92 ml, $4x10^{-4}$ moldm-3, 0.0015 mmol) and trifluoroacetone (0.97 g, 0.77 ml, 8.6 mmol) were added to a 0 °C solution of the alkene **502** (0.23 g, 0.78 mmol) dissolved in acetonitrile (12 ml). Solid $NaHCO_3$ (0.52 g, 6.3 mmol) mixed with oxone (2.41 g, 3.9 mmol) was then added to the solution portion-wise over 1 h. The reaction mixture was stirred a further 2 hr before sodium sulphate (~1 g) and dichloromethane (30 ml) were added. The mixture was filtered and the filtrate was concentrated under vacuum to yield an inseparable 3:1 mixture of the *epoxides* **515** and **516** (0.23 g, 95%). Data for isomeric mixture: Major isomer **515**: δ_H (300 MHz, $CDCl_3$; Me_4Si): 2.43 (3H, s, Ar 4-Me), 3.53 (1H, d, *J*12.9, *H*H-5), 3.55 (2H, s, H-3, H-4), 3.68 (1H, d, *J*12.9, H*H*-5), 4.07 (1H, dd, *J*5.7, 4.2, H-2), 4.27 (1H, dd, *J*11.4, 5.7, C*H*HO), 4.41 (1H, dd, *J*11.4, 4.2, CH*H*O), 4.69 (2H, br s, NH_2), 7.32 (2H, d, *J*8.0, 2xAr H-3), 7.65 (2H, d, *J*8.0, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 19.63 (CH_3), 47.19 (CH_2), 52.93 (CH), 54.68 (CH_2), 57.77 (CH), 62.71 (CH), 125.64 (2 × CH), 127.64 (2 × CH), 132.88 (C), 141.85 (C), 154.53 (C); Minor isomer **516**: δ_H (300 MHz, $CDCl_3$; Me_4Si): 2.45 (3H, s, Ar 4-Me), 3.27 (1H, dd, *J*11.7, 1.8, *H*H-5), 3.65 (1H, dd, *J*3.0, 1.8, H-4), 3.69 (1H, dd, *J*9.3, 4.5, 1.8, H-2), 3.72 (1H, dd overlapping, *J*3.0, 1.8, H-3), 3.75 (1H, d, *J*11.7, H*H*-5), 3.14 (1H, dd, *J*10.5, 9.3, C*H*HO), 4.73 (2H, s br, NH_2), 4.84 (1H, dd, *J*10.5, 4.5, CH*H*O), 7.35 (2H, d, *J*8.0, 2xAr H-3), 7.74 (2H, d, *J*8.0, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.56 (CH_3), 50.50 (CH_2), 54.82 (CH), 57.66 (CH), 57.90 (CH), 63.48 (CH_2), 127.83 (2 × CH), 129.89 (2 × CH), 133.52 (C), 144.15 (C), 156.12 (C).

6.14.2 (*S*)-1-(Toluene-4-sulfonyl)-2-benzoylcarbamoyloxymethyl -2,5-dihydro-1*H*-pyrrole 517



Benzoyl isocyanate (0.11 g, 0.095 ml, 0.76 mmol) was added to a 0 °C solution of the alcohol **492** (0.12 g, 0.46 mmol) in dichloromethane (2.5 ml). The reaction mixture was stirred for 5 h

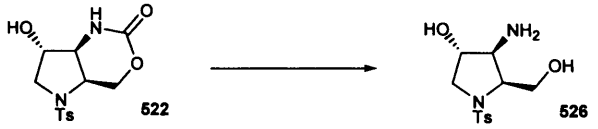
before being diluted with ethyl acetate (10 ml) and washed with water (20 ml). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (50:50, ethyl acetate: petroleum-ether) to afford the *carbamate* **417** (0.166 g, 91%) as a white foam. $[\alpha]^{20}_D$: -136.5 (c=1.07 MeOH); ν_{max} (solid state)/ cm^{-1}: 1726s (C=O), 1485s, 1339s, 1181s, 1158s, 1091m, 1009s; δ_H (300 MHz; $CDCl_3$; Me_4Si): 2.40 (3H, s, Ar 4-Me), 4.08-4.14 (1H, m, *H*H-5), 4.17-4.24 (1H, m, H*H*-5), 4.33 (1H, dd, *J*11.1, 4.8, C*H*HO), 4.49 (1H, dd, *J*11.1, 4.5, CH*H*O), 4.70-4.72 (1H, m, H-2), 5.630 (1H, ddd, *J*6.3, 3.9, 2.1, H-4), 5.74 (1H, dd, *J*6.3, 1.8, H-3), 7.30 (2H, d, *J*8.1, 2xAr H-3), 7.47 (2H, app t, *J*7.8, 2xPh-H*meta*), 7.58 (1H, app t, *J*7.8, Ph-H*para*), 7.70 (2H, d, *J*8.1, 2xAr H-2), 7.85 (2H, d, *J*7.8, 2xPh-H*ortho*), 8.51 (1H, br s, NH); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.47 (CH_3), 55.74 (CH_2), 65.76 (CH), 67.34 (CH_2), 126.23 (CH), 127.41 (3 × CH), 127.72 (2 × CH), 128.79 (2 × CH), 129.84 (2 × CH), 132.85 (CH), 132.97 (C), 134.00 (C), 143.86 (C), 150.93 (C), 164.97 (C); (ES) m/z (%): 236 (M-OCONHCOPh, 100%), 401 (58, $M+H^+$), 418 (25, $M+NH_4^+$); Accurate mass (FAB): Found 401.1171 ($M+H^+$ $C_{20}H_{21}O_5N_2S$ requires 401.1171).

6.14.3 (2*S*, 3*S*, 4*S*)-1-(Toluene-4-sulfonyl)-4-hydroxy-2,3-(oxazin-2-one)-pyrrolidine 522

t-BuOK (0.20 g, 0.66 mmol) was added to a solution of the epoxide **515** (0.2 g, 0.66 mmol; 3:1 mixture of isomers **515** : **516**) dissolved in *n*PrOH (8 ml). The solution was stirred for 5 min before ^{t}BuOCl (0.073 g, 0.66 mmol) was added and the reaction mixture was stirred for a further 2 h before the solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (1: 99, methanol: ethyl acetate) to give the *bicyclic carbamate* **522** (0.064 g, 31%; 43% based on **515**) as a white solid. Mp: 216-219 °C; $[\alpha]^{20}_D$: -115.3 (c=1.04 MeOH); ν_{max} (solid state)/ cm^{-1}: 3262br (NH/OH), 1702s (C=O), 1596w, 1408w, 1341m, 1157s, 1065m, 958w; δ_H (300 MHz, MeOD): 2.41 (3H, s, Ar 4-Me), 3.40 (1H, d, *J*12.0, *H*H-5), 3.55 (1H, dd, *J*12.0, 3.3, H*H*-5), 3.73 (1H, d, *J*5.4, H-3), 3.91-3.94 (2H, br m, H-4, H-2), 4.36 (1H, d, *J*12.0, C*H*HO), 4.67 (1H, dd, obscured by HOD peak, *J*12.0, 2.4, CH*H*O), 7.37 (2H, d, *J*8.1, 2xAr H-3), 7.74 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; MeOD): 22.36 (CH_3), 56.03 (CH), 57.06 (CH_2), 62.27 (CH), 69.17 (CH_2), 75.24 (CH), 129.89 (2 × CH), 131.57 (2 × CH), 136.47 (C), 146.21 (C), 157.07 (C); (ES) m/z (%): 252 ($M+H^+$-(OH+CONH), 100%), 313 (33, $M+H^+$), 236 (30, $M+H^+$-($OHNHCO_2$)); Accurate mass (FAB): Found 313.0858 ($M+H^+$ $C_{13}H_{17}O_5N_2S$ requires

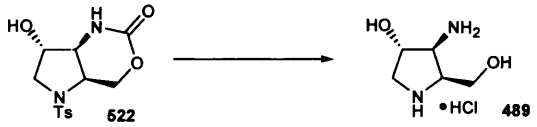
313.0858). Anal. Found: C, 49.82; H, 4.91; N, 8.80; $C_{13}H_{16}O_5N_2S$ requires: C, 49.99; H, 5.16; N, 8.97.

6.14.4 (2*S*, 3*S*, 4*S*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-3-amino-4-hydroxy-pyrrolidine 526



The bicyclic carbamate **522** (0.026 g, 0.08 mmol) was dissolved in MeOH (0.1 ml) and water (0.34 ml) and underwent hydrolysis as described in experiment 6.13.2 using LiOH (0.016 g, 0.66 mmol). The mixture was heated (90 °C) for 1.5 h before the solution was cooled and the solvent was removed under reduced pressure. The resulting crude mixture was purified by flash column chromatography on silica (20:80, methanol: ethyl acetate) to give the *aminopyrrolidine* **526** (0.016 g, 68%). Mp: 54-56 °C; $[\alpha]^{20}_D$: -18.9 (c=1.0 MeOH); ν_{max} (solid state)/ cm^{-1}: 3361br (NH/OH), 2944w, 1597m, 1402w, 1335m, 1090m, 1025m, 816w; δ_H (400 MHz, D_2O): 2.44 (3H, s, Ar 4-Me), 3.02 (1H, dd, *J*10.4, 7.0, *H*H-5), 3.11 (1H, app t, *J*7.0, H-3), 3.77 (1H, dd, *J*10.4, 7.0, H*H*-5), 3.81 (1H, ddd, *J*7.0, 4.8, 3.2, H-2), 3.87 (1H, dd, *J*12.4, 3.2, C*H*HO), 3.92 (1H, dd, *J*12.4, 4.8, CH*H*O), 4.27 (1H, app q, *J*7.0, H-4), 7.48 (2H, d, *J*8.0, 2xAr H-3), 7.77 (2H, d, *J*8.0, 2xAr H-2); δ_C (100.6 MHz; D_2O): 20.69 (CH_3), 52.47 (CH_2), 57.96 (CH), 60.48 (CH_2), 61.30 (CH), 73.33 (CH), 127.55 (2 × CH), 130.11 (2 × CH), 133.31 (C), 145.71 (C); (ES) m/z (%): 287 ($M+H^+$, 100%); Accurate mass (FAB): Found 287.1065 ($M+H^+$ $C_{12}H_{19}O_4N_2S$ requires 287.1066).

6.14.5 (2*S*, 3*S*, 4*S*)-2-Hydroxymethyl-3-amino-4-hydroxypyrrolidine 489

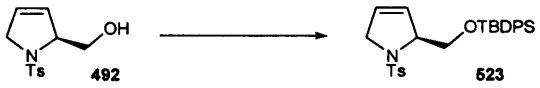


The bicyclic carbamate **522** (0.031 g, 0.098 mmol) was subjected to the general tosyl deprotection procedure (see experiment 6.2.9) using sodium metal (0.0056 g, 0.24 mmol) in liquid ammonia (~5 ml). After the addition of sodium was complete the reaction was warmed to room temperature and the liquid ammonia was allowed to evaporate. The crude residue was dissolved into water and transferred to a round bottom flask. Water was removed under reduced pressure and the resulting crude material was purified by flash column chromatography on silica (100% methanol) to give the *amine* **489** (0.0061 g, 41%) as a yellow oil. To aid analysis dilute HCl (aq) was added to the amine in water until the solution was approximately pH 2~3 yielding the corresponding hydrochloride salt of **489**. $[\alpha]^{20}_D$: +7.8 (c=0.61 MeOH); ν_{max} (solid state)/ cm^{-1}: 3326w (OH/NH), 2022w, 1599w, 1396w, 1347w,

1157m, 1037m, 978m, 767m; δ_H (400 MHz, D_2O): 3.36 (1H, dd, *J*12.8, 4.4, *H*H-5), 3.73 (1H, dd, *J*12.8, 5.2, H*H*-5), 3.98 (1H, dd, *J*12.4, 3.6, CH*H*O), 4.02 (1H, dd, *J*7.2, 4.0, H-3), 4.07 (1H, dd, *J*12.4, 3.6, CH*H*O) 4.31 (1H, d app t, *J*7.2, 3.6, H-2), 4.63-4.74 (1H, m obscured by HOD peak, H-4); δ_C (100.6 MHz; D_2O): 49.63 (CH_2), 56.58 (CH_2), 57.08 (CH), 58.37 (CH), 71.94 (CH); (ES) m/z (%): 133 ($M+H^+$, 100%); Accurate mass (FAB): Found 133.0976 ($M+H^+$ $C_5H_{13}O_2N_2$ requires 133.0977).

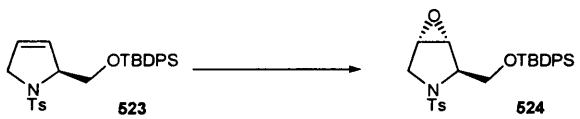
6.15 Stereoselective synthesis of the *anti*-epoxide 515

6.15.1 (*S*)-1-(Toluene-4-sulfonyl)-2-(*tert*-butyl diphenylsilanyloxymethyl)-2,5-dihydro-1*H*-pyrrole 523



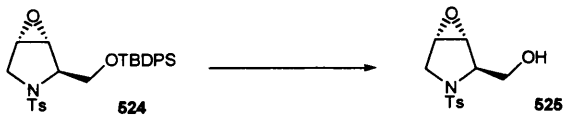
Imidazole (0.045 g, 0.67 mmol) and DMAP (0.0081 g, 0.066 mmol) were added to a 0 °C solution of the alkene **492** (0.14 g, 0.556 mmol) dissolved in dichloromethane (5 ml) and the solution was stirred for 15 min. ^{t}Butyldiphenylsilyl chloride (0.18 g, 0.17 ml, 0.67 mmol) was added and the mixture stirred for a further 4 h. The reaction mixture was diluted with dichloromethane (10 ml) and was washed with water (10 ml). The organic layer was dried ($MgSO_4$), and the solvent removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (10:90, ethyl acetate: petroleum-ether) to give the *protected alcohol* **523** (0.22 g, 81%) as a white solid. Mp: 69-71 °C; $[\alpha]^{20}_D$: - 172.6 (c= 1.0 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3055m, 2928m, 1593m, 1464m, 1428m, 1346s, 1264s, 1093s, 1029w, 823m, 765s; δ_H (300 MHz, $CDCl_3$; Me_4Si): 1.07 (9H, s, $C(CH_3)_3$), 2.42 (3H, s, Ar 4-Me), 3.77 (1H, dd, *J*9.9, 7.2, C*H*HO), 4.02 (1H, dd, *J*9.9, 3.9, CH*H*O), 4.10-4.14 (2H, m, 2xH-5), 4.43-4.46 (1H, m, H-2), 5.69 (1H, ddd, *J*6.3, 3.6, 1.8, H-3), 5.76 (1H, d app q, *J*6.3, 2.4, H-4), 7.27 (2H, d, *J*8.1, 2xAr H-3), 7.37-7.48 (6H, m, 6xPh-H), 7.62 (2H, d, *J*8.1, 2xAr H-2), 7.63-7.69 (4H, m, 4xPh-H); δ_C (75 MHz; 300 MHz, $CDCl_3$; Me_4Si): 19.21 (C), 21.49 (CH_3), 26.83 (3 × CH_3), 55.93 (CH_2), 67.07 (CH_2), 68.03 (CH), 125.65 (CH), 127.38 (2 × CH), 127.66 (4 × CH), 128.56 (CH), 128.57 (2 × CH), 129.66 (2 × CH), 135.61 (4 × CH), 133.31 (C), 137.14 (2 × C), 143.24 (C); (ES) m/z (%): 414 (M-Ph, 100%), 509 (23, $M+NH_4^+$), 492 (16 $M+H^+$); Accurate mass (FAB): 492.2029 Found ($M+H^+$ $C_{28}H_{34}O_3N_1SSi^{28}$ requires 492.2029).

6.15.2 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-(*tert*-butyldiphenylsilanyloxymethyl)-3,4-epoxypyrrolidine 524



The alkene **523** (0.07 g, 0.16 mmol) underwent epoxidation following the procedure outlined in experiment 6.14.1, using Na_2EDTA (0.75 ml, $4x10^{-4}$ $moldm^{-3}$, 0.00032 mmol), trifluoroacetone (0.19 g, 0.16 ml, 1.77 mmol), sodium hydrogen carbonate (0.11 g, 1.29 mmol) and oxone (0.50g, 0.81 mmol) in acetonitrile (3 ml). The reaction was stirred for 2 h before sodium sulphate (0.5 g) was added. The solution was diluted with dichloromethane (10 ml), filtered and the filtrate was concentrated under vacuum. The resulting crude material was purified by flash column chromatography on silica (10:90, ethyl acetate: petroleum-ether) to afford the *epoxide* **524** (0.048 g, 60%) as a white solid. Mp: 118-119 °C; $[\alpha]^{20}_D$: - 86.0 (c= 1.18 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 1748w, 1430w, 1343s, 1235m, 1156s, 1090s, 1045s, 1010m, 928w, 847s; δ_H (300 MHz, $CDCl_3$; Me_4Si): 1.07 (9H, s, $C(CH_3)_3$), 2.43(3H, s, Ar 4-Me), 3.55 (1H, d, *J*3.0, H-3 or H-4), 3.57 (1H, d, *J*12.6, *H*H-5), 3.58 (1H, d *J*3.0, H-3 or H-4), 3.67 (1H, d, *J*12.6, H*H*-5), 3.91 (1H, dd, *J*10.5, 5.4, C*H*HO), 3.93 (1H, dd, *J*10.5, 6.6, CH*H*O), 3.88-3.98 (1H, m overlapping, H-2), 7.28 (2H, d, *J*8.1, 2xAr H-3), 7.39-7.48 (6H, m, 6xPh-H), 7.60-7.69 (6H, m, 4xPh-H, 2xAr H-2); δ_C (75 MHz; 300 MHz, $CDCl_3$; Me_4Si): 19.14 (C), 21.55 (CH_3), 26.83 (3 × CH_3), 49.60 (CH_2), 55.06 (CH), 57.66 (CH), 61.72 (CH), 64.87 (CH_2), 127.51 (2 × CH), 127.85 (4 × CH), 129.50 (2 × CH), 129.90 (CH), 129.95 (2 × CH), 132.72 (C), 132.92 (C), 135.20 (C), 135.56 (3 × CH), 143.43 (C); (ES) m/z (%): 414 (M-Ph, 100%), 509 (23, $M+NH_4^+$), 430 (M-Ph, 100%), 525 (22, $M+NH_4^+$), 508 (7, $M+H^+$); Accurate mass (FAB): 508.1977 Found ($M+H^+$ $C_{28}H_{34}O_4NSSi$ requires 508.1978); Anal. Found: C, 66.14; H, 6.56; N, 2.60; $C_{28}H_{33}O_4NSSi$ requires: C, 66.29; H, 6.55; N, 2.75.

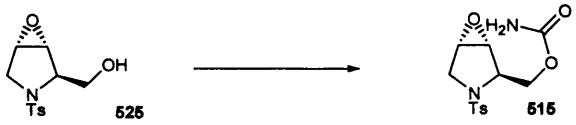
6.15.3 (2*R*, 3*R*, 4*S*)-1-(toluene-4-sulfonyl)-2-hydroxymethyl-3,4-epoxypyrrolidine 525



Tetra-butylammonium fluoride (0.081 ml of a 1 $moldm^{-3}$ solution in THF, 0.081 mmol) was added to a 0 °C solution of the epoxide **524** (0.037 g, 0.073 mmol) dissolved in THF (1.9 ml). The reaction mixture was stirred for 2 h before the solvent was removed under vacuum. The resulting crude material was purified by flash column chromatography on silica (50:50, ethyl acetate: petroleum-ether) to give the *alcohol* **525** (0.016 g, 84%) as a white solid. Mp: 127-129 °C (Lit.[187], 131-133 °C); $[\alpha]^{20}_D$: -91.0 (c= 1.53 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3503br (OH), 1332s, 1317s, 1219m, 1155s, 1092m, 1008m, 848s, 816m, 670s; δ_H (300 MHz, $CDCl_3$; Me_4Si): 2.43 (3H, s, Ar 4-Me), 3.51 (1H, d, *J*3.0, H-3 or H-4), 3.57 (1H, d, *J*3.0, H-3 or H-4), 3.58 (1H, d overlapping, *J*12.6, H*H*-5), 3.68 (1H, d, *J*12.6, *H*H-5), 3.80 (1H, dd, *J*12.6, 6.0, C*H*HO), 3.88 (1H, dd, *J*12.6, 4.2, CH*H*O), 3.90 (1H, dd, *J*6.0, 4.2, H-2), 7.31 (2H, d, *J*8.1,

2xAr H-3), 7.66 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; 300 MHz, $CDCl_3$; Me_4Si): 21.56 (CH_3), 49.51 (CH_2), 54.98 (CH), 57.21 (CH), 62.47 (CH), 63.15 (CH_2), 127.60 (2 × CH), 129.63 (2 × CH), 134.52 (C), 143.86 (C); (ES) m/z (%): 252 ($M+H^+-H_2O$, 100%), 155 (45, $[CH_3C_4H_6SO_2]^+$), 270 (7, $M+H^+$); Accurate mass (FAB): 270.0800 Found ($M+H^+$ $C_{12}H_{16}O_4NS$ requires 270.0800); Anal. Found: C, 53.34; H, 5.40; N, 5.03; $C_{12}H_{15}O_4NS$ requires: C, 53.50; H, 5.61; N, 5.20.

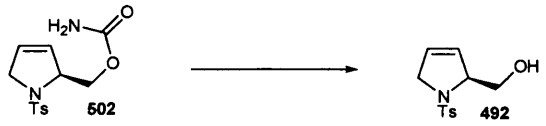
6.15.4 (2*R*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-(carbamoyloxymethyl)-3,4-epoxypyrrolidine 515



The alcohol **525** (0.0056 g, 0.021 mmol) was dissolved in dichloromethane (0.1 ml) and formation of the carbamate was achieved following the procedure described in experiment 6.11.2 using trichloroacetylisocyanate (0.036 g, 0.0023 ml, 0.031 mmol). After 8 h TLC analysis confirmed all starting material had been consumed. The crude material was dissolved in methanol (0.1 ml) and hydrolysis was carried out using potassium carbonate (1.34 g, 0.5 $moldm^{-3}$, 9.7 mmol). After 18 h the reaction was worked up to yield the *carbamate* **515** (0.0054 g, 78%) as a white solid. NMR data as for 6.14.1, additional data: Mp: 164-166 °C; $[\alpha]^{20}_D$: -35.94 (c= 0.96 MeOH); ν_{max} (solid state)/ cm^{-1}: 3455w (NH), 1730s (C=O), 1707ss, 1602ss, 1452sw, 1398sm, 1314ss, 1160ss, 1079sw, 979sm, 853m, 688s; (ES) m/z (%): 252 ($M-OCONH_2$, 100%), 155 (62, $SO_2C_6H_4CH_3$), 313 (53, $M+H^+$); Accurate mass (FAB): Found 313.0858 ($M+H^+$ $C_{13}H_{17}O_5N_2S$ requires 313.0858).

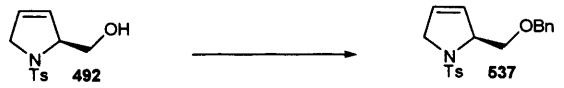
6.16 Synthesis of the (2*S*, 3*R*, 4*R*)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 491

6.16.1 (*S*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-2,5-dihydro-1*H*-pyrrole 492



An aqueous solution of NaOH (2.2 ml of a 0.5M solution) was added to carbamate **502** (0.11 g, 0.37 mmol) dissolved in propanol (4.4 ml). The reaction mixture was refluxed for 1 h before the solvent was removed under reduced pressure. The resulting aqueous material was extracted with ethyl acetate (3x10 ml), the organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield the *alcohol* **492** (0.08 g, 87%) as an off-white solid. Data as for experiment 6.11.1.

6.16.2 (*S*)-1-(Toluene-4-sulfonyl)-2-benzyloxymethyl-2,5-dihydro-1*H*-pyrrole 537



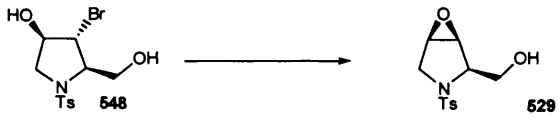
A solution of the alcohol **492** (0.11 g, 0.42 mmol) in DMF (2 ml) was added to sodium hydride (0.025 g of a 60% dispersion in oil, 0.63 mmol) also in DMF (0.5 ml). The mixture was stirred for 30 min before benzyl bromide (0.11 g, 0.075 ml, 0.63 mmol) was added. The reaction was stirred for a further 6 h before being quenched with water (5 ml). The mixture was extracted with ethyl acetate (3x10 ml), the organic layers were combined, dried ($MgSO_4$) and the solvent removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (10:90, ethyl acetate: petroleum-ether) to afford the *benzyl alcohol* **537** as a clear oil (0.12 g, 82%). $[\alpha]^{20}_D$: -189.4 (c=1.09 MeOH); ν_{max} (solid state)/ cm^{-1}: 2917w, 1723w, 1493m, 1452m, 1333s, 1237m, 1156s, 1112m, 1043m, 824s, 809s; δ_H (300 MHz; $CDCl_3$; Me_4Si): 2.42 (3H, s, Ar 4-Me), 3.57 (1H, dd, *J*9.3, 7.8, C*H*HO), 3.92 (1H, dd, *J*9.3, 3.9, CH*H*O), 4.10 (1H, ddd, *J*9.0, 4.5, 2.4, *H*H-5), 4.17 (1H, dd app t, *J*9.0, 4.5, 1.8, H*H*-5), 4.51-4.56 (1H, m, H-2), 4.57 (2H, s, $PhCH_2$), 5.67 (1H, ddd, *J*6.3, 3.6, 1.8, H-3), 5.77 (1H, d app t, *J*6.3, 4.5, H-4), 7.27-7.38 (7H, m, 5xPh-H, 2xAr H-3), 7.70 (2H, d, *J*8.4, 2xAr H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.49 (CH_3), 56.79 (CH_2), 69.44 (CH), 73.57 (CH_2), 73.65 (CH_2), 125.55 (CH), 127.44 (2 × CH), 127.61 (3 × CH), 128.35 (2 × CH), 128.52 (CH), 129.72 (2 × CH), 134.35 (C), 138.17 (C), 143.53 (C); (ES) m/z (%): 222 (100%), 344 (63, $M+H^+$), 252 (57, $M-CH_3C_6H_4$); Accurate mass (FAB): 344.1320 Found ($M+H^+$ $C_{19}H_{22}O_3NS$ requires 344.1320)

6.16.3 (2*R*, 3*R*, 4*R*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-3-bromo-4-hydroxypyrrolidine 548

NBS (1.37 g, 7.70 mmol) was added to a solution of the alcohol **492** (0.97 g, 3.85 mmol) in THF (27.7 ml) and water (3.18 ml) and the mixture stirred in the dark for 4 h. The reaction mixture was diluted with diethyl ether (50 ml) and washed with water (50 ml). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (40:60, ethyl acetate: petroleum-ether) to yield the *bromohydrin* **548** (0.54 g, 41%) as a white solid. The yield of **548** was increased to 0.78 g, (59%) by recrystallisation of a mixed fraction with chloroform. Mp: 164-168 °C; $[\alpha]^{20}_D$: -43.6 (c= 1.09 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3175w, 2877w, 1596m,

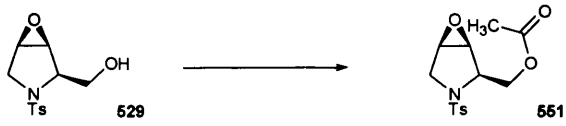
1455m, 1348m, 1165s, 1074m, 1032m, 822m, 663s ; δ_H (300 MHz, $CDCl_3$; Me_4Si): 2.45 (3H, s, Ar 4-Me), 3.65 (2H, d, *J*3.0, CH_2O), 3.82 (1H, dd, *J*12.0, 1.8, *H*H-5), 4.01 (1H, ddd, *J*3.6, 3.3, 1.8, H-4), 4.08 (1H, br s, H-3), 4.15 (1H, dd, *J*12.0, 3.6, H*H*-5), 4.19 (1H, br s, H-2), 7.36 (2H, d, *J*8.1, 2xAr H-3), 7.74 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; 300 MHz, $CDCl_3$; Me_4Si): 21.49 (CH_3), 53.51 (CH), 55.31 (CH_2), 65.03 (CH_2), 71.48 (CH), 77.52 (CH), 127.60 (2 × CH), 129.63 (2 × CH), 134.52 (C), 143.86 (C); (ES) m/z (%): 352 ($M(Br^{81})+H^+$, 100%), 350 (90, $M(Br^{79})+H^+$), 334 (42, $M(Br^{81})-H_2O$), 332 (40, $M(Br^{79})-H_2O$); Accurate mass (FAB): 350.0061 Found ($M+H^+$ $C_{12}H_{17}O_4NSBr^{79}$ requires 350.0062); Anal. Found: C, 41.12; H, 4.82; N, 3.82; $C_{12}H_{16}O_4NSBr$ requires: C, 41.15; H, 4.60; N, 3.99.

6.16.4 (2*R*, 3*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-3,4-epoxypyrrolidine 529



Potassium *tert*-butoxide (0.062 g, 0.55 mmol) was added to a solution of bromohydrin **548** (0.18 g, 0.50 mmol) in toluene (5 ml) and the reaction mixture stirred for 2 h. The mixture was diluted with diethyl ether (10 ml) and the organic extract washed with water (10 ml). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure to give the *epoxide* **529** (0.11 g, 84%) as a white solid. Mp: 128-131 °C (Lit.[187], 129-132 °C); $[\alpha]^{20}_D$: -104.9 (c= 1.45 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3547m (OH), 2961w, 2896w, 1597w, 1405w, 1336s, 1306m, 1160s, 1092s, 1038s, 864s, 817s, 707m; δ_H (300 MHz, $CDCl_3$; Me_4Si): 2.33 (1H, br s, OH), 2.46 (3H, s, Ar 4-Me), 3.27 (1H, dd, *J*11.7, 1.8, *H*H-5), 3.56 (1H, app td, *J*5.2, 2.1, H-2), 3.61 (1H, dd, *J*3.0, 1.8, H-4), 3.76 (1H, dd, *J*3.0, 2.1, H-3), 3.80 (1H, d, *J*11.7, H*H*-5), 3.98 (1H, dd, *J*11.7, 5.2, C*H*HO), 4.15 (1H, dd, *J*11.7, 5.2, CH*H*O), 7.36 (2H, d, *J*8.1, 2xAr H-3), 7.72 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; 300 MHz, $CDCl_3$; Me_4Si): 21.55 (CH_3), 50.87 (CH_2), 53.81 (CH), 58.42 (CH), 61.66 (CH), 62.96 (CH_2), 127.73 (2 × CH), 129.92 (2 × CH), 133.19 (C), 144.25 (C); (ES) m/z (%): 292 ($M+Na^+$, 100%), 270 (49, $M+H^+$); Accurate mass (FAB): 270.0800 Found ($M+H^+$ $C_{12}H_{16}O_4NS$ requires 270.0800); Anal. Found: C, 53.38; H, 5.77; N, 5.05; $C_{12}H_{15}O_4NS$ requires: C, 53.50; H, 5.61; N, 5.20.

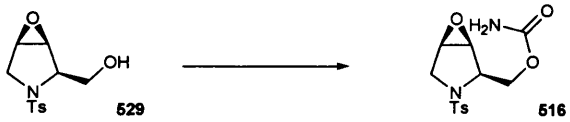
6.16.5 (2*R*, 3*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-ethylacetyl-3,4-epoxypyrrolidine 551



Potassium *tert*-butoxide (0.225, 2.06 mmol) was added to a solution of the alcohol **529** (0.64 g, 1.82 mmol) in ethyl acetate (20 ml) and the reaction mixture was stirred for 2 h. The

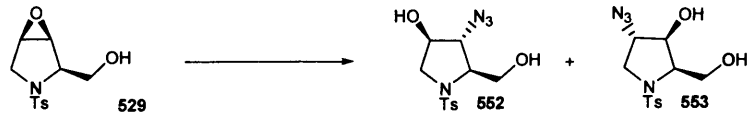
mixture was then washed with water (30 ml), the organic layer dried ($MgSO_4$) and the solvent removed under reduced pressure. The resulting crude material was purified using flash column chromatography on silica (50:50, ethyl acetate: petroleum-ether) to give the *acetate* **551** (0.45 g, 80%). Mp: 148-150 °C (Lit.[187], 154-158 °C (from benzene, hexane)); $[\alpha]^{20}_D$: -149.6 (c= 1.79 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 2925w, 1748s, 1598w, 1332m, 1236m, 1156s, 1090m, 1040m, 852m, 809m, 675m; δ_H (300 MHz, $CDCl_3$; Me_4Si): 2.09 (3H, s, CH_3), 2.44 (3H, s, Ar 4-Me), 3.28 (1H, dd, *J*11.7, 1.8, *H*H-5), 3.65-3.74 (3H, m, H-2, H-3, H-4), 3.74 (1H, d overlapping, *J*11.7, H*H*-5), 4.14 (1H, dd, *J*10.8, 9.6, C*H*HO), 4.83 (1H, dd, *J*10.8, 4.5, CH*H*O), 7.35 (2H, d, *J*8.1, 2xAr H-3), 7.73 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; 300 MHz, $CDCl_3$; Me_4Si): 20.76 (CH_3), 21.52 (CH_3), 50.42 (CH_2), 54.92 (CH), 57.62 (2 × CH), 62.84 (CH_2), 127.37 (2 × CH), 129.74 (2 × CH), 133.27 (C), 144.18 (C), 170.35 (C); (ES) m/z (%): 252 (M-CH_3CO_2, 100%), 155 (50, $CH_3C_6H_4SO_2^+$), 334 (48, M+Na^+); Accurate mass (FAB): 312.0905 Found (M+H^+ $C_{14}H_{18}O_5NS$ requires 312.0906); Anal. Found: C, 53.90; H, 5.33; N, 4.40; $C_{14}H_{17}O_5NS$ requires: C, 54.00; H, 5.50; N, 4.49.

6.16.6 (2*R*, 3*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-(carbamoyloxymethyl)-3,4-epoxypyrrolidine 516



The alcohol **529** (0.057 g, 0.21 mmol) was dissolved in dichloromethane (0.45 ml) and formation of the carbamate was achieved following the procedure described in experiment 6.11.2 using trichloroacetylisocyanate (0.38 g, 0.024 ml, 0.32 mmol). After 1 h TLC analysis confirmed all starting material had been consumed. The crude material was dissolved in methanol (1 ml) and hydrolysis was achieved using potassium carbonate (0.088 g, 0.5 moldm^{-3}, 0.64 mmol). After 18 h the reaction was worked up to yield the *carbamate* **516** as a white solid (0.043 g, 65%). NMR data as for 6.14.1, additional data: Mp: 185-188 °C; $[\alpha]^{20}_D$: -122.32 (c= 0.89 MeOH); ν_{max} (solid state)/ cm^{-1}: 3450w (NH_2), 3355w, 2974w, 1698m (C=O), 1666s, 1599w, 1482w, 1414m, 1340s, 1156s, 1119w, 1092s, 855s; (ES) m/z (%): 313 (M+H^+, 100%), 252 (81, M-$OCONH_2$); Accurate mass (FAB): Found 313.0859 (M+H^+ $C_{13}H_{17}O_5N_2S$ requires 313.0858).

6.16.7 (2*S*, 3*R*, 4*S*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-3-azido-4-hydroxypyrrolidine 552 and (2*R*, 3*S*, 4*S*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-3-hydroxy-4-azidopyrrolidine 553



Sodium azide (0.12 g, 1.84 mmol) was added a solution of the epoxide **529** (0.098 g, 0.36 mmol) in acetone (10.2 ml) and water (1.3 ml). Ammonium chloride (0.019 g, 0.84 mmol) was added and the solution was heated at reflux (80 °C) for 18 h. After cooling, acetone was removed under reduced pressure and the resulting aqueous layer was washed with ethyl acetate (3x20 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (100% diethyl ether) to give the azide **552** (0.66 g, 58%) as a white solid, together with the azide **553** (0.008 g, 7%) as a white oil and a mixed fraction (0.036, 32%; mix of **552** : **553**). Data for **552**: Mp: 94-96 °C; $[\alpha]^{20}_D$: -78.3 (c= 1.24 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3260br (OH), 2927w, 2098s (N_3), 1597w, 1343m, 1239w, 1163s, 1089m, 1032s, 973w, 823m, 663s; δ_H (300 MHz, $CDCl_3$; Me_4Si): 2.46 (3H, s, Ar 4-Me), 3.33 (1H, dd, *J*10.8, 4.8, *H*H-5), 3.54-3.60 (2H, m, H-2, H*H*-5), 3.83 (1H, dd, *J*11.7, 2.1, C*H*HO), 3.92-3.95 (2H, m, H-3, H-4), 4.21 (1H, dd, *J*11.7, 3.6, CH*H*O), 7.36 (2H, d, *J*8.1, 2xAr H-3), 7.73 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; 300 MHz, $CDCl_3$; Me_4Si): 21.59 (CH_3), 55.66 (CH_2), 63.86 (CH_2), 66.10 (CH), 69.48 (CH), 73.06 (CH), 127.66 (2 × CH), 129.91 (2 × CH), 132.50 (C), 144.40 (C); (ES) m/z (%): 313 ($M+H^+$ 100%), 330 (42, $M+NH_4^+$); Accurate mass (FAB): 313.0970 Found ($M+H^+$ $C_{12}H_{17}O_4N_4S$ requires 313.0971); Anal. Found: C, 46.04; H, 5.02; N, 17.84; $C_{12}H_{16}O_4N_4S$ requires: C, 46.14; H, 5.16; N, 17.94. Data for **553**: $[\alpha]^{20}_D$: +5.2 (c= 0.36 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3269br (OH), 2101s (N_3), 1599m, 1328s, 1268m, 1151s, 1089s, 1031m, 991m, 864m; δ_H (300 MHz, $CDCl_3$; Me_4Si): 2.46 (3H, s, Ar 4-Me), 3.21 (1H, dd, *J*11.4, 4.2, H-5), 3.47 (1H, ddd, *J*6.0, 4.2, 3.9, H-2), 3.87 (1H, dd, *J*11.4, 5.4, H-5), 3.95-4.04 (3H, m, H-3, H-4, C*H*HO), 4.26 (1H, dd, *J*12.3, 3.9, CH*H*O), 7.36 (2H, d, *J*8.1, 2xAr H-3), 7.71 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; 300 MHz, $CDCl_3$; Me_4Si): 21.58 (CH_3), 51.20 (CH_2), 61.17 (CH), 61.94 (CH_2), 64.36 (CH), 77.29 (CH), 127.57 (2 × CH), 129.95 (2 × CH), 132.88 (C), 144.41 (C); (ES) m/z (%): 642 ($2M+NH_4^+$ 100%), 313 (68, $M+H^+$), 330 (46, $M+NH_4^+$); Accurate mass (FAB): 313.0972 Found ($M+H^+$ $C_{12}H_{17}O_4N_4S$ requires 313.0971).

6.16.8 (2*S*, 3*R*, 4*R*)-1-(Toluene-4-sulfonyl)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 554

10% Palladium on charcoal catalyst (6.3 mg) was added to a solution of the azide **552** (0.021 g, 0.067 mmol) in THF (4 ml) and water (2 ml). The mixture was stirred for 3 h under hydrogen at atmospheric pressure (balloon). The reaction mixture was diluted with THF (10

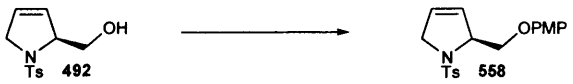
ml), filtered and the filtrate was concentrated under reduced pressure to yield the *amine* **554** (0.018 g, 97%) as a white solid. Mp: 92-93 °C; $[\alpha]^{20}_D$: - 62.3 (c= 1.16 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3284br (OH/NH), 2924br, 1597m, 1492w, 1455w, 1333s, 1182w, 1152s, 1089m, 1010m, 948w, 812m, 659s; δ_H (300 MHz, D_2O): 2.45 (3H, s, Ar 4-Me), 3.14 (1H, app t, *J*5.6, H-3), 3.195-3.26 (2H, m, *H*H-5, H-2), 3.49 (1H, d app t, *J*9.6, 5.6, H-4), 3.53 (1H, dd, *J*10.5, 5.6, H*H*-5), 3.75 (1H, dd, *J*12.0, 3.6, CH*H*O), 3.82 (1H, *J*12.0, 5.4, CH*H*O), 7.43 (2H, d, *J*8.1, 2xAr H-3), 7.72 (2H, d, *J*8.1, 2xAr H-2); δ_C (75 MHz; D_2O): 20.68 (CH_3), 53.25 (CH_2), 58.81 (CH), 62.01 (CH_2), 67.08 (CH), 74.22 (CH), 127.44 (2 × CH), 130.25 (2 × CH), 131.52 (C), 145.78 (C); (ES) m/z (%): 111 (100%), 287 (42, $M+H^+$); Accurate mass (FAB): 287.1065 Found ($M+H^+$ $C_{12}H_{19}O_4NS$ requires 287.1066).

6.16.9 (2*S*, 3*R*, 4*R*)-2-Hydroxymethyl-3-amino-4-hydroxy-pyrrolidine 491

The pyrrolidine **554** (0.018 g, 0.064 mmol) was subjected to the general tosyl deprotection procedure (see experiment 6.2.9) using sodium metal (0.0044 g, 0.19 mmol) in liquid ammonia (~5 ml). After the addition of sodium was complete the reaction was warmed to room temperature and the liquid ammonia was allowed to evaporate. The crude residue was dissolved into water and transferred to a round bottom flask; water was removed under reduced pressure and the resulting crude material was purified by flash column chromatography on silica (100% methanol bubbled with NH_3) to give the *amine* **491** (0.0032 g, 38%) as a clear oil. To aid analysis dilute HCl (aq) was added to the amine in water until the solution was approximately pH 2~3 affording the hydrochloride salt of **491**. $[\alpha]^{20}_D$: +26.9 (c=0.32 MeOH); ν_{max} (solid state)/ cm^{-1}: 3334br (OH/NH), 2901br, 1599br, 1408w, 1102m, 1026m, 970w, 922m; δ_H (400 MHz, D_2O): 3.38 (1H, dd, *J*12.4, 4.0, *H*H-5), 3.61 (1H, dd, *J*12.4, 4.0, H*H*-5), 3.60- 3.62 (1H, m overlapping, H-3), 3.81 (1H, app td, *J*5.1, 4.4, H-2), 3.87 (1H, dd, *J*12.0, 5.1, C*H*HO), 3.97 (1H, dd, *J*12.0, 4.4, CH*H*O), 4.56 (1H, app q, *J*4.0, H-4); δ_C (100.6 MHz; MeOD): 50.22 (CH_2), 56.86 (CH), 58.69 (CH_2), 62.64 (CH), 72.14 (CH).

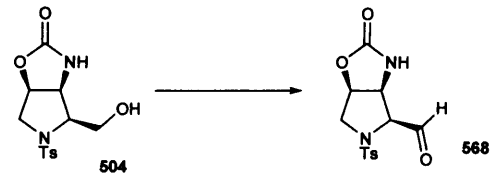
6.17 Investigations into the synthesis of the (2*S*, 3*R*, 4*S*)-2-hydroxymethyl-3-amino-4-hydroxypyrrolidine 490

6.17.1 (*S*)-1-(Toluene-4-sulfonyl)-2-(4-methoxyphenoxymethyl)-2,5-dihydro-1*H*-pyrrole 558



The alcohol **492** (0.10 g, 0.41 mmol), 4-methoxyphenol (0.15 g, 1.22 mmol) and triphenylphosphine (0.14 g, 0.53 mmol) were dissolved in THF (1.5 ml). To this solution DEAD (0.092 g, 0.083 ml, 0.53 mmol) was added and the reaction mixture was refluxed for 3.5 h. The mixture was cooled and the solvent was removed under reduced pressure. The resulting crude material was purified using flash column chromatography on silica (100% toluene) to give the *PMP ether* **558** (0.11 g, 76%) as an off-white solid. Mp: 124-128 °C; $[\alpha]^{20}_{D}$: -236.7 (c=0.99 MeOH); ν_{max} (solid state)/ cm^{-1}: 2910w, 1597w, 1508s, 1461m, 1331s, 1237s, 1098s, 1068m, 1035s, 902w, 825s; δ_H (300 MHz; $CDCl_3$; Me_4Si): 2.42 (3H, s, Ar 4-Me), 3.78 (3H, s, PMP 4-OMe), 3.94 (1H, app t, *J*9.0, C*H*HO), 4.12 (1H, ddd, *J*14.7, 4.2, 2.1, *H*H-5), 4.21 (1H, ddt, *J*14.7, 2.1, 1.8, H*H*-5), 4.41 (1H, dd, *J*9.0, 3.9, CH*H*O), 4.69-4.71 (1H, m, H-2), 5.73 (1H, ddd, *J*6.3, 2.1, 1.8, H-3), 5.83 (1H, ddd, *J*6.3, 4.2, 2.1, H-4), 6.87 (4H, m, 4xPMP-H), 7.30 (2H, d, *J*8.1, Ar H-3), 7.73 (2H, d, *J*8.1, Ar H-2); δ_C (75 MHz; $CDCl_3$; Me_4Si): 21.52 (CH_3), 55.73 (CH_3), 55.86 (CH_2), 66.75 (CH), 71.69 (CH_2), 114.63 (2 × CH), 115.53 (2 × CH), 125.99 (CH), 127.47 (2 × CH), 128.18 (CH), 129.82 (2 × CH), 134.20 (C), 143.69 (C), 152.62 (C), 154.02 (C); (ES) m/z (%): 360 (M+H+, 100%), 236 (M-$CH_3OC_6H_4O$); Accurate mass (FAB): 360.1270 Found ($M+H^+$ $C_{19}H_{22}O_4NS$ requires 360.1279).

6.17.2 (2*S*, 3*S*, 4*R*)-1-(Toluene-4-sulfonyl)-2-carbaldehyde-3,4-(oxazol-2-one)-4-hydroxypyrrolidine 568



The Dess-Martin periodinane (0.075 g, 0.18 mmol) was added to a solution of the alcohol **504** (0.028 g, 0.089 mmol) in dichloromethane (1 ml). The reaction mixture was stirred for 2 h before being diluted with diethyl ether (5 ml). The mixture was sonnicated, filtered and the filtrate was concentrated under reduced pressure. The resulting crude material was purified using flash column chromatography on silica (80:20, ethyl acetate: petroleum-ether) to give the *aldehyde* **568** (0.015 g, 54%, contaminated with ~10% side product from DMP) as a white powder. δ_H (300 MHz; $CDCl_3$; Me_4Si): 2.39 (3H, s, Ar 4-Me), 2.95 (1H, dd, *J*11.7, 5.4, *H*H-5), 3.34 (1H, dd, *J*6.3, 3.0, H-2), 3.84 (1H, d, *J*11.7, H*H*-5), 4.52 (1H, ddd, *J*7.2, 6.3, 1.8, H-3), 4.97 (1H, dd, *J*7.2, 5.4, H-4), 5.75 (1H, brs, NH), 7.32 (2H, d, *J*8.1, 2xAr H-3), 7.70 (1H, dd, *J*8.1, 2xAr H-2), 9.72 (1H, d, *J*3.0, CHO).

6.17.3 (2*S*, 3*R*, 4*R*)-1-(Toluene-4-sulfonyl)-4-hydroxy-2,3-(oxazin-2-one)-pyrrolidine 528

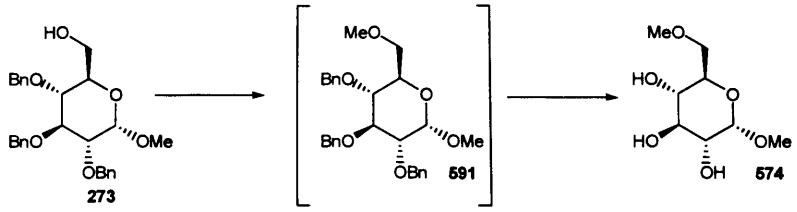
Triphosgene (0.0045 g, 0.015 mmol) was added to a 0 °C solution of the amine **554** (0.013 g, 0.045 mmol) in dichloromethane (0.1 ml). The solution was stirred at room temperature for 48 h before the reaction mixture was diluted with dichloromethane (1 ml) and washed with water (2 ml). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure. The resulting crude material was purified using flash column chromatography on silica (100% ethyl acetate) to yield the *cyclic carbamate* **528** (0.0040 g, 25%) as a white powder. δ_H (300 MHz; MeOD): 2.42 (3H, s, Ar 4-Me), 2.94 (1H, app td, *J*9.7, 5.0, H-2), 3.17 (1H, dd, *J*10.6, 7.2, *H*H-5), 3.43 (1H, dd, *J*10.6, 8.4, H*H*-5), 3.51 (1H, app t, *J*9.7, H-3), 3.71 (1H, ddd, *J*9.7, 8.4, 7.2, H-4), 4.39 (1H, app t, *J*9.7, C*H*HO), 4.70 (1H, dd, *J*9.7, 5.0, CH*H*O), 7.40 (2H, d, *J*8.1, 2xAr H-3), 7.66 (2H, d, *J*8.1, 2xAr H-2); δ_C (100.6 MHz; MeOD): 20.13 (CH_3), 54.98 (CH), 55.02 (CH_2), 60.28 (CH), 69.85 (CH), 71.12 (CH_2), 127.87 (2 × CH), 129.98 (2 × CH), 131.34 (C), 144.93 (C), 154.33 (C); (ES) m/z (%): 313 ($M+H^+$, 100%), 335 (15, $M+Na^+$).

6.17.4 (2*S*, 3*R*, 4*R*)-1-(Toluene-4-sulfonyl)-2,3-(oxazin-2-one)-4-hydroxyacetylpyrrolidine 573

Triethylamine (0.0014 g, 0.0019 ml, 0.014mmol) was added to a 0 °C solution of the cyclic carbamate **528** (0.004 g, 0.013 mmol) in dichloromethane (0.1 ml). The solution was stirred for 20 min before acetic anhydride (0.0013 g, 0.0018 ml, 0.019 mmol) and DMAP (0.73 mg, 0.64 μmol) were added and stirring continued for 18 h. The mixture was diluted with dichloromethane (1 ml) and washed with water (2 ml). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure. The resulting crude material was purified using flash column chromatography on silica (80:20, ethyl acetate: petroleum-ether) to give the *acetate* **573** (0.0029 g, 64%) as an off-white oil. δ_H (300 MHz; MeOD): 1.96 (3H, s, CH_3), 2.35 (3H, s, Ar 4-Me), 3.31 (1H, dd, *J*11.7, 3.6, *H*H-5), 3.59 (1H, dd, *J*11.7, 5.7, H*H*-5), 3.63-3.67 (1H, m, H-2), 3.85 (1H, app t, *J*4.2, H-3), 4.25 (2H, d, *J*6.0, CH_2O), 4.79-4.81 (1H, m obscured by HOD peak, H-4), 7.32 (2H, d, *J*8.1, 2xAr H-3), 7.63 (2H, d, *J*8.1, 2xAr H-2); (ES) m/z (%): 355 ($M+H^+$, 100%), 236 (99, M-($OCOCH_3$+OCONH)), 360 (51), 372 (28, $M+NH_4^+$)

6.18 Derivatives of the sugar moiety

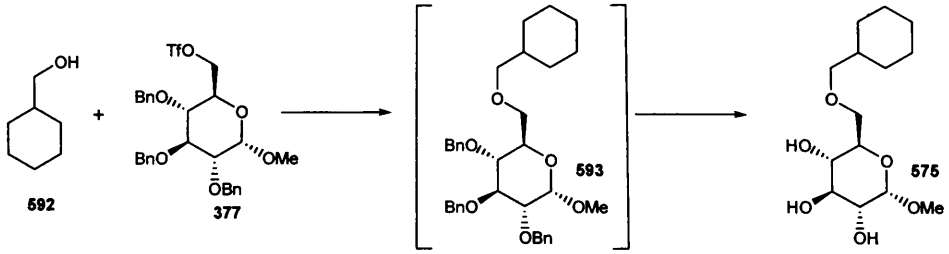
6.18.1 Methyl-[O^6-(methyl)-α-D-glucopyranoside] 574



The alcohol **273** (0.27 g, 0.57 mmol) dissolved in DMF (5 ml) was added dropwise to sodium hydride (0.033 g of a 60% dispersion in oil, 1.43 mmol) in DMF (1 ml) at 0 °C. The reaction was warmed to room temperature and stirred for 45 min before methyl iodide (0.16 g, 0.072 ml, 1.14 mmol) was transferred to the reaction mixture. The reaction stirred at room temperature for 24 h before a further portion of sodium hydride (0.044 g, 1.14 mmol) and methyl iodide (0.016 g, 0.072 ml, 1.14 mmol) was added. The solution was stirred a further 4 h before being quenched with saturated aqueous ammonium chloride (10 ml). The aqueous layer was washed with ethyl acetate (3x10 ml), the organic layers were combined, dried ($MgSO_4$) and solvent was removed under reduced pressure. The resulting crude product was purified by flash column chromatography on silica (20:80, ethyl acetate: petroleum-ether) to give the *methylated sugar moiety* **591** (0.12 g, 44%).

The methylated sugar moiety **591** (0.082g, 0.17 mmol) was dissolved in ethanol (9.0 ml) and acetic acid (1.26 ml) and was subjected to the general hydrogenolysis procedure (outlined in experiment 6.2.7) using $Pd(OAc)_2$ (0.034 g, 0.15 mmol). The reaction mixture was left stirring under a hydrogen atmosphere for 2 h before being filtered. The filtrate was concentrated under vacuum and the resulting crude product purified by flash column chromatography on silica (10:90, methanol: ethyl acetate) to give the *methylated sugar* **574** (0.031 g, 86%) as a colourless oil. $[\alpha]^{20}_D$: +112.6 (c=0.28 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3394br (OH), 1606s, 1053s, 739s; δ_H (400 MHz; D_2O): 3.34 (1H, app t, *J*7.5, H-4), 3.35 (3H, s, OMe), 3.37 (3H, s, OMe), 3.50 (1H, dd, *J*7.5, 2.7, H-2), 3.61 (1H, app t, *J*7.5, H-3), 3.62 (1H, dd, *J*8.4, 3.6, H-6), 3.65 (1H, dd, *J*8.4, 1.5, H-6), 3.69 (1H, ddd, *J*7.5, 3.6, 1.5, H-5), 4.74 (1H, d, *J*2.7, H-1); δ_C (100.6 MHz; D_2O): 55.19 (CH_3), 58.51 (CH_3), 69.66 (CH), 70.13 (CH), 70.98 (CH_2), 71.16 (CH), 72.99 (CH), 99.35 (CH); MS (ES) m/z (%): 226 ($M+NH_4^+$, 100%); Accurate mass (FAB): Found 209.1026 ($M+H^+$ $C_8H_{17}O_6$ requires 209.1025).

6.18.2 Methyl-[O^6-(cyclohexylmethoxy)-α-D-glucopyranoside] 575



The alcohol **592** (0.0092 g, 0.010 ml, 0.081 mmol) dissolved in THF (0.5 ml) was added to a 0 °C solution of sodium hydride (0.00033 g of a 60% dispersion in oil, 0.15 mmol) in THF (0.2 ml). The solution was stirred for 20 min before the triflate **377** (0.053 g, 0.1 mmol) dissolved in THF (0.5 ml) was added. The reaction mixture was stirred at 60 °C for 2 h before being quenched with saturated ammonium chloride (5 ml). Water (5 ml) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3x10 ml). The organic layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude mixture was purified by flash column chromatography on silica (15:85, ethyl acetate: petroleum-ether) to give the *cyclohexylmethoxy ether* **593** as a colourless oil (0.024 g, 44%).

The cyclohexylmethoxy ether **593** (0.022g, 0.039 mmol) was dissolved in ethanol (2 ml) and acetic acid (0.29 ml) and was subjected to the general hydrogenolysis procedure (outlined in experiment 6.2.7) using $Pd(OAc)_2$ (0.0079 g, 0.035 mmol). The reaction mixture was left stirring under a hydrogen atmosphere for 2 h before being filtered. The filtrate was concentrated under vacuum and the resulting crude product was purified by flash column chromatography on silica (100% ethyl acetate) to give the *alkylated sugar* **575** (0.0091 g, 83%) as a colourless oil. $[\alpha]^{20}_D$: +64.8 (c=0.13 MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1}: 3679br (OH), 3065s, 2928m, 2855m, 1601m, 1342w, 1259s, 1169w, 1114m, 1048m; δ_H (400 MHz; D_2O): 0.83-0.92 (2H, m, CH_2), 1.09-1.24 (3H, m, CH_2+C*H*H), 1.54-1.69 (4H, m, CH, C*H*H, CH_2), 3.29 (1H, dd, *J*7.5, 5.1, C*H*HO), 3.39 (1H, dd, *J*7.5, 6.9, CH*H*O), 3.37 (3H, s, OMe), 3.38 (1H, app t, *J*7.5, H-4), 3.50 (1H, dd, *J*7.5, 2.7, H-2), 3.60 (1H, app t, *J*7.5, H-3), 3.58-3.63 (1H, m overlapping, H-6), 3.67-3.74 (2H, m, H-5, H-6), 4.73 (1H, d, *J*2.7, H-1); δ_C (100.6 MHz; D_2O): 25.32 (2 × CH_2), 26.14 (2 × CH_2), 29.39 (CH_2), 29.29 (CH_2), 37.02 (CH), 55.17 (CH_3), 69.35 (CH), 69.85 (CH), 70.35 (CH_2), 71.14 (CH), 73.11 (CH), 77.25 (CH_2), 99.27 (CH); MS (ES) m/z (%): 308 ($M+NH_4^+$, 100%), 598 (63, $2M+NH_4^+$), 291 (18, $M+H^+$); Accurate mass (FAB): Found 291.1808 ($M+H^+$ $C_{14}H_{27}O_6$ requires 291.1808).

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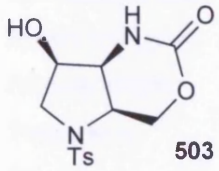
Appendix

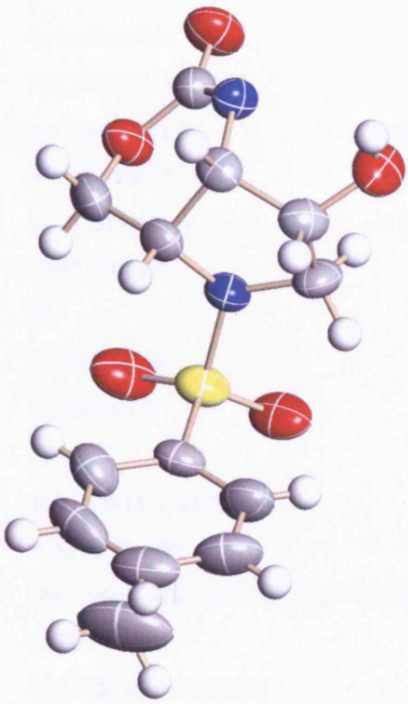
SPECIAL NOTE

ITEM SCANNED AS SUPPLIED
PAGINATION IS AS SEEN

X-Ray Structures



503



There are two unique molecules in the unit cell, the difference between them is minimal as shown in the fit drawing below. There is also a disordered solvent molecule that is not resolved.

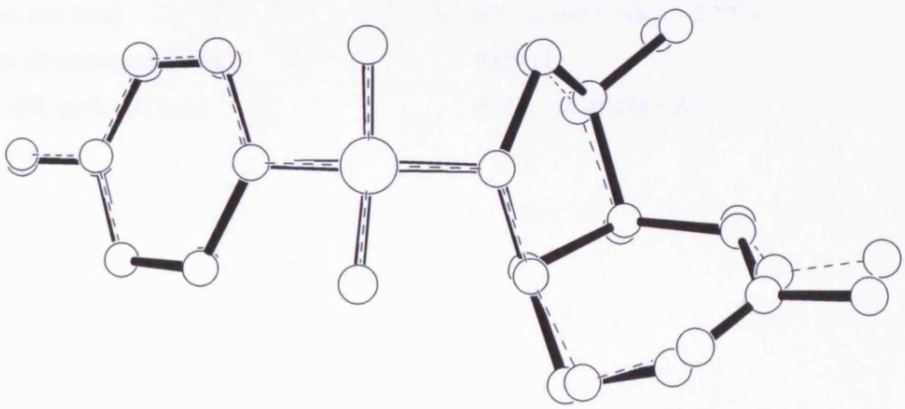
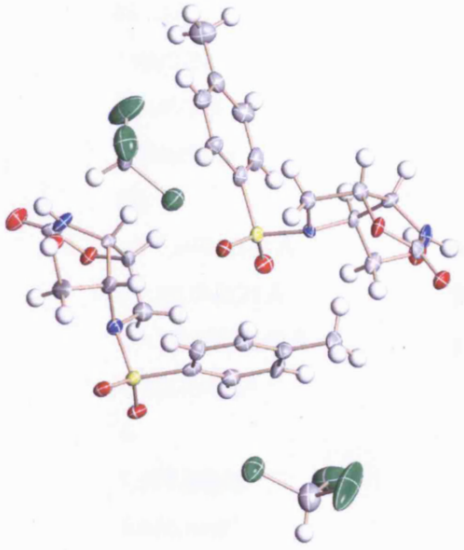


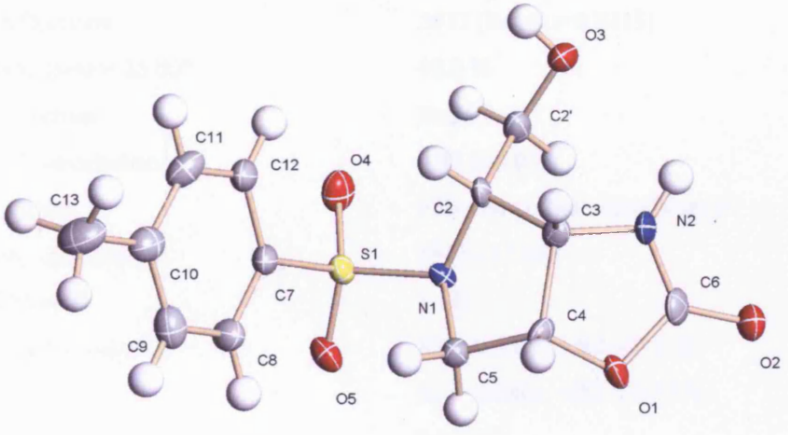
Table 1. Crystal data and structure refinement for **503**.

Identification code	03061	
Empirical formula	C13 H15 N2 O5 S	
Formula weight	311.33	
Temperature	290(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 5.6923(7) Å	α= 90°.
	b = 15.7807(17) Å	β= 90°.
	c = 33.089(4) Å	γ = 90°.
Volume	2972.3(6) $Å^3$	
Z	8	
Density (calculated)	1.391 Mg/m^3	
Absorption coefficient	0.240 mm^{-1}	
F(000)	1304	
Crystal size	0.30 x 0.18 x 0.03 mm^3	
Theta range for data collection	1.43 to 25.00°.	
Index ranges	-6<=h<=6, -18<=k<=18, -39<=l<=39	
Reflections collected	21703	
Independent reflections	5232 [R(int) = 0.0577]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5232 / 0 / 401	
Goodness-of-fit on F^2	0.986	
Final R indices [I>2sigma(I)]	R1 = 0.0582, wR2 = 0.1165	
R indices (all data)	R1 = 0.0833, wR2 = 0.1274	
Absolute structure parameter	0.05(11)	
Largest diff. peak and hole	0.252 and -0.284 $e.Å^{-3}$	

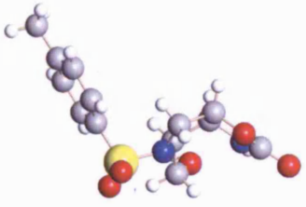
504



The asymmetric unit has two unique molecules (=two unique $CHCl_3$) the unique molecules are different only in slight orientation of the TsN group



Labelled drawing showing 50% displacement ellipsoids



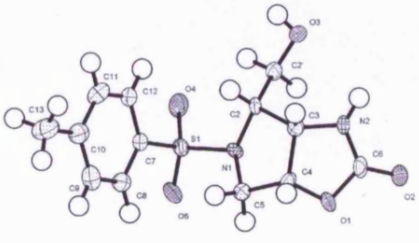
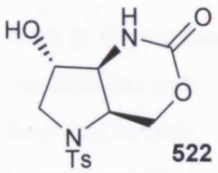


Table 2. Crystal data and structure refinement for **504**.

Identification code	03121	
Empirical formula	C14 H17 Cl3 N2 O5 S	
Formula weight	431.71	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 7.5405(10) Å	α= 90°.
	b = 23.062(3) Å	β= 90.174(2)°.
	c = 10.2684(14) Å	γ = 90°.
Volume	1785.7(4) $Å^3$	
Z	4	
Density (calculated)	1.606 Mg/m^3	
Absorption coefficient	0.658 mm^{-1}	
F(000)	888	
Crystal size	0.09 x 0.15 x 0.19 mm^3	
Theta range for data collection	1.77 to 25.00°.	
Index ranges	-8<=h<=8, -27<=k<=26, -12<=l<=12	
Reflections collected	12999	
Independent reflections	5937 [R(int) = 0.0413]	
Completeness to theta = 25.00°	99.8 %	
Absorption correction	Empirical	
Max. and min. transmission	0.93 and 0.76	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5937 / 1 / 455	
Goodness-of-fit on F^2	1.121	
Final R indices [I>2sigma(I)]	R1 = 0.0813, wR2 = 0.2133	
R indices (all data)	R1 = 0.0865, wR2 = 0.2176	
Absolute structure parameter	0.12(12)	
Largest diff. peak and hole	1.301 and -0.605 $e.Å^{-3}$	



522

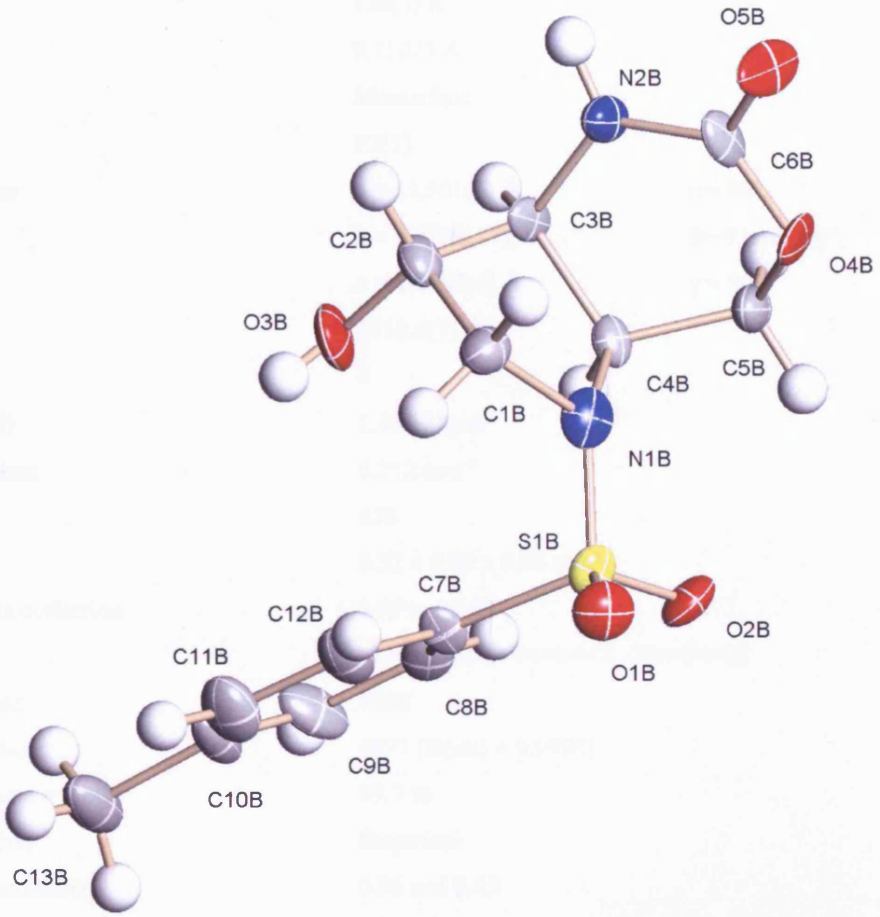
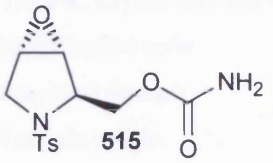


Fig shows one of the unique molecules with the atom label scheme and 50% displacement ellipsoids

Table 3. Crystal data and structure refinement for **522**.

Identification code	03181	
Empirical formula	C13 H16 N2 O5 S	
Formula weight	312.34	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 11.501(3) Å	α= 90°.
	b = 6.2268(17) Å	β= 91.192(5)°.
	c = 19.785(6) Å	γ = 90°.
Volume	1416.6(7) $Å^3$	
Z	4	
Density (calculated)	1.464 Mg/m^3	
Absorption coefficient	0.252 mm^{-1}	
F(000)	656	
Crystal size	0.32 x 0.09 x 0.03 mm^3	
Theta range for data collection	1.77 to 25.00°.	
Index ranges	-13<=h<=13, -7<=k<=7, -23<=l<=23	
Reflections collected	9300	
Independent reflections	4877 [R(int) = 0.0988]	
Completeness to theta = 25.00°	99.7 %	
Absorption correction	Empirical	
Max. and min. transmission	0.96 and 0.45	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4877 / 31 / 383	
Goodness-of-fit on F^2	1.010	
Final R indices [I>2sigma(I)]	R1 = 0.1071, wR2 = 0.2389	
R indices (all data)	R1 = 0.1435, wR2 = 0.2614	
Absolute structure parameter	0.12(19)	
Largest diff. peak and hole	0.688 and -0.522 $e.Å^{-3}$	



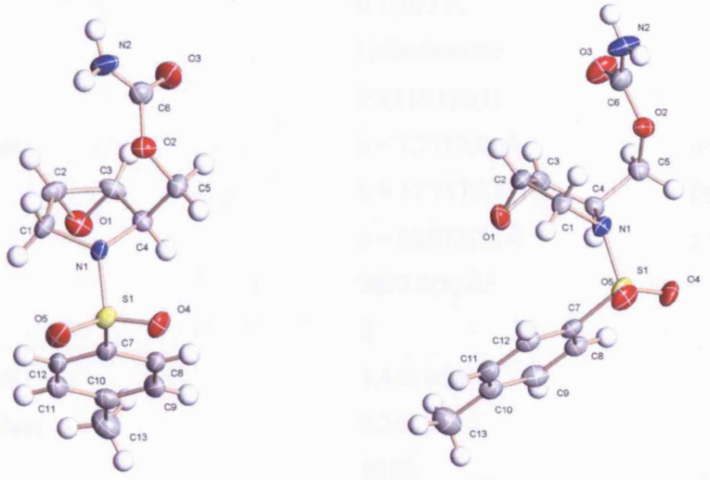


Fig 1 shows two views of one of the two unique molecules in the asymmetric unit with the atom label scheme and 50% displacement ellipsoids

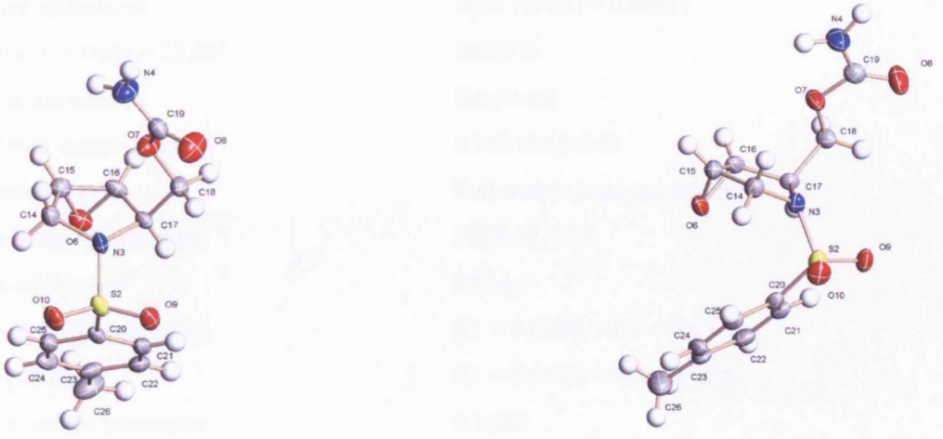
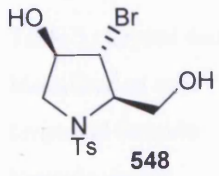


Fig 2 shows two views of the other unique molecules in the asymmetric unit

Table 4. Crystal data and structure refinement for **515**.

Identification code	04002	
Empirical formula	C13 H16 N2 O5 S	
Formula weight	312.34	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 7.7577(8) Å	α= 90°.
	b = 17.7377(19) Å	β= 90°.
	c = 20.872(2) Å	γ = 90°.
Volume	2872.0(5) $Å^3$	
Z	8	
Density (calculated)	1.445 Mg/m^3	
Absorption coefficient	0.249 mm^{-1}	
F(000)	1312	
Crystal size	0.39 x 0.10 x 0.03 mm^3	
Theta range for data collection	1.51 to 25.00°.	
Index ranges	-9<=h<=9, -21<=k<=20, -24<=l<=24	
Reflections collected	20984	
Independent reflections	5053 [R(int) = 0.0491]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	Empirical	
Max. and min. transmission	0.962 and 0.848	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5053 / 0 / 379	
Goodness-of-fit on F^2	0.914	
Final R indices [I>2sigma(I)]	R1 = 0.0384, wR2 = 0.0673	
R indices (all data)	R1 = 0.0482, wR2 = 0.0699	
Absolute structure parameter	0.12(6)	
Largest diff. peak and hole	0.343 and -0.316 $e.Å^{-3}$	



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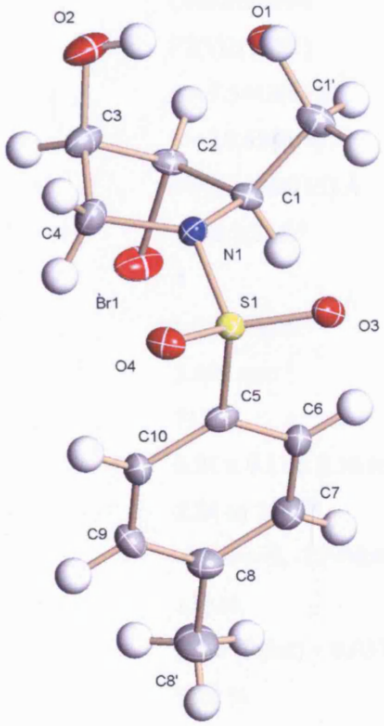


The molecular structure with the atom label scheme and 50% displacement ellipsoids

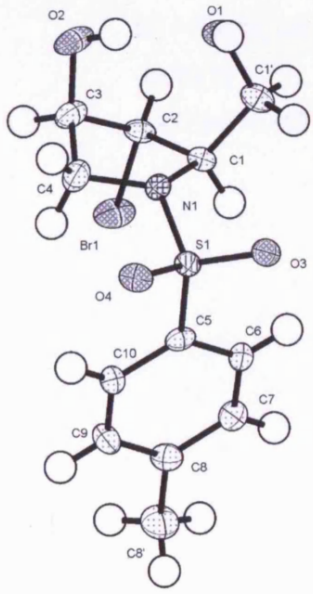
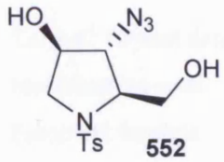
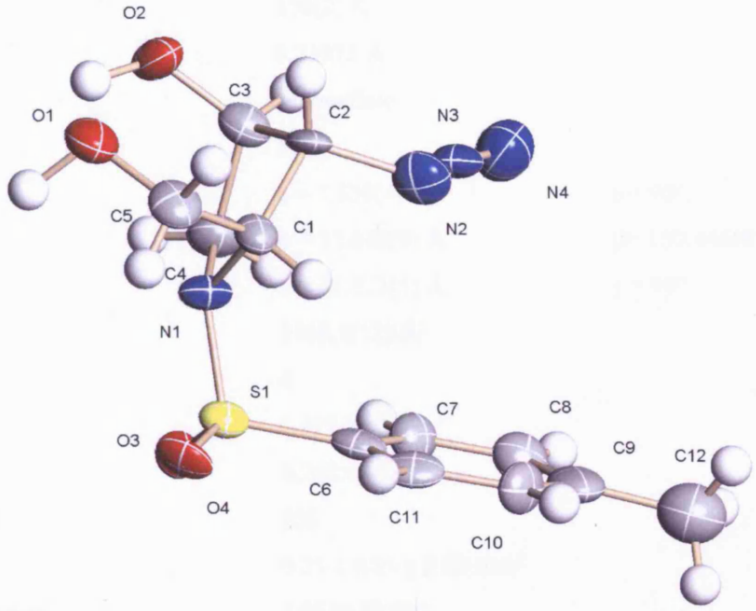


Table 5. Crystal data and structure refinement for **548**.

Identification code	04058	
Empirical formula	C12 H16 Br N O4 S	
Formula weight	350.23	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 7.5443(6) Å	α= 90°.
	b = 10.6588(9) Å	β= 90°.
	c = 17.4366(15) Å	γ = 90°.
Volume	1402.1(2) $Å^3$	
Z	4	
Density (calculated)	1.659 Mg/m^3	
Absorption coefficient	3.089 mm^{-1}	
F(000)	712	
Crystal size	0.21 x 0.17 x 0.16 mm^3	
Theta range for data collection	2.24 to 25.99°.	
Index ranges	-9<=h<=9, -13<=k<=13, -21<=l<=21	
Reflections collected	11010	
Independent reflections	2752 [R(int) = 0.0337]	
Completeness to theta = 25.99°	99.9 %	
Absorption correction	Empirical	
Max. and min. transmission	0.87 and 0.76	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2752 / 0 / 175	
Goodness-of-fit on F^2	1.063	
Final R indices [I>2sigma(I)]	R1 = 0.0255, wR2 = 0.0657	
R indices (all data)	R1 = 0.0273, wR2 = 0.0661	
Absolute structure parameter	-0.003(7)	
Largest diff. peak and hole	0.455 and -0.482 $e.Å^{-3}$	



552



Figs show the two unique molecules with the atom label scheme and 50% displacement ellipsoids

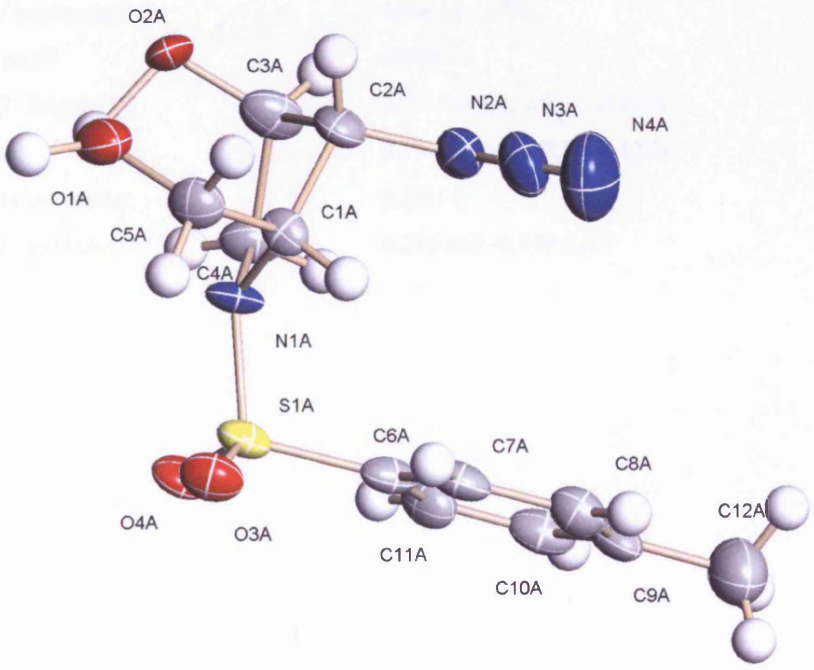
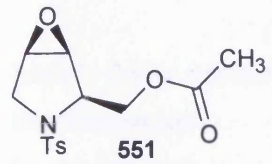
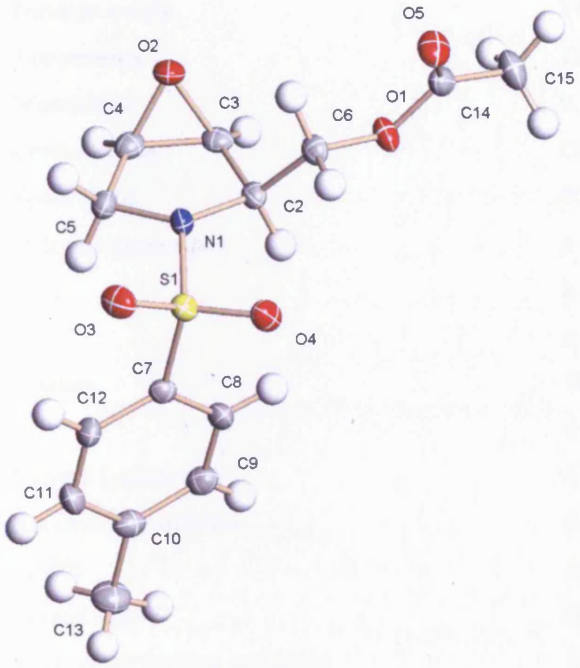


Table 6. Crystal data and structure refinement for **552**.

Identification code	04121	
Empirical formula	C12 H16 N4 O4 S	
Formula weight	312.35	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 7.539(4) Å	α= 90°.
	b = 17.643(9) Å	β= 100.446(8)°.
	c = 11.223(5) Å	γ = 90°.
Volume	1468.1(12) Å^3	
Z	4	
Density (calculated)	1.413 Mg/m^3	
Absorption coefficient	0.242 mm^{-1}	
F(000)	656	
Crystal size	0.21 x 0.11 x 0.03 mm^3	
Theta range for data collection	1.85 to 23.00°.	
Index ranges	-8<=h<=8, -19<=k<=19, -12<=l<=12	
Reflections collected	8666	
Independent reflections	4086 [R(int) = 0.1143]	
Completeness to theta = 23.00°	100.0 %	
Absorption correction	Empirical	
Max. and min. transmission	0.95 and 0.63	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4086 / 1 / 379	
Goodness-of-fit on F^2	0.958	
Final R indices [I>2sigma(I)]	R1 = 0.0684, wR2 = 0.1231	
R indices (all data)	R1 = 0.1147, wR2 = 0.1376	
Absolute structure parameter	0.19(14)	
Largest diff. peak and hole	0.210 and -0.349 e.Å^{-3}	



551



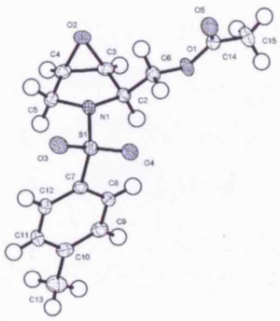


Fig shows the atom label scheme and 50% displacement ellipsoids

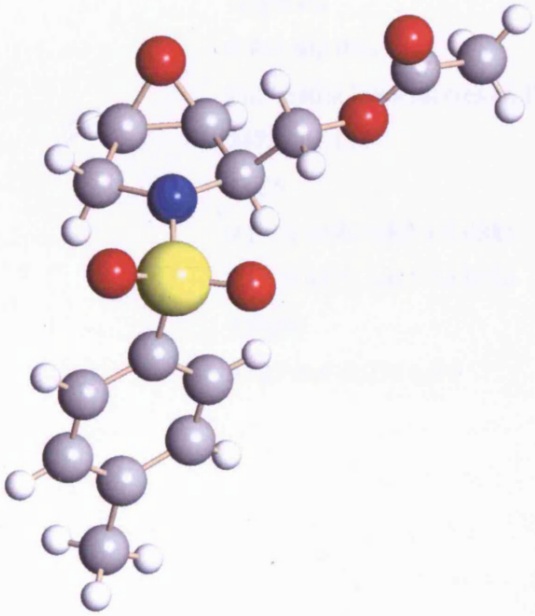


Table 7. Crystal data and structure refinement for **551**.

Identification code	04127	
Empirical formula	C14 H17 N O5 S	
Formula weight	311.35	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 6.822(5) Å	α= 90°.
	b = 8.603(6) Å	β= 90°.
	c = 24.035(16) Å	γ = 90°.
Volume	1410.5(17) $Å^3$	
Z	4	
Density (calculated)	1.466 Mg/m^3	
Absorption coefficient	0.251 mm^{-1}	
F(000)	656	
Crystal size	0.31 x 0.09 x 0.09 mm^3	
Theta range for data collection	1.69 to 24.99°.	
Index ranges	-8<=h<=8, -10<=k<=10, -28<=l<=28	
Reflections collected	10083	
Independent reflections	2472 [R(int) = 0.0601]	
Completeness to theta = 24.99°	99.8 %	
Absorption correction	Empirical	
Max. and min. transmission	0.962 and 0.815	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2472 / 0 / 192	
Goodness-of-fit on F^2	1.055	
Final R indices [I>2sigma(I)]	R1 = 0.0390, wR2 = 0.0881	
R indices (all data)	R1 = 0.0423, wR2 = 0.0914	
Absolute structure parameter	0.04(9)	
Largest diff. peak and hole	0.227 and -0.274 $e.Å^{-3}$	

Enzyme Bioassay Method

Experiments undertaken by E. Evinson at the Institute of Grassland Research, Aberystwyth

The basis for the majority of the glucosidase assays is the reaction between the enzyme solution and the selected substrate, causing the release of the *para*-nitrophenol conjugated group, which has a yellow colour in solution that can be quantified spectrophotometrically. The amyloglucosidase assay works on the principle that glucose monomers are released from starch chains in the presence of the enzyme – glucose is then detected using Trinder glucose reagent, which gives a red colour when reacted with glucose solution. In both assays, the extent of enzyme inhibition is measured with respect to water or the sample solvent, which is used as a blank (assume 0% inhibition with dH_2O).

Enzyme	Source	pH	Conc. (U/ml)	Substrate
α-D-glucosidase	*Saccharomyces cerevisiae*	6.0	1.5	PNP-α-D-glucopyranoside
α-D-glucosidase	*Bacillus sterothermophilus*	6.8	5.0	PNP-α-D-glucopyranoside
α-D-glucosidase	Rice (*Oryzae sativa*)	4.0	5.0	PNP-α-D-glucopyranoside
β-D-glucosidase	Almond (*Prunus* sp.)	5.0	0.2	PNP-β-D-glucopyranoside
α-D-galactosidase	Green coffee beans (*Coffea* sp.)	6.5	0.25	PNP-α-D-galactopyranoside
β-D-galactosidase	Bovine liver	7.3	0.2	PNP-β-D-galactopyranoside
α-D-mannosidase	Jack bean (*Canavalia ensiformis*)	4.5	0.2	PNP-α-D-mannopyranoside
α-L-fucosidase	Bovine kidney	5.5	0.5	PNP-α-L-fucopyranoside
Naringinase	*Penecillium decumbens*	4.0	1.0	PNP-α-D-rhamnopyranoside
N-acetyl-β-D-glucosaminidase	Bovine kidney	4.25	0.2	PNP-N-acetyl-β-D-glucosaminide
N-acetyl-β-D-glucosaminidase	Jack bean (*Canavalia ensiformis*)	7.0	0.25	PNP-N-acetyl-β-D-glucosaminide
N-acetyl-β-D-hexosaminidase	*Aspergillus oryzae*	5.0	0.25	PNP-N-acetyl-β-D-glucosaminide
Amyloglucosidase	*Aspergillus niger*	4.5	0.5	1% soluble starch & Trinder reagent

All enzymes and substrate compounds were purchased from Sigma (including the Trinder reagent). Substrate solutions were made up to 5 m*M* concentration using McIlvaine citrate-phosphate buffer, at the optimum pH for the enzyme as suggested by the supplier. Once made up, all solutions were stored at 4 °C and discarded after a month if not used and they were allowed to warm up to room temperature before use. The Trinder glucose detection reagent (required for the amyloglucosidase assay) was diluted in 100 ml of dH_2O before use

and was stored and handled as suggested by the supplier. The starch solution was made using McIlvaine buffer, pH 4.5 – the solution sometimes needed to be boiled or autoclaved to get the starch into solution (it makes a cloudy, thinly-gelatinous solution on heating, that remains stable but needs mixing well after refrigeration). The other assays also require 0.4*M* glycine solution, pH 10.4, which was made using dH_2O and pH-adjusted using NaOH pellets.

The assay method is designed for use with 96-well microtitre plates, which are read using a microplate reader at 405 nm (550 nm for the amyloglucosidase assay). All samples (and blanks) were assayed in triplicate.

Method (all assays except amyloglucosidase)

The following were combined (in this order):

10 µl enzyme solution

10 µl sample solution/water/sample solvent (containing 10µg sample)

50 µl substrate solution

The reaction mixture was incubated at room temperature – the length of time required depending on the concentration and activity of the enzyme (usually 5 – 20 minutes). In practice, the enzyme solutions were tested when made up to determine the time needed to give approximately 0.5 – 1.0 U absorbance at the end of the reaction period (after the glycine solution had been added). When the reaction period was complete, 70 µl glycine solution was added, and the absorbance was measured at 405 nm.

Amyloglucosidase assay

This assay follows the same method as the other assays (see above), except 100 µl of Trinder solution was added at the end of the reaction period. The colour reaction needed at least 10 minutes to complete at room temperature, after which the absorbance was read at 550 nm.

Calculating % inhibition

Enzyme inhibition was calculated with respect to the blank samples:

First, the mean of the triplicate assays per sample was calculated.

Then, % inhibition = (100 - [(Mean absorbance sample / Mean absorbance blank) x 100])