Studies on Ring Closure Heterometathesis And the Synthesis of Novel Glycosidase Inhibitors

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By

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Statement

The accompanying thesis submitted for the degree of Ph.D. entitled "Studies on Ring Closure Heterometathesis and the Synthesis of Novel Glycosidase Inhibitors" is based on work conducted by the author in the Department of Chemistry at the University of Leicester between the period October 1999 to November 2004.

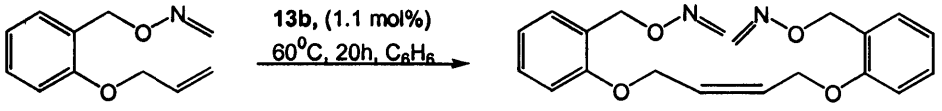
All the work recorded in this thesis is original unless otherwise acknowledged in the text or references. None of the work has been submitted for another degree in this or any other university.

Signed:  Date: .....

Abstract

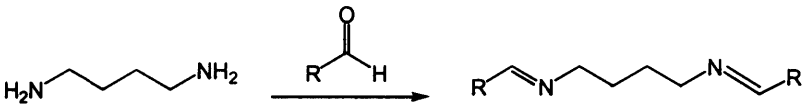
Studies on Ring Closure Heterometathesis and the Synthesis of Novel Glycosidase Inhibitors by Vanessa L. Maxwell

Compound **I** was synthesised to study intramolecular ring closing metathesis of an oxime and an olefin. In the event an intermolecular reaction took place to give **II**



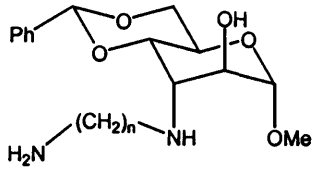
I II

A series of bis imines **IV** were synthesised and also subjected to ring closure metathesis conditions using the Grubbs catalyst. No cyclisation was observed.



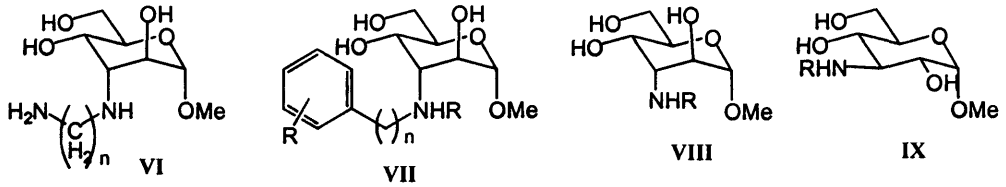
III IV

Three diamino sugar derivatives **V** were prepared by epoxide opening of the corresponding 2,3 manno (up) epoxide. Benzylidene deprotection was not possible.



V n =2, 3, 4

A further six examples of deprotected diamino altro derivatives **395b-g** were prepared and tested for glycosidase inhibition and the best results were obtained when n = 4 and n =5 where IC_{50} values gave 0.15 mM and 0.63 mM respectively for β-galactosidase from bovine liver. Nine examples of the benzylamino altro derivatives were prepared and tested for glycosidase inhibition. IC_{50} values obtained gave 70 μM and 90 μM where n =3, R = H and n = 4, R = H respectively for β-galactosidase from bovine liver.



VI VII VIII IX

Opening the manno epoxide produced seven examples and the structure of the products were proved with an X-ray structure where R = morpholine. The stereochemistry of the other examples were assigned following ^{13}C comparison. By contrast opening of the allo epoxide gave attack at C-3 and this fact was determined by an X-ray structure on R = furanyl. ^{13}C comparison indicated that the other examples had followed attack at C-3.

Acknowledgements

First and foremost I would like to thank my Lord and Saviour; through him all things are possible. I would like to give a special thank you to my supervisor Dr. Paul Jenkins for his assistance, encouragement and guidance. I also wish to thank Merck Sharp and Dohme Research Laboratories for their funding. Two other members of staff who deserve thanks are Dr. Sandeep Handa and Dr. Steve Baker who have gone out of their way to encourage and teach.

I am grateful to all my friends and colleagues past (Will, Mel, Simon and Mona) and present (Manny and the usual suspects), for the exceptional atmosphere within the lab, promoting hard work in a very relaxed and friendly environment. I am also indebted to all the technical staff, as without them nothing would ever get done!!

Finally I would like to thank my mother and my father(s) for their unconditional love and the Woodburn family for their support.

If it moves, it's biology. If it stinks, it's chemistry. If it doesn't work, it's physics. ---Unknown/Anonymous

To my dearest mother – I love you

Contents

Abbreviations

ADMET	acyclic diene metathesis
AIDS	Acquired Immuno Deficiency Syndrome
Bn	benzyl
BOC	*t*-butoxycarbonyl
Bzl	benzoyl
Cy	cyclohexyl
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DEAD	diethylazodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
Ee	enantiomeric excess
EI	electron ionisation
ES	electrospray
FAB	fast atom bombardment
GLC	gas liquid chromatography
HMPA	hexamethylphosphoric triamide
HPLC	high performance liquid chromatography
IPA	propan-2-ol
J	coupling constant
LTA	lead tetraacetate
Ms	methane sulfonyl
NMO	*N*-morpholine oxide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
PCC	pyridinium chlorochlorate
PDC	pyridinium dichromate
PMB	*para*-methoxybenzyl
PPTS	pyridinium *p*-toluenesulfonate
RCM	ring closing metathesis
ROMP	ring opening metathesis polymerisation
RSA	retro synthetic analysis

Rt	room temperature
TBDMS	tert-butyldimethylsilyl
TBDPD	tert-butyldiphenylsilyl
TBS	tributylsilyl
TFAA	trifluroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
TPP	triphenylphosphine
Ts	para-toluenesulfonyl

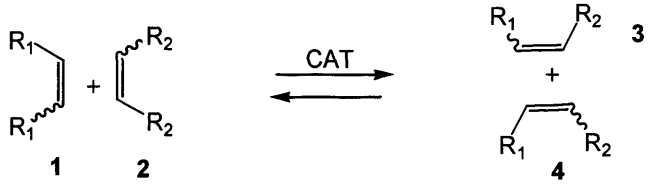
Chapter One

Hetero-Metathesis

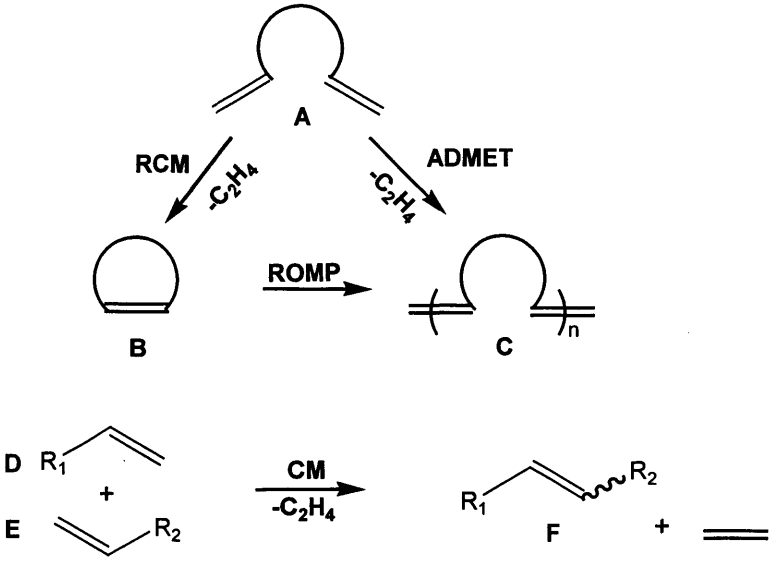
1.1 Introduction

Reliable methods for the formation of carbon-carbon bonds are the most important reactions used in organic synthesis. Olefin metathesis is becoming one of the most useful and frequently applied transformations in organic chemistry giving synthetic chemists a new route to synthesising carbon-carbon double bonds.

Olefin metathesis is the metal-catalysed redistribution of carbon-carbon double bonds. In other words, the metathesis constitutes a catalytic method for both cleaving and forming C-C double bonds, by the conversion of olefins **1** and **2** into olefins **3** and **4**.



Several fundamental types of metathesis reactions for monoolefins or diolefins are shown in Scheme 1. Diene **A** can undergo ring-closing metathesis (RCM) to form the cyclic alkene **B** with the release of ethene. This type of metathesis has received a great deal of attention for the synthesis of medium or large sized rings[1]. The ring-closed product **B** can undergo ring-opening metathesis polymerisation (ROMP), which involves the metathetic opening of strained cyclic olefins to give polymeric compounds of the type **C**. If the substrate is a diene then **C** can be formed via acyclic diene metathesis polymerisation (ADMET). Intermolecular olefin metatheses between two alkenes **D**+**E** undergo cross metathesis (CM) to form a new olefinic product **F**.



Scheme 1: General types of olefin metathesis

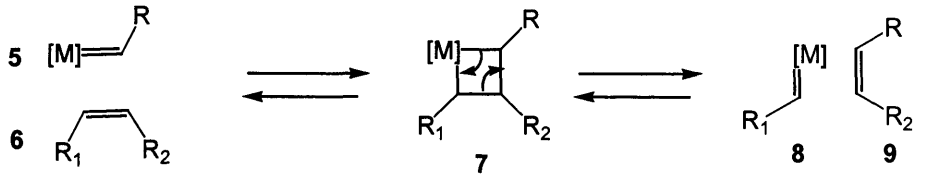
CM, ADMET and ROMP were the first examples of metathesis studied. The catalysts used in these reactions were then applied to ring closure reactions and eventually improved catalyst systems for these reactions were developed.

1.2 Background and Catalyst Systems

In this review we will cover early studies on polymerisation metathesis in general and then concentrate on recent work involving RCM.

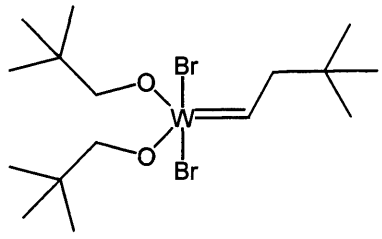
1.2.1 Early Studies on Metathesis Reactions

Anderson and Merckling first put the concept of olefin metathesis forward in 1955. They described the polymerisation of norbornene by titanium (II) species formed *in situ*.[2] 1964 saw Natta and co-workers describe the ring opening polymerisation of cyclic olefins by homogeneous catalysts.[3] Calderon *et al.*[4] and Mol *et al.*[5] who observed an exchange of alkylidene groups during the metathesis of labelled olefins carried out many studies which contributed to the understanding of olefin metathesis reactions over the years, but it was the scheme developed by Chauvin that was found to most consistent with experimental evidence.[6] Chauvin states that olefin metathesis proceeds by a [2+2] cycloaddition between a metal carbene **5** and a C-C double bond **6** to produce a metallocyclobutane **7** which breaks down by cycloreversion to give carbene **8** and olefin **9**.



Chauvin's mechanistic explanation of olefin metathesis influenced work on catalyst development in that it provided both a design rationale and a way to begin to understand catalyst activity. Efforts to synthesise alkylidene and metallacyclobutane complexes led to the discovery of the first single component homogeneous catalyst during the 1980s and 1990s. These new catalysts included bis (cyclopentadienyl)titanocyclobutanes,[7] various dihalo – alkoxide – alkylidene complexes of tungsten **10** and **11** developed by Osborn[8] and Schrock[9] respectively in 1988 by the thermal abstraction of an α-hydrogen from the alkyl moiety. Further work by Schrock and co-workers introduced the alkylidene-molybdenum catalyst **12**[10]. Shortly after this Grubbs

et al. described the preparation of ruthenium-alkylidene catalysts such as **13a**[11] from the addition of diphenylcyclopropene to $RuCl_2(PPh_3)_3$ and **13b**[12] from phenyl diazomethane and $RuCl_2(PPh_3)$.



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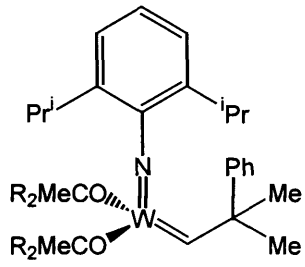
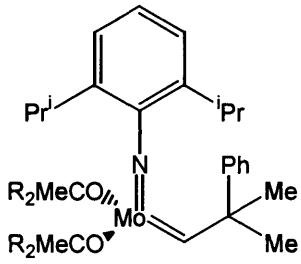
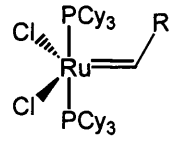


11a, R = Me
11b, R = CF_3



12a, R = Me
12b, R = CF_3



13a, R = $CH{=}CPh_2$
13b, R = Ph

The development of catalysts that react preferentially with olefins in the presence of other functional groups is imperative. Functional groups in the substrate or solvent can interfere with catalytic activity in different ways. These include binding competitively to the active metal centre and deactivating the catalyst, or reacting directly with the metal centre and destroying the active species. The advent of single component catalysts allowed the relationships between structure and reactivity to be more clearly defined. The catalysts were observed to react more selectively with olefins as the metal centres varied from left to right and bottom to top on the periodic table. This trend is portrayed for titanium, tungsten, molybdenum and ruthenium in Table 1.[13]

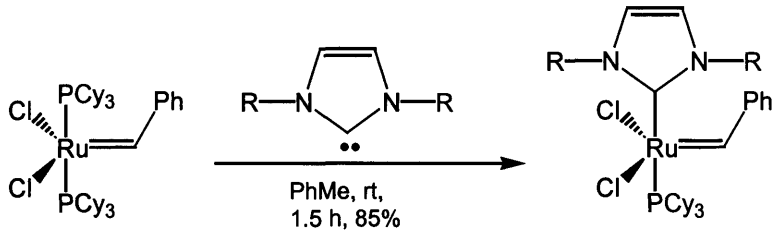
Titanium	**Tungsten**	**Molybdenum**	**Ruthenium**
Acids	Acids	Acids	**Olefins**
Alcohols, Water	Alcohols, Water	Alcohols, Water	Acids
Aldehydes	Aldehydes	Aldehydes	Alcohols, Water
Ketones	Ketones	**Olefins**	Aldehydes
Esters, Amides	**Olefins**	Ketones	Ketones
Olefins	Esters, Amides	Esters, Amides	Esters, Amides

Increasing reactivity

Table 1: Functional group reactivity of early and late transition metal olefin catalysts.

Titanium and Tungsten catalysts (Table 1) will readily olefinate ketones and esters, whereas molybdenum is more reactive towards olefins, even though they react with aldehydes and other polar or protic groups. Ruthenium on the other hand reacts preferentially with carbon-carbon double bonds over most other species.

A new family of ruthenium-based complexes **13c**[14] have been prepared starting from **13b**. These air- and water-tolerant complexes have been shown to exhibit an increased ring-closing metathesis at elevated temperatures when compared to that of the parent complex **13b** and the previously developed complex **13a**.

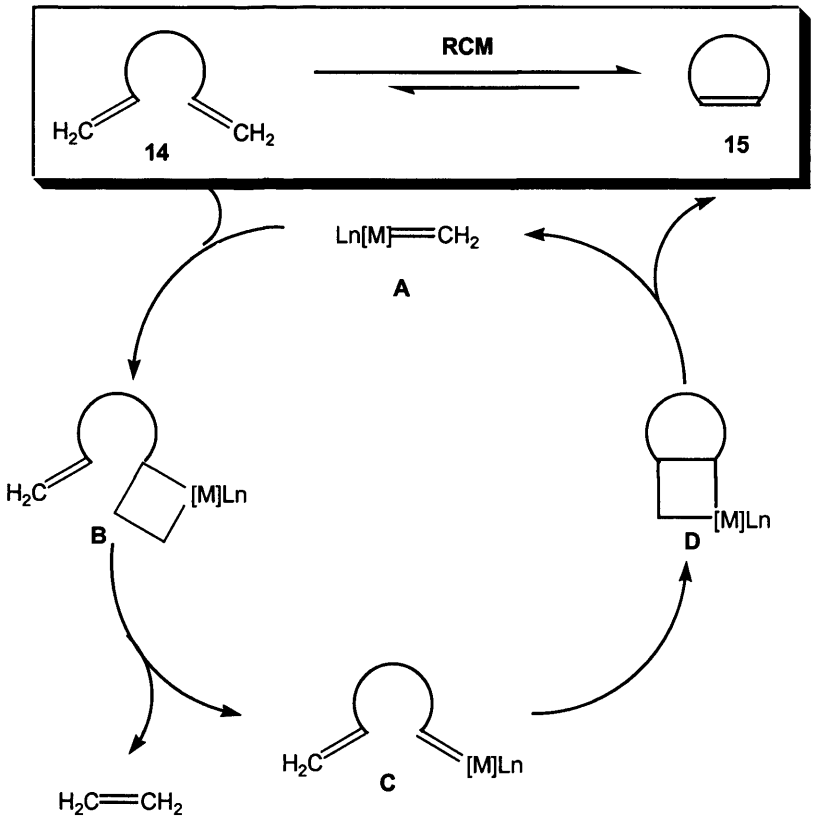


13b **13c**

R = C_6H_2-2,4,6-$(CH_3)_3$

1.3 Ring Closing Metathesis

Among all the categories shown in Scheme 1, RCM reactions, which lead to cyclic products, have found the widest application in synthesis. An extension of the generally accepted Chavin mechanism for olefin metathesis, portrays how an RCM reaction proceeds *via* a sequence of alternating [2+2] cycloadditions and cycloreversions between the metal alkylidene and a metallocyclobutane species (Scheme 2).



Scheme 2

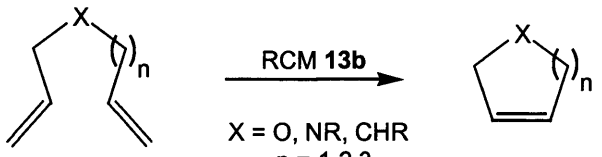
Catalyst **A** undergoes a [2+2] cycloaddition between its alkylidene moiety and a terminal olefin **14** to give a metallocyclobutane **B**. Cycloreversion affords metal-alkylidene **C** with the generation of ethene. Another [2+2] cycloaddition takes place to give the ring closed intermediate **D**, which undergoes a cycloreversion to give the cyclic olefin **15** and regeneration of the catalytic species.

The above cycle of any RCM reactions are in principle reversible. The overall process is useful in preparative terms due to the following:

- The forward reaction is entropically favourable since RCM cuts one molecule into two.
- The release of the volatile by-product ethene helps shifts the equilibrium towards the cycloalkene.
- The reverse reaction is kinetically hindered if the product has a more highly substituted double bond than the substrate, because most catalysts are sensitive to steric factors.
- ADMET can compete with RCM of a diene substrate, but is reduced if the reaction is performed at high dilution.[15]

1.3.1 Synthesis of Medium Sized (5-9) Rings

Even though the first example of RCM was reported by Tsuji,[16] its use as a effective tool can be followed through a series of papers in the early 1990s which saw Grubbs and co-workers[17] display ring closing of dienes to produce five-, six-, and seven-membered rings **17**. Using catalyst **13b**, they were able to promote cyclisation of a number of dienes **16** which contained a range of functional groups.



16 **17**

The synthesis of seven- and in particular, eight-membered rings from open chain precursors *via* RCM was a challenging extension of previous RCM reactions. Factors contributing to this inherent problem may be the competing metathesis based polymerisation of reactants and or products and to the strain in many eight membered rings.[17] Table 2 shows a series of metathesis reactions leading to eight-membered rings. It is clear from the cyclisations of **18** and **22** that RCM is favoured when the *trans* fused product is formed. The analogous products **21** and **25** are less favoured due to the increased strain of the *cis* fused system. A significant amount of side products resulting from intermolecular metathesis in addition to starting material was recovered.

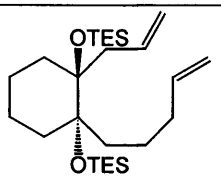
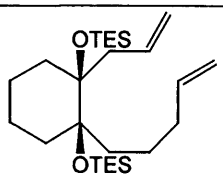
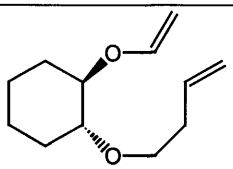
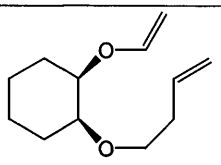
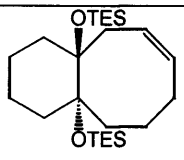
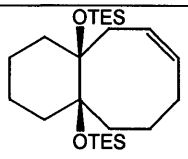
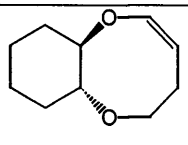
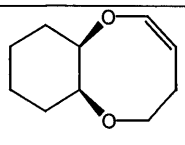
Substrate	 **18**	 **20**	 **22**	 **24**
Product	 **19**	 **21**	 **23**	 **25**
Conditions **Yield**	5% mol **13b**, 4h, 25°C, C_6H_6, 75%	5% mol **13b**, 20h, 25°C, C_6H_6, 33%	5% mol **13b**, 2h, 55°C, C_6H_6, 60%	5% mol **13b**, 2h, 55°C, C_6H_6, 20%

Table 2: Catalytic RCM synthesis of eight-membered rings

Further investigation by Grubbs and co-workers uncovered a limitation to the Ru based catalyst **13b**. Table 3 shows comparative activity of **13b** with Schrock's Mo catalyst **12b** when cyclising highly substituted dienes to achieve tri- and tetra-substituted

olefins.[18] The introduction of a conformational constraint in the form of different substituents leads to some conformations no longer being accessible as shown by the substrates in Table 3. This is known as the Thorpe Ingold effect and greatly enhances the ability of such systems to undergo the desired cyclisation.[15] Even though both catalysts are able to bring about the tri-substituted products **27** in excellent yield, the Ru catalyst **13b** was not able to ring close the bulky *gem* di-substituted dienes **28** and **30**. The ruthenium catalyst is able to compensate for its lower reactivity by an increased tolerance towards functional groups compared to that of molybdenum.

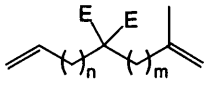
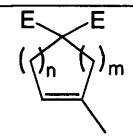
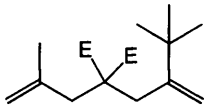
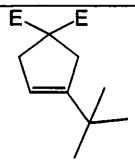
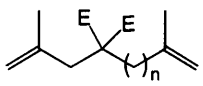
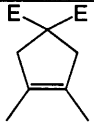
Substrate		**Product**		**Yield %**	
				12b	**13b**
	**26a**, n=1,m=1 **26b**, n=1, m=2 **26c**, n=2, m=2		**27a** **27b** **27c**	100 100 100	93 97 96
	**28**		**29**	96	0
	**30a** **30b**		**31a** **31b**	93 61	0 0

Table 3: Formation of tri- and tetra substituted alkenes; comparison of the efficiency of catalysts **12b** and **13b** (E=COOMe)

A system combining the positive features of each catalyst is highly desirable, and a contribution by Grubbs *et al.* increased the utility of the ruthenium complex by improving its activity.[14] They prepared ruthenium-based complexes such as **13c,** co-ordinated with 1,3-dimestiyl-4,5-dihydroimidazol-2-ylidene ligands as shown previously. These complexes exhibited a high ring-closing metathesis activity similar to that of the molybdenum complex **12b,** yet retained its remarkable air and water stability of its parent benzylidene ruthenium complex **13b**.

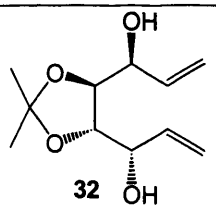
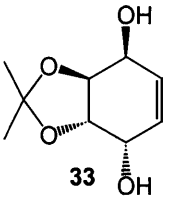
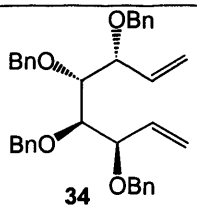
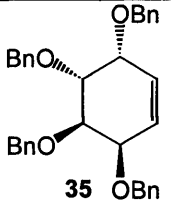
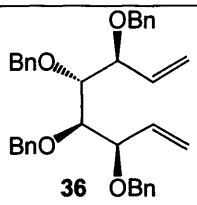
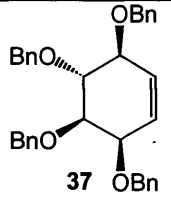
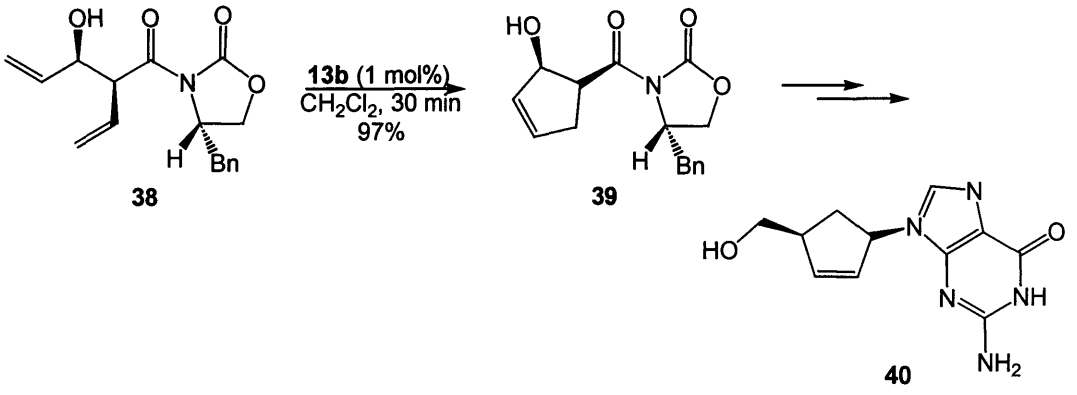
Substrate	Catalyst	Mol%	Time (h)	Product	Yield
 **32**	**12a**	20	20	 **33**	0
	13b	5	2		0
	13c	1.5	2		69
 **34**	**12a**	5	1	 **35**	91
	13c	5	3		85
 **36**	**12a**	5	1	 **37**	92
	13b	5	60		32
	13c	5	2		89

Table 4: Comparative investigations of the reactivity of different metathesis catalyst **12a, 13b/c,**

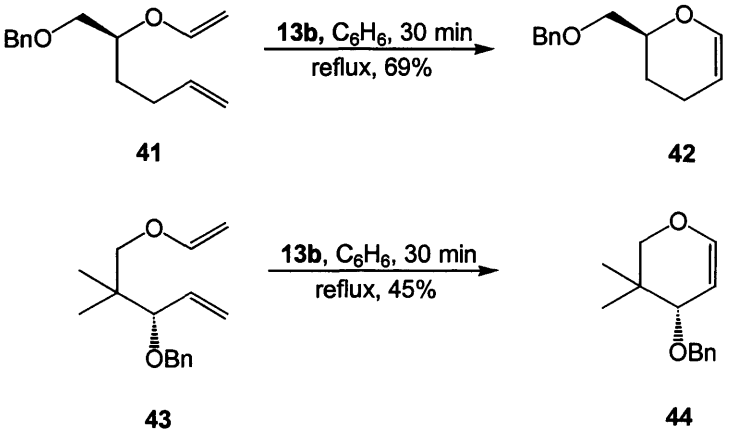
It can be seen from Table 4 the increased activity of catalyst **13c**. Fűrstner and co-workers described the comparative investigation of catalysts **12a**, **13b** and **13c** involved in RCM of dienes **32**, **34**, and **36** to produce the naturally occurring conduritol derivatives **33**, **35** and **37** respectively.[19] They illustrated the effectiveness of the new ruthenium catalyst **13c** and the molybdenum catalyst **12a** for the ring closure of bulky tetrabenzylated dienes **34** and **36**. The superiority of **13c** is clearly demonstrated when it was able to furnish **33** from the unprotected diol **32**.

Due to its remarkable tolerance to a wide variety of functional groups such as, silyl ethers, acetals, amides, sulphonamides, silanes, and heteroatoms the ruthenium complexes **13b** and **13c** have been involved in a wide variety of synthetic applications. Crimmins and King[20] reported a synthetic approach to a precursor of the HIV transcriptase inhibitor carbovir **40**. The highly functionalised chiral diene **38**, formed by aldol methodology cyclised to produce the cyclopentanol **39** when reacted with the ruthenium catalyst **13b** in dichloromethane. Removal of the chiral auxiliary gave the corresponding diol which is the key intermediate in the synthesis of carbovir **40**.

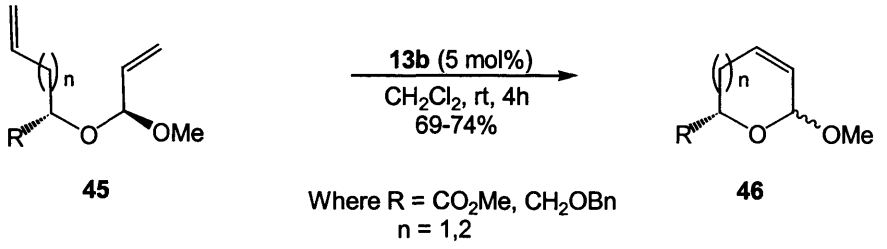
Heterometathesis



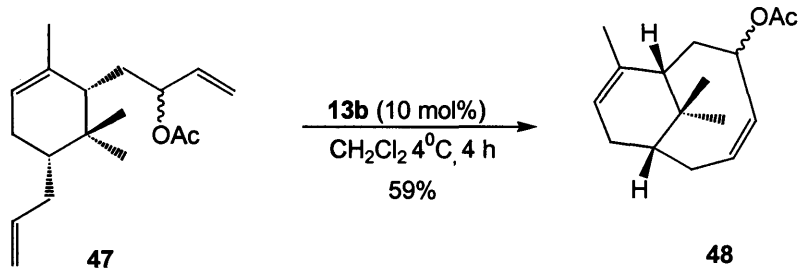
Sturino and Wang have reported the ring closing metathesis of certain vinyl ethers **41** and **43**, to the dihydropyrans **42** and **44** with Ru catalyst **13b** in moderate yields, contrary to previous findings.[21]



The synthesis of α,α' substituted dihydropyrans employing **13b** to catalyse RCM has been demonstrated by Rutjes and co-workers. [22] Diene **45** was transformed to the corresponding heterocyclic product **46** in good yield. These compounds can be used in the synthesis of a variety of natural products.

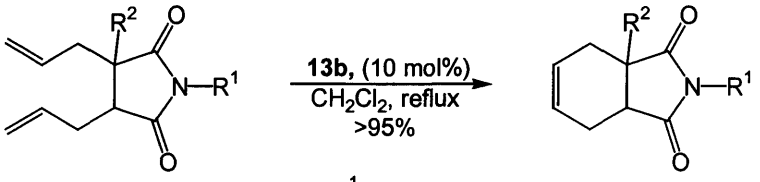


Clearly, bridged-ring systems, such as the one found in Taxol represent a formidable challenge for synthesis. An A, B – ring fragment pertaining to taxol was successfully synthesised by Blechert et al. by invoking a RCM reaction, which produced **48** in moderate yield.[23]



47 48

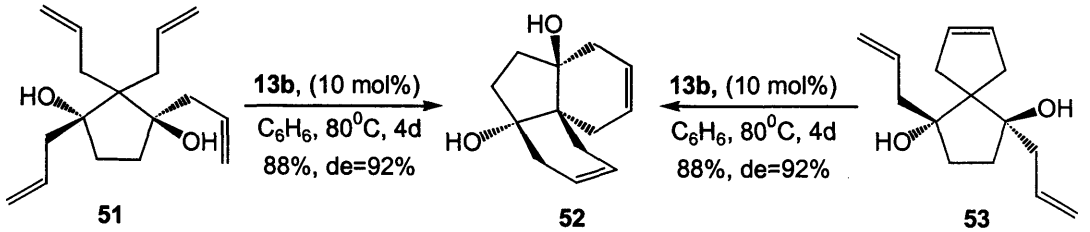
A limitation of the Ru catalysts **13a, b** and **c**, is that their activity is reduced by the presence of free amines, therefore the amine functionality should be suitably protected before RCM takes place. However, the activity of the Ru catalyst is unaffected by amide functionality. Dyatkin was able to transform the diene moiety **49** to the bicycle **50** in an excellent yield of > 95%, when the substrate was treated with 10 mol% Ru catalyst **13b.** Surprisingly, when the R^2=allyl no spirocyclic product was formed, even though the two ring closure reactions are in direct competition.[24]



49 50

Where R^1 = alkyl, aryl
R^2 = alkyl, aryl, allyl

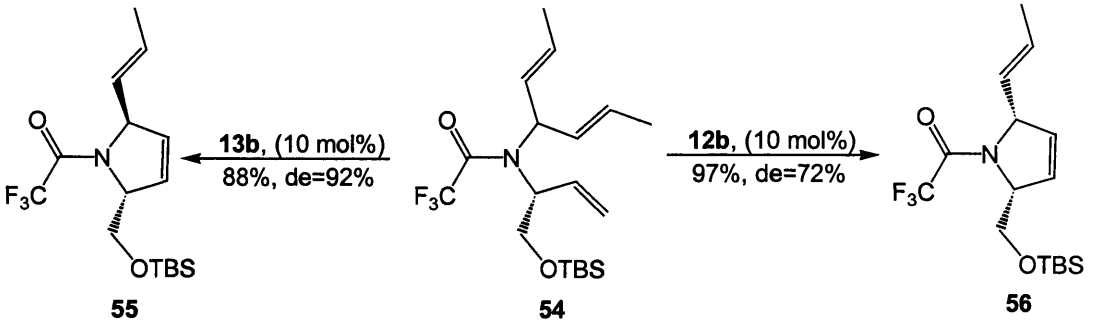
Harrity and co-workers have illustrated that bicyclic and tricyclic angularly fused systems are formed in preference to spirocyclic systems when the two processes are in direct competition. Tetraene **51** was subjected to 10% mol Ru catalyst **13b** for 48 hours to produce the tricyclic **52** in high yield. To confirm that the tricyclic product is preferred over the spirocyclic product, Harrity and co-workers reacted the spirocyclic compound **53** using the same reaction conditions to the give the same tricyclic product as before.[25]



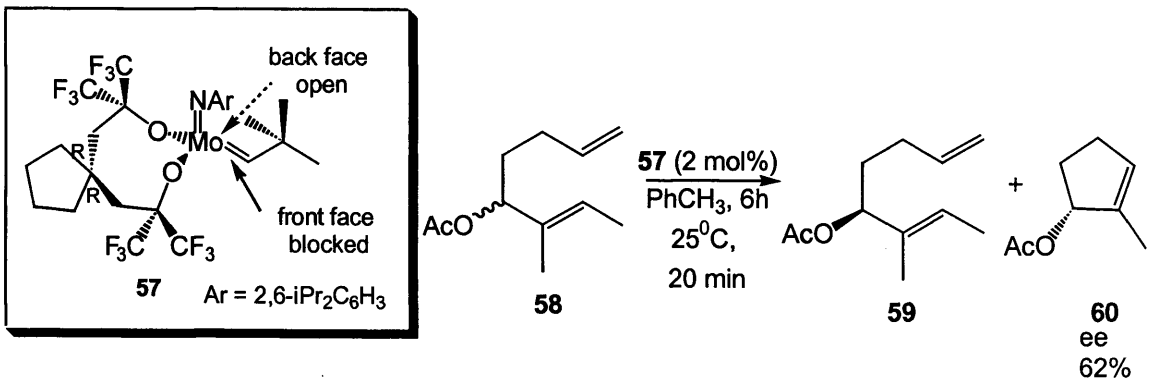
51 52 53

1.3.2 Asymmetric Ring Closure Metathesis

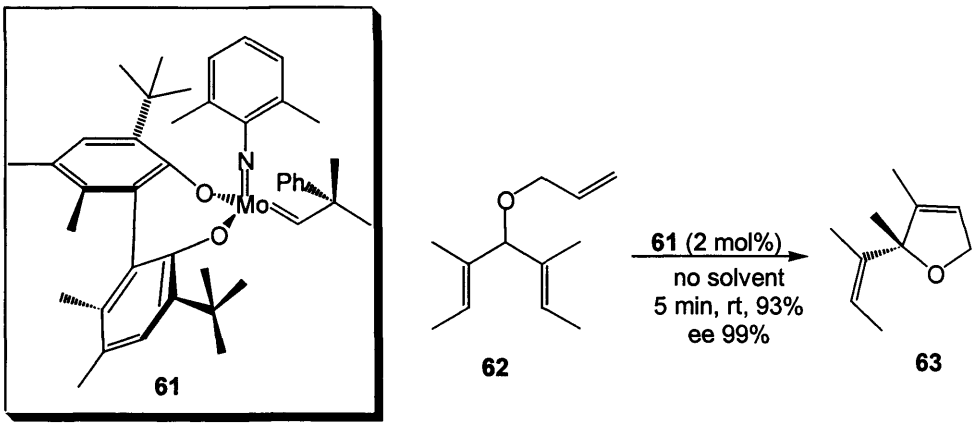
Due to the potential of RCM, asymmetric variants are of particular importance. Blechert and co-workers have explored the use of RCM that uses an existing chiral centre to control the direction of cyclisation with a prochiral diene **54**. They have found cyclisation to give the five-membered ring was highly catalyst-specific and that the ruthenium catalyst gave the product with anti-orientation of the two substituents **55**, while the syn isomer was obtained using the molybdenum catalyst **56**. They believed that the catalyst-specificity is attributed to the different spatial arrangements of the respective ligands during cyclisation.[26]



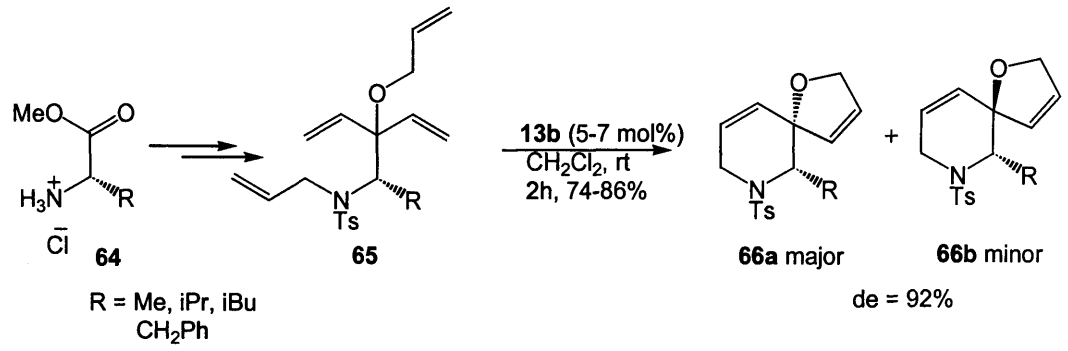
The RCM of racemic dienes catalysed by the chiral molybdenum alkylidene complex **57** has been reported by Grubbs and co-workers.[27] The group developed a chiral molybdenum alkylidene catalyst **57** where one face of the Mo-C double bond is blocked by the ligand substituents, thus one enantiomer of the substrate reacts faster than the other hence kinetic resolution. They treated the racemic diene **58** with 2 mol% of complex **57** at 25^{0}C for 20 minutes, at which time 90% of it was consumed. The unreacted starting material was recovered and the enantiomeric excess calculated to be 84% of the *S* isomer.[27]



Hoveyda and Schrock reported the first catalytic and enantioselective synthesis of chiral heterocycles employing chiral catalyst **61**. Treatment of the diene ether **62** with 2 mol% of **61** with no solvent led to the formation of the derived dihydrofuran **63**.[28]



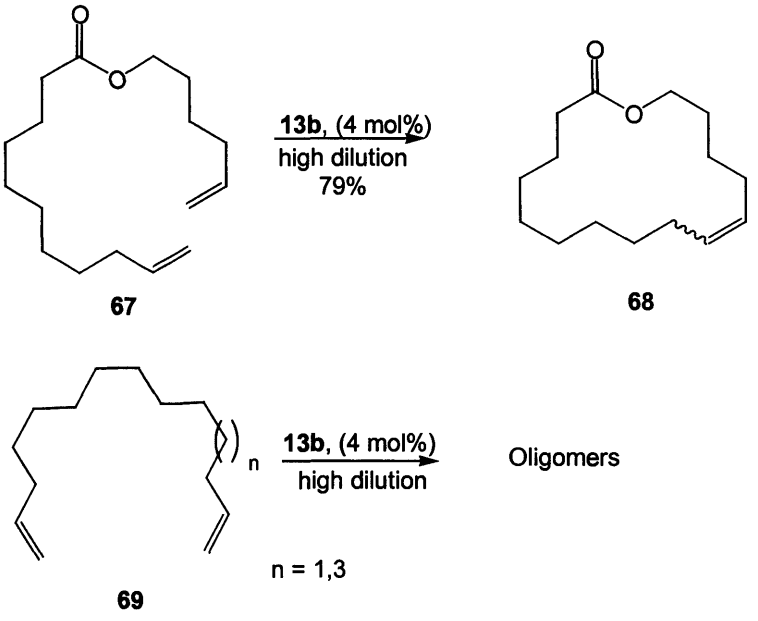
The first diasteroselective double bond RCM reaction producing spirocyclic compounds has been reported by Wallace *et al.*.[29] Chiral tetraenes **65**, prepared from amino acid derivatives **64**, were cyclised on treatment with Ru catalyst **13b** at room temperature for two hours to give chiral spirocycles **66a** and **66b**.



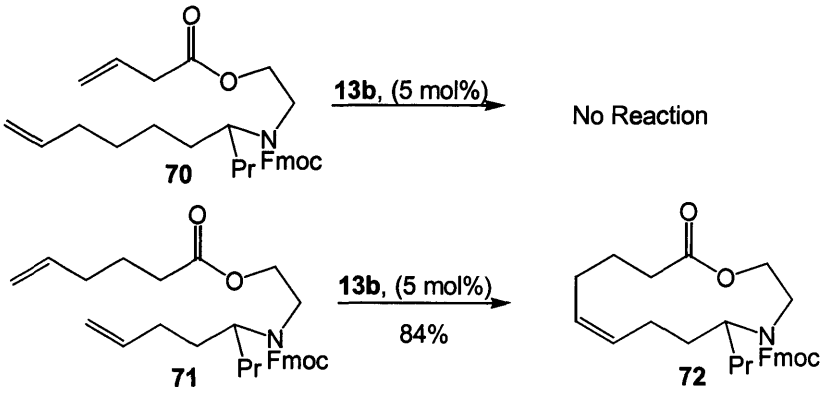
1.3.3 Macrocyclic RCM and Natural Products

Previous assumptions concerning macrocyclic RCM stated that macrocyclisation via RCM required substrates that were conformationally predisposed. Fürnster has been able to list a set of key parameters for successful RCM.[15]

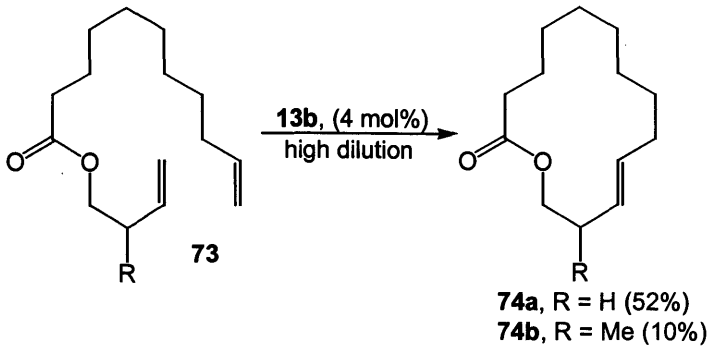
- The presence of a polar functional group (amide, ester, ether, ketone, urethane, sulphonamide etc...) is a requirement for smooth macrocyclisation by RCM. This is illustrated below, as 18-membered acyclic lactone **67** is cleanly cyclised to the 16-membered cyclic lactone **68** in good yield, whereas the analogous carbon chain **69** affords a mixture of oligomers.



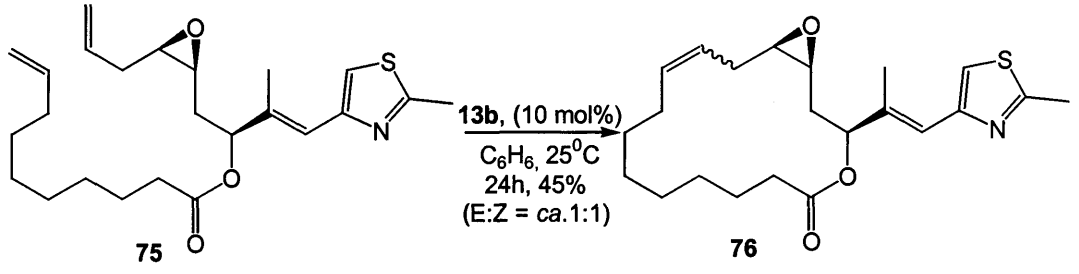
- The site of ring closure is another important requirement. There must be an appropriate distance between the polar group and the alkene to be metathesised. This is shown below. Diene **70** does not cyclise, but by changing the site of reaction, **71** ring closes to give the heterocycle **72** in 84% yield.



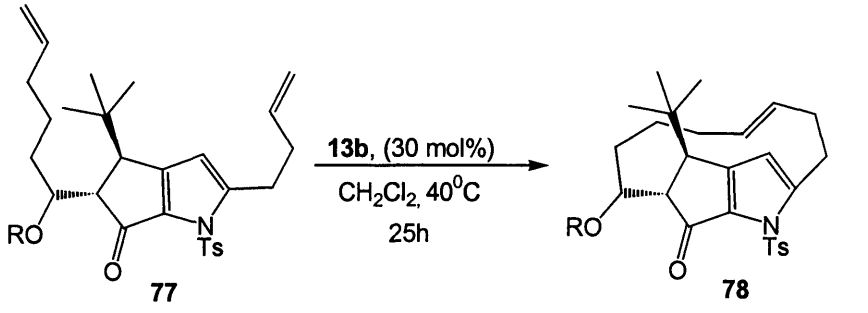
- Low steric hinderance close to the double bond increases the yield of cyclisation. Thus the methyl branched compound **74b** is formed in significantly lower yield than **74a**.



Danishefsky and co-workers have employed RCM to the construction of the macrocyclic skeleton of the epothilones, a class of natural products that exhibit potent biological activity.[30] The metathesis of the highly functionalised diene **75** proceeded smoothly to produce the 16-membered macrocycle as a mixture of E- and Z-stereoisomers **76**, when treated with the Ru catalyst **13b**.

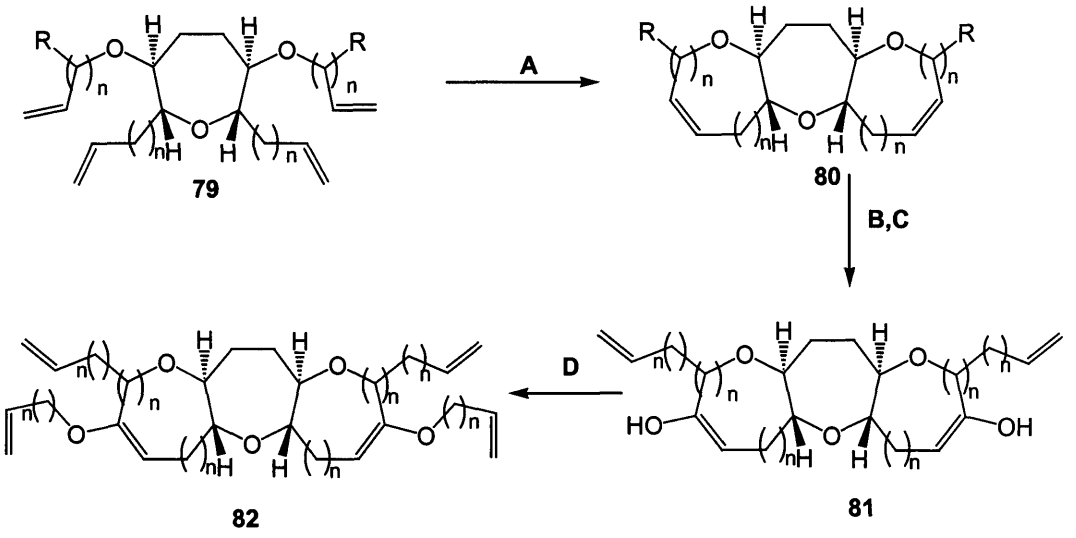


An example of the ability of RCM to synthesise the macrotricyclic core of roseophilin has been illustrated.[31] After a conformational control element **R** had been introduced to help bring the terminal olefins of **77** closer together (thus lowering the enthalpy barrier during ring formation) RCM was able to form the anchor chain of the target molecule **78**.

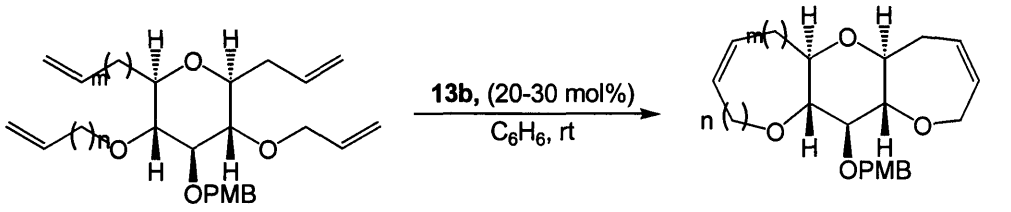


R = H, (0%, only dimer)
R = TIPS (60%)

Polycyclic ethers are abundant in natural products and strategies for their synthesis have been developed. Clark and Hamelin have designed a novel method for the efficient construction of **84a-d.**[32] The stepwise production of polycyclic ethers, which could provide a whole library of polycyclic ethers is illustrated. Step A involves RCM across the diene moiety of **79**. The functionalisation of the new ring systems (step B) followed by side chain functionalisation produces **81**. Step D represents the introduction of a new side chain to regenerate a tetraene **82**.



The tetraenes prepared from D-glucal were cyclised in the presence of **4b** to afford **73a-d**



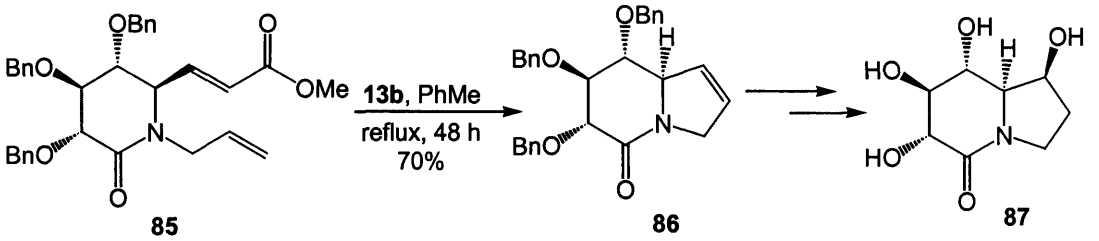
83

84a, n = 0, m = 1, 71%
84b, n = 1 m = 1, 88%
84c, n = 1, m = 2, 77%
84d, n = 3, m = 1, 65%

1.4 RCM in Carbohydrate Derivatives

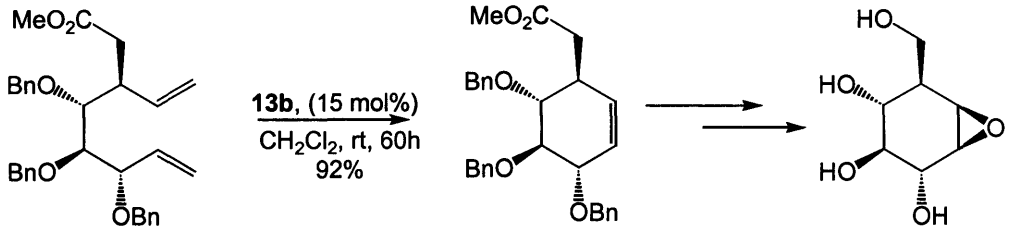
Carbohydrates provide an excellent template for a variety of syntheses due to their intrinsic functionality and chirality. The advent of well defined catalysts for RCM, and in particular the functional group tolerant catalysts such as **13**, has generated a huge interest within the field of carbohydrate chemistry leading to a variety of carbo- and heterocyclic products, and many total syntheses.

Overkleeft and co-workers described the cyclisation of the aza-sugar **85** using Ru catalyst **13b**, in refluxing toluene to give the bicyclic lactam **86** in 70% yield and subsequently converted it into the glycosidase inhibitor **87**. This proved to be a novel approach using a substrate where one of the olefinic groups is an α-β-unsaturated ester, despite the harsh reaction conditions. These conditions were necessary since the by-product is methyl acrylate instead of the more volatile ethene.[33]



85 86 87

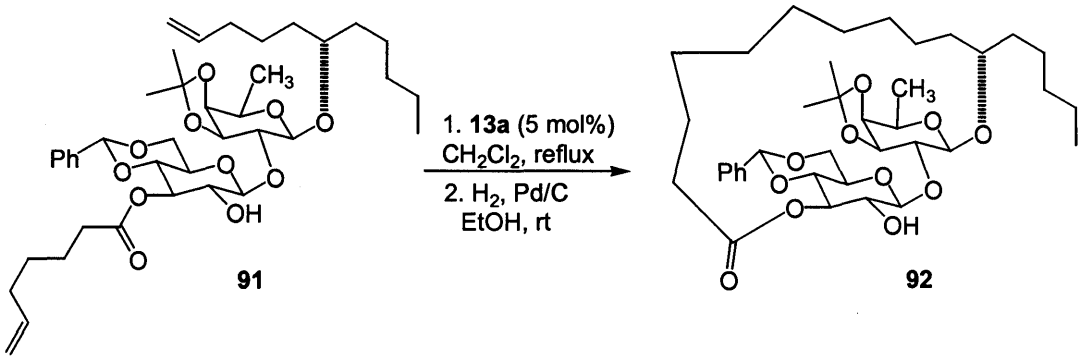
Ziegler and Wang exposed the diene **88** to Ru-catalysed RCM, which led to the efficient formation of the cyclohexene **89** in 92% yield. This was then converted to the final target (+)-cyclophellitol **90** a β-glucosidase inhibitor originally isolated from the culture broth of the mushroom *Phellinus sp* in a few further steps.[34]



88 89 90

(+)-cyclophellitol

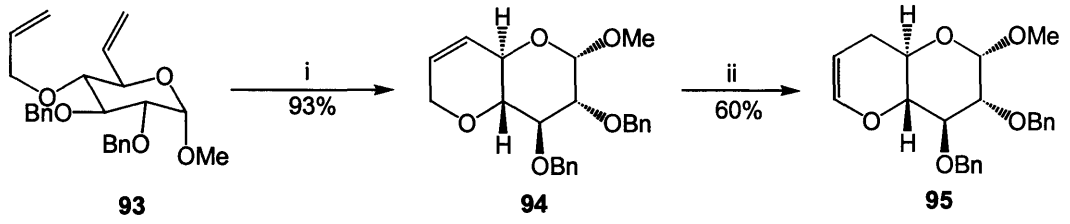
Fürnster and Muller employed RCM instead of macrolactonisation to cleanly cyclise the diene **91** to the desired 19-membered ring on reaction with Ru catalyst **13a** as a mixture of *E* and *Z* isomers. Subsequent hydrogenation gave the disaccharide fragment trichlorin A **92**, a cytotoxic resin glycoside, isolated from *Ipomoea tricolour* in 71% yield over both steps.[35]



91 92

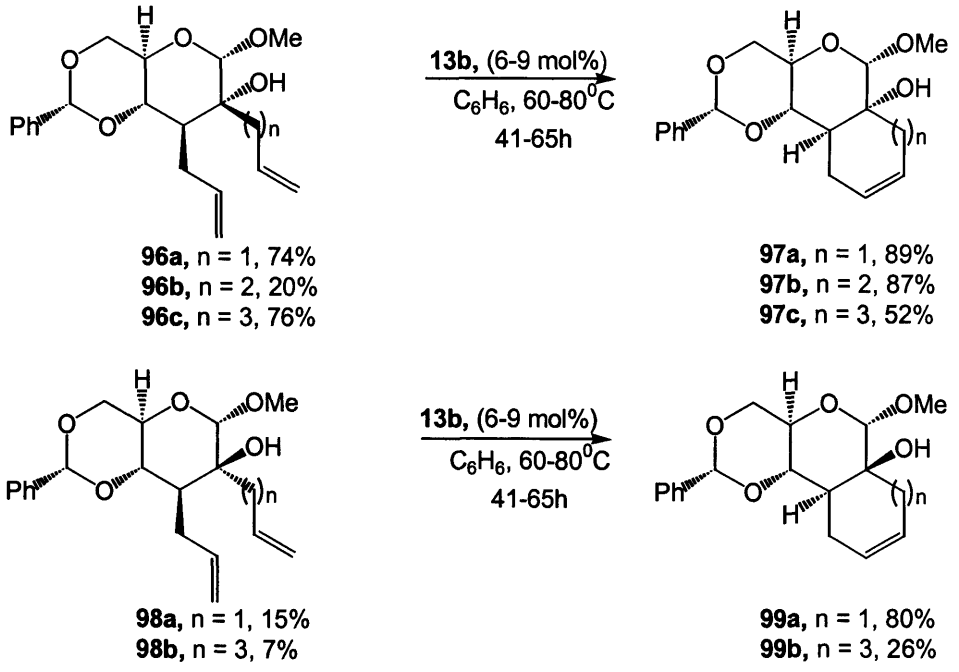
Van Boom and co-workers have described a novel synthesis of both *cis* and *trans* fused pyranopyran systems using RCM of glycal derived dienes. It is well known that trans-fused polytetrahydropyrans are necessary structural elements of several marine

toxins such as brevetoxins and ciguatoxin. They took diene **93** obtained from α-1,2-anhydro 3,4,6-tri-*O*-benzyl-D-glucose in several steps and converted it to the trans-fused allylic bicyclic ether **94** by RCM (Scheme 3). Isomerisation of the double bond produced **95** as a single isomer in 60% yield.[36]

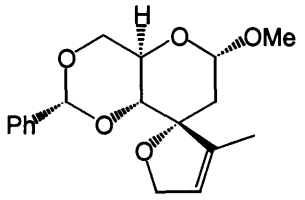


Scheme 3. **Reagents and Conditions;** i) **13a** (3 mol%), PhMe, rt; ii) PPh_3RhCl, DBU, EtOH, 50°C

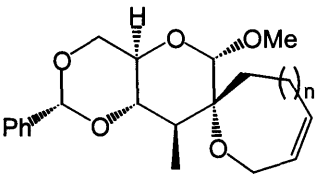
Enantiomerically pure quaternary centres along with spiro compounds are difficult to synthesise especially when the starting materials are sugars. Research by Jenkins and co-workers have employed RCM to carbohydrate annulation reactions to produce tricyclic systems. Five-, six-, seven- and eight-membered annulated sugars along with spiro systems have been prepared in good yields.[37]



The dienes **96** and **98** were obtained by reacting an unsaturated ketone with either vinyl- or allyl grignard. The RCM reaction of **96** and **98** with catalyst **13b** gave tricyclic systems **97** and **99** in good yields. The same group also prepared spiro-fused dihydropyran derivatives such as **100** and **101**.[38]

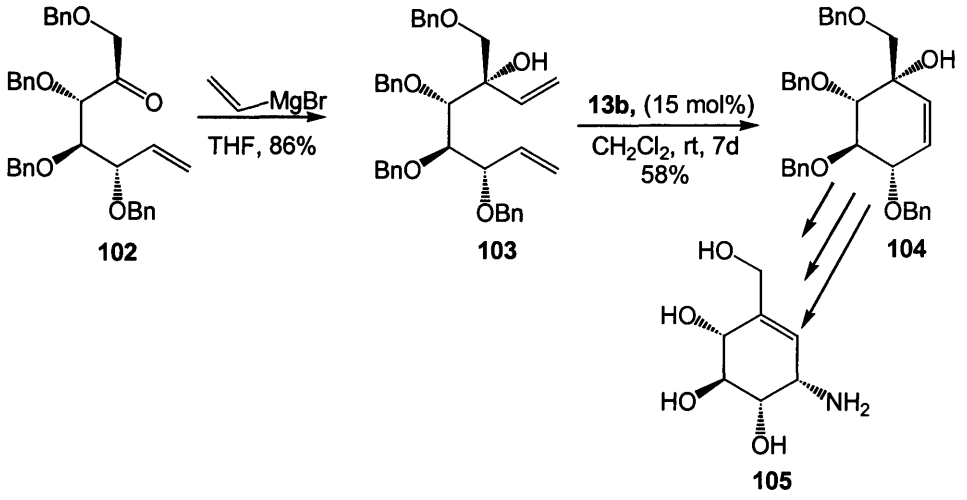


100



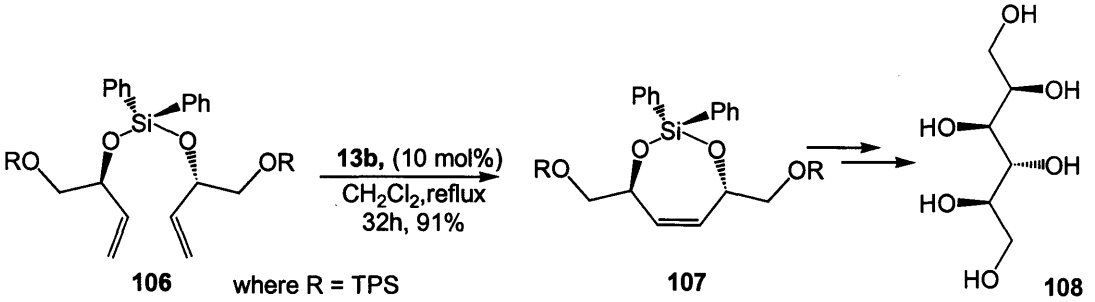
101a, n = 1, 52%
101b, n = 2, 33%

A close derivative of valiolamine has been reported by Vasella and co-workers.[39] They took D-glucose and converted it to the acyclic ketone **102** in a number of steps (Scheme 4). The ketone was alkylated with a vinyl grignard reagent to afford **103**, which underwent RCM in the presence of **13b** catalyst to produce the chiral carbocycle **104**. Three further steps afforded (+)-valienamine **105** in an overall yield of 47%.



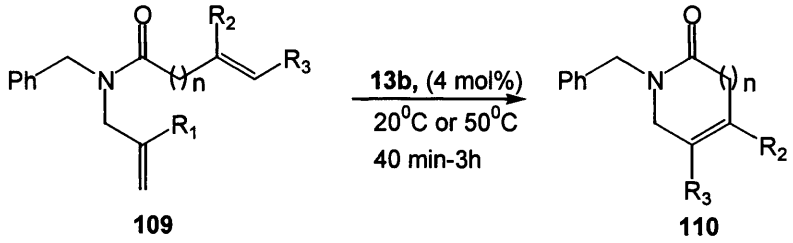
Scheme 4

Simple chiral olefins have been employed by Evans and Murthy in the preparation of carbohydrates. They portrayed that RCM of a silicon tethered diolefin **106** afforded a symmetric 1,4-diol **107**. Dihydroxylation of the new olefinic moiety by Sharpless protocol and removal of the protecting groups gave the reduced carbohydrate D-altitrol **108**.[40]



1.5 Synthesis of Nitrogen-Containing Systems

Various studies in recent years have demonstrated the usefulness of RCM for constructing nitrogen heterocycles. Due to the fact that many biologically active substances are nitrogen-containing compounds, the method enhances synthetic chemistry. The preparation of nitrogen containing compounds can be traced to 1992 and 1993 when Grubbs and co-workers published several papers.[41] The writers presented the nitrogen functional group compatibility of the catalyst **12b** and **13b**, along with their ability to form five-, six- and seven membered rings **109**.

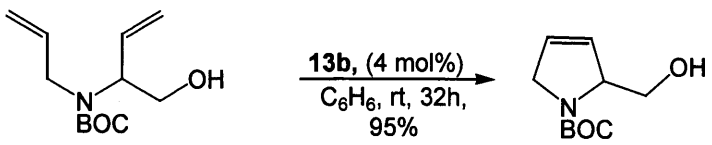


109 **110**

n	R_1	R_2	R_3	Yield (%)
0	H	H	Me	74
0	H	Me	Me	77
1	H	H	Et	80
2	H	H	H	87
2	Me	H	H	81

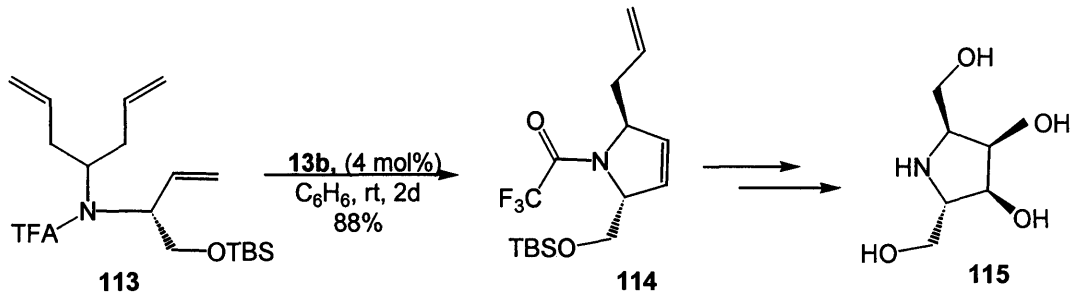
There are a large number of examples of RCM involving substrates, which contain a diene attached to one or more nitrogen atom(s). These types of substrates give access to a number of useful classes of compounds such as mono- and bicyclic pyrrolidine, pyrrolidionone, piperidionone and piperidine ring systems. Many of these heterocycles are obtained from amino acids and offer a significant potential as peptido-mimetics. Some of these compounds are also important intermediates for the synthesis of alkaloids and azasugars.

Huwe and Blechert have reported a novel synthesis of azasugars **112**[42] involving the RCM of a vinyl glycine methyl ester **111** using catalyst **13b** in 95% yield.

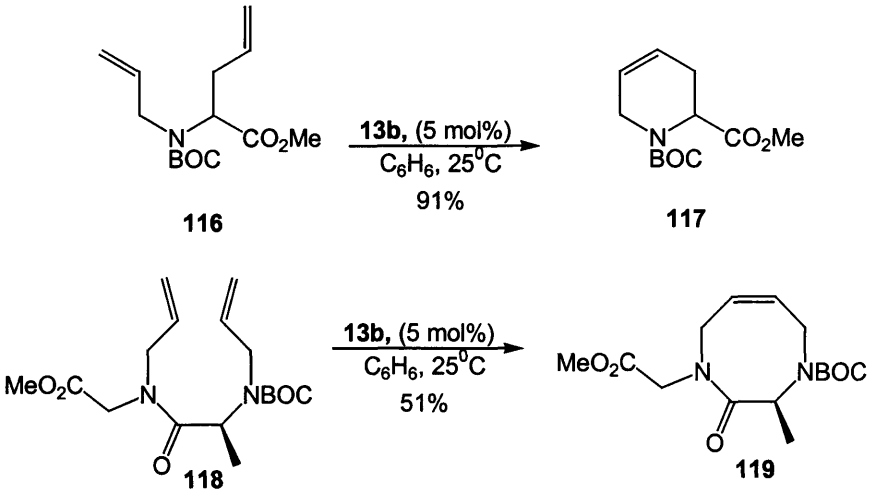


111 **112**

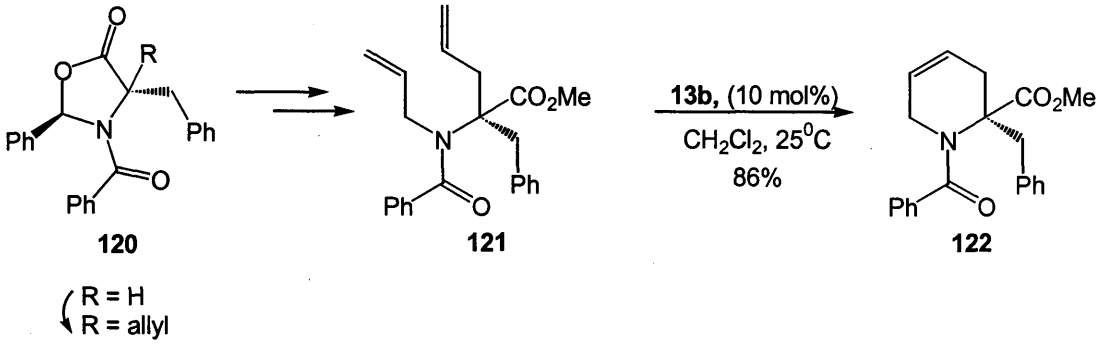
In another application the same group applied the Sharpless dihydroxylation method to derive 1-deoxy azasugars. They also used an analogous strategy toward the synthesis of the homoazasugar **115**.[43]



Grubbs and co-workers have employed the Ru catalyst in peptide and heterocyclic chemistry as illustrated.[44] They reported the use of catalyst **13b**, in the synthesis of cyclic amino acids **117** and in the synthesis of an eight-membered Ala-Gly dipeptide **119**.

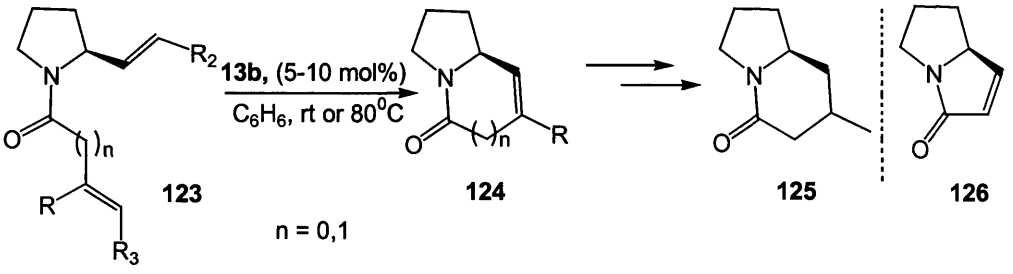


The reaction was extended by Abell and co-workers, who used a combination of Seebach's oxazolidinone chemistry and RCM in the synthesis of a phenylalanine mimic **120** (Scheme 5). T his tetrahydropyridine system was designed to probe the constraints imposed by the six membered ring on the torsion angle between the α carbon and the nitrogen. X-ray analysis demonstrated that this type of system has potential as a β-turn mimic.[45]



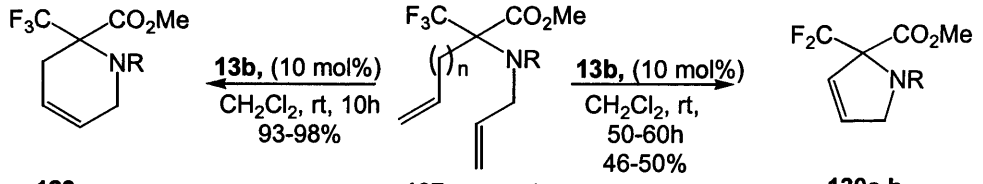
Scheme 5

A number of fused nitrogen heterocycles, including pyrrolizidines, quinolizidines and indolizidines can be readily prepared via RCM. Arisawa and co-workers described the RCM of chiral dienes **123**, which are readily available from L-proline. Further reactions gave (-)-coniceine **125**, which is the simplest indolizidine alkaloid and (*S*)-pyrrolam A **126.**[46]



123 **124** **125** **126**

Osipov and Dixneuf reported the synthesis of fluorine-containing cyclic amino acid derivatives via RCM. An intramolecular RCM reaction was attempted on the 1,6 dienes **127a, c** to afford the α-CF_3 dehydropipecolinate derivatives **129a, c**. The prolinate derivatives **130a, b** were also formed in reasonable yield.[47] Compounds of this type have potential application in the modification of peptide drugs.



129a,c **127a,c** n = 1, **128a,b** n = 0 **130a,b**

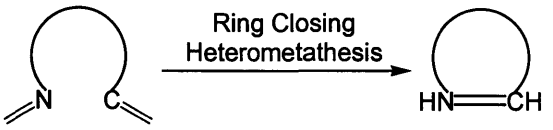
a. R = SO_2Ph
b. R = CO_2Bn
c. R = CO_2Bu^t

1.6 Summary

Olefin metathesis has become a standard synthetic method because of the wide variety of applications that are now feasible and the ease with which catalysts can be used. The mild conditions under which most reactions can be performed, along with the high functional-group tolerance, is shown by the diverse types of new alkenes that can be made. The synthesis of *N*-containing compounds such as heterocycles and peptides has benefited from these features.

An alternative approach to olefin metathesis would be where on or both of the double bonds contains a heteroatom such as **131** going to **132**. This type of metathesis

which we have termed heterometathesis is not known. The mechanism behind heterometathesis is undetermined. Using prior knowledge of the mechanism of RCM reactions, one could put forward a sequence of steps we would expect for this process. Each step in the mechanism would pose an interesting question in organometallic chemistry and also produce a new catalytic cycle.



131 **132**

The objective of the results and discussion is to probe this new metathesis reaction, firstly to see if it is possible and then to determine its scope and applications if it is successful.

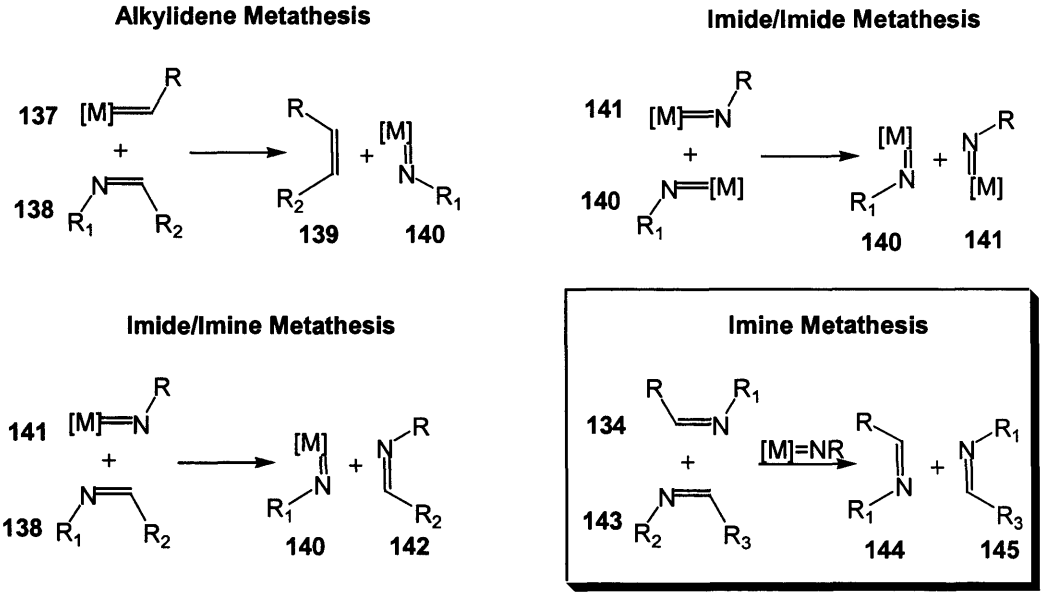
1.7 Imine Metathesis

Catalytic double bond metathesis is a valuable reaction, both in polymer and small molecule synthesis, but has been limited almost exclusively to carbon-carbon bond formation. Imine metathesis therefore extends the metathesis methodology to the formation of double bonds containing heteroatoms. It is the catalytic reaction of two different imines **133** and **134** to give a statistical mixture of all possible =NR exchange products **135** and **136**

It offers many potential advantages over traditional approaches to carbon-heteroatom bond formation, including, selectivity, various functional group tolerances, increased reaction rates and mild reaction conditions.

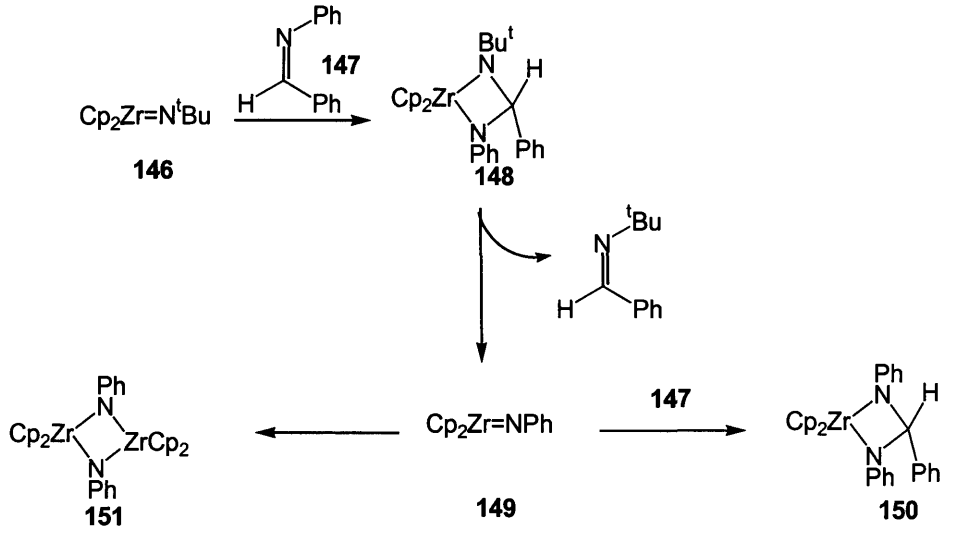
Reports of catalytic metathesis of azaheteroalkenes are rare. Meyer *et al.*[48] have incorporated the use of molybdenum bis (imide) complex of the type $(DME)Cl_2Mo(=NR)_2$ (where $R=C_6H_3{}^{i}Pr_2$-2,6) originally prepared by Schrock and co-workers[10] to examine

several classes of =NR transfer reactions, which include three stiochiometric and one catalytic.

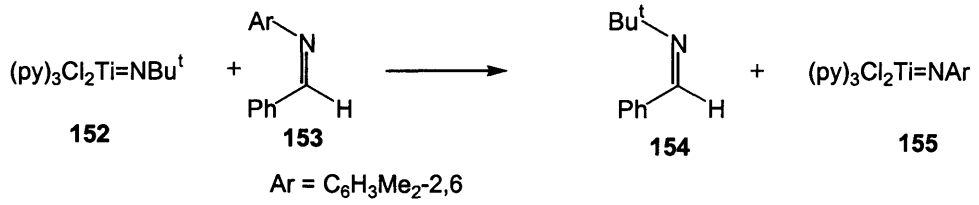


The stoichiometric reactions include alkylidene/imine metathesis, which is a Wittig-like reaction of an imine **138** with an alkylidene **137** to give an olefin **139** and a metal imide **140**. Imide/imide metathesis where the =NR groups between metal centres are exchanged and imide/imine metathesis which involves the reaction of a metal imide **141** and a single imine **138**. The catalytic imine metathesis which is similar to olefin metathesis is the catalytic reaction of two different imines **134** and **143** to give a mixture of all possible =NR exchange products **144** and **145**. The final reaction differs from the stoichiometric ones in that the imine substrates do not directly react with each other.

Alkylidene /imine metathesis was first reported by Schrock *et al.*[49] who used a series of tantalum alkylidene complexes. Bergman and co workers[50] have described both imide/imine and imine metathesis using the early transition-metal imide complex (η-$C_5H_5)_2Zr(=NBu^t)(THF)$ **146** to react with *N*-phenylimines **147** to give diazametallacycles **148** *via* a [2+2] cycloaddition which underwent further metathetical exchange with **147**. The catalytic utility of complex **146** suffers from significant limitations. The reaction is inhibited by high imine concentrations and the formation of an inactive dimer **151.**



Metal imide/organic imine metathesis has been furnished using a number of titanium imido complexes. Mountford *et al.*[51] were able to convert one equivalent of the *N*-2,6-dimethylphenyl benzaldehyde imine **153** to **154** and the arylimido complex **155** using $[Ti(NBu^t)Cl_2(py)_3]$.

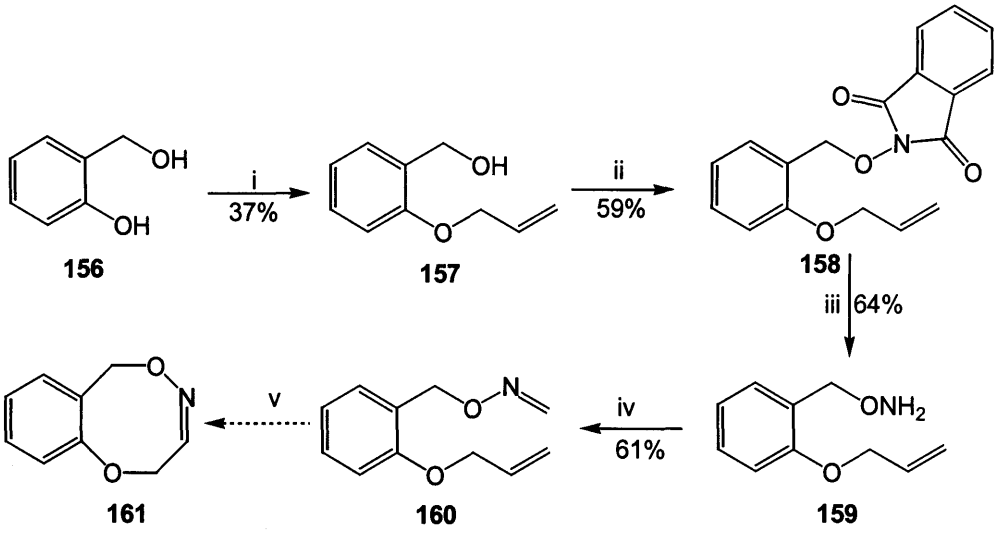


It would be interesting to apply the concept of imine metathesis to a number of substrates to see whether intramolecular imine metathesis is viable.

1.8 Results and Discussion

1.8.1 Studies on Intramolecular Metathesis Involving an Oxime and an Olefin

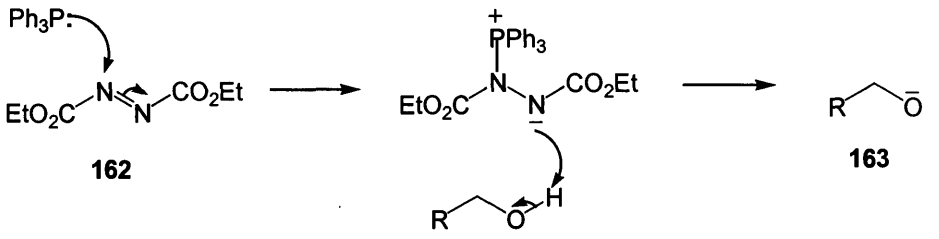
In order to investigate the formation of carbon-nitrogen double bonds via catalytic double bond metathesis we required a substrate with one or two carbon-nitrogen double bonds. As a first study of this idea we decided to synthesise a metathesis substrate with a carbon-carbon double bond and a carbon-nitrogen double bond. A convenient way to synthesise the carbon-nitrogen double bond was to produce an oxime by reaction of an *O* substituted hydroxylamine with formaldehyde. This leads to a substrate with a carbon-carbon double bond and a nitrogen-carbon double bond. In reaction Scheme 6 we see our practical studies of this idea. *Ortho*-hydroxybenzyl alcohol **156** was allylated in the presence of potassium carbonate according to the method of La Chapelle and co-workers,[52] a colourless oil **157** was produced in 37% yield. The proton NMR spectra of this product showed the characteristic one proton dq centred at 5.27ppm corresponding to CH=CH*H trans*. A one proton dq centred at 5.39ppm relating to CH=C*H*H *cis* proton and a ddt at 6.03ppm pertaining to CH_2CH=CH_2. The signals for the 4 aromatic protons and the benzyl protons remain relatively unchanged compared to that of the starting material.



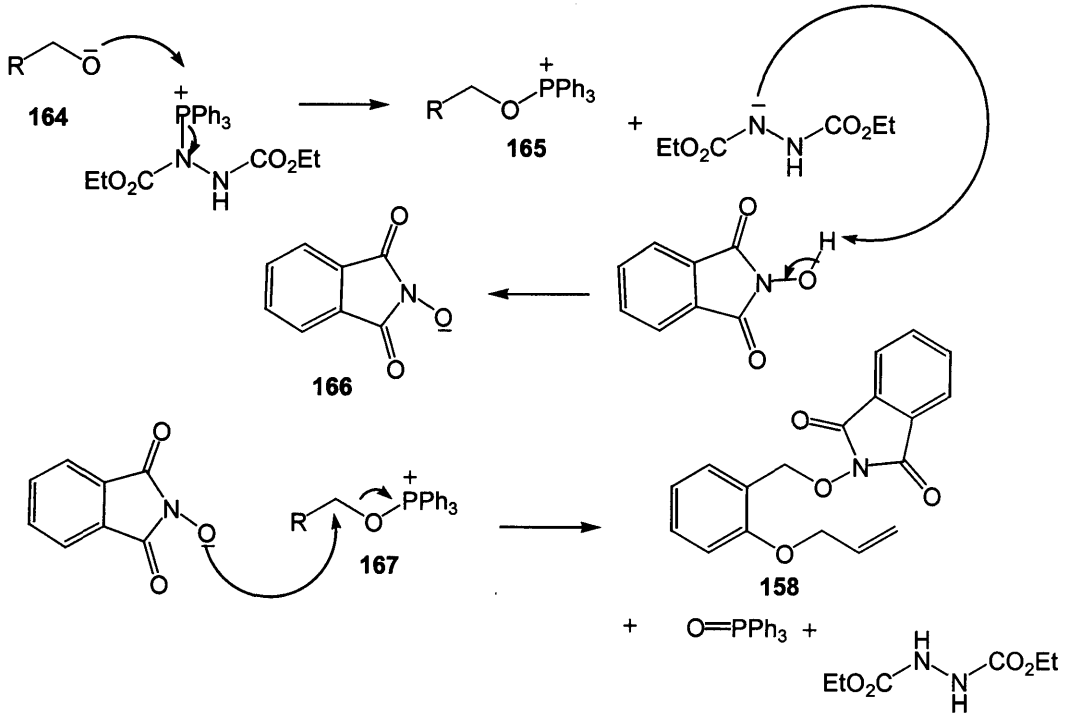
Scheme 6, Reagents and Conditions; i) allyl bromide, K_2CO_3, acetone, Δ, 5 h; ii) *N*-hydroxyphthalimide, DEAD, PPh_3, THF, rt, 24 h; iii) hydrazine hydrate, EtOH, Δ, 24 h; iv) formaldehyde, pyridine, Δ for 4 h then rt, 24 h

The benzyl alcohol **157** was reacted further with *N*–hydroxyphthalimide in a Mitsunobu type displacement, the mechanism of this reaction is shown. The main function of DEAD and triphenylphosphine in this reaction is to make the alcohol group of the substrate a good leaving group. The first step of the reaction is between DEAD and

triphenylphosphine, this is believed to produce ylide **162** which is then thought to deprotonate the alcohol to produce alkoxide **163**.



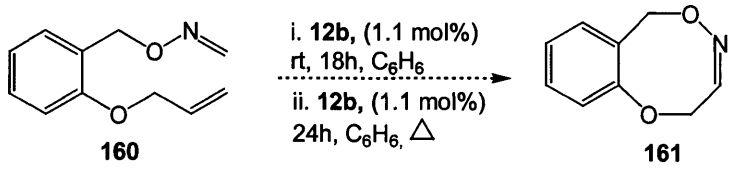
The alkoxide ion can then attack the positively charged phosphorous to produce the alkoxy phosphonium salt **165**. The anion formed during this process then deprotonates the *N*-hydroxyphthalimide which then displaces triphenylphosphine oxide from the phosphine salt furnishing **158**.



The driving force for this process is the formation of the stable triphenylphosphine oxide a situation comparable to the Wittig mechanism. The substituted phthalimide **158** was purified by column chromatography to rid the triphenylphosphine oxide to give 59% of a white solid which showed four new aromatic signals and a molecular ion in the mass spec of 310. The infrared showed the appearance of the phthalimide carbonyl frequency and the disappearance of the band at 3300 cm^{-1}. In the ^{13}C we observed 1 extra quaternary carbonyl and 3 extra aromatic carbons. These pieces of evidence taken together clearly show we have produced the phthalimide. The phthalimide group was then reacted with hydrazine hydrate using the procedure of Ing Manske.[53] This involved nucleophilic attack

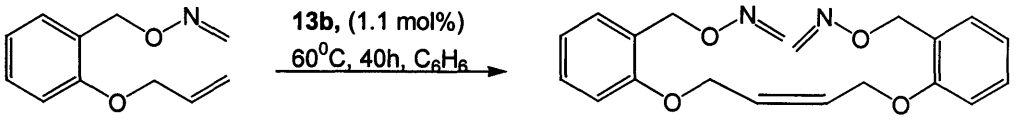
of the amino group of the hydrazine, an especially favoured process due to the alpha effect.[54] A second nucleophilic attack at the carbonyl of the phthalimide led to a reaction pathway whose ultimate product is a maleic hydrazine derivative **159**. The substituted hydroxylamine produced was then reacted with formaldehyde in pyridine. In this reaction, the hydroxylamine exhibited an alpha effect making it an efficient nucleophile which attacked the formaldehyde, subsequent loss of water produced the product **160**, which was confirmed by NMR spectroscopy by the appearance of two one proton doublets downfield at 6.54 ppm and 6.96 ppm and the extra CH_2 signal in the ^{13}C.

The attempted cyclisation reaction of **160** using **12b** was unsuccessful. Thin layer chromatography (TLC) of the crude reaction mixture showed starting material was present. Thus the reaction was repeated employing the use of heat. The reaction was still futile producing an unidentifiable brown gum.



160 **161**

Substrate **160** was taken with the Grubbs ruthenium catalyst **13b** and stirred at 60^0C for 40 hours. Following purification, starting material was recovered and another substance. Upon further analysis *via* mass spectrometry which contained a MNa^+ peak at 377 it seems that **160** reacted across the double bond to produce the dimer **168**.

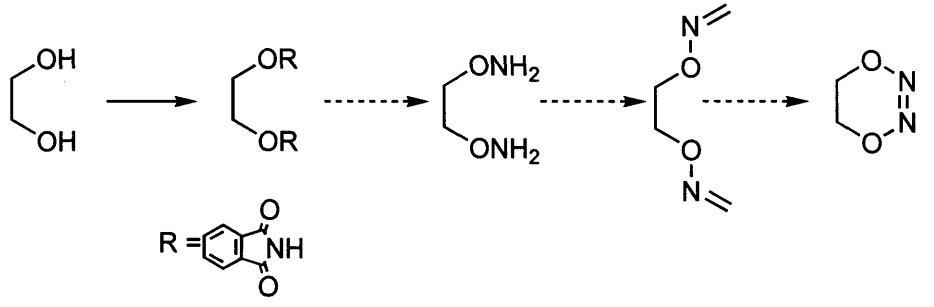


160 **168**

The reaction may not have given the ring closed product due to oxime being less reactive than the olefin, therefore needing a far longer reaction time or an activating group attached to the oxime. This experiment shows that the normal olefin is far more reactive to metathesis than the oxime since we see preferential reaction of the C=C bond instead of the metathesis involving the oxime. Since this may be a reoccurring problem we next turned our attention to substrates which contained two oxime bonds and then two imine double bonds to see if reaction was possible without the competing olefin metathesis reaction.

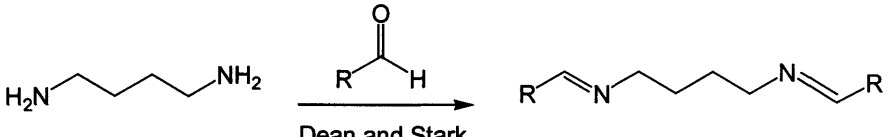
1.8.2 Studies on Heterometathesis Using Two Oximes

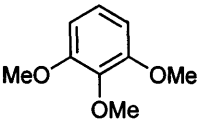
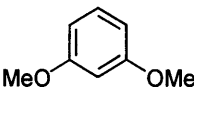
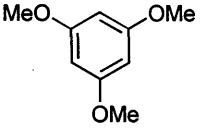
To simplify the system a six-membered ring system was sought, whereby we would get reaction between two oximes moieties instead of an oxime and an olefin. The synthesis was only successful up until the first step, cleaving both the phthalimide groups proved troublesome. Thus work ceased in this area.



1.8.3 Studies on Heterometathesis Using Two Iminies

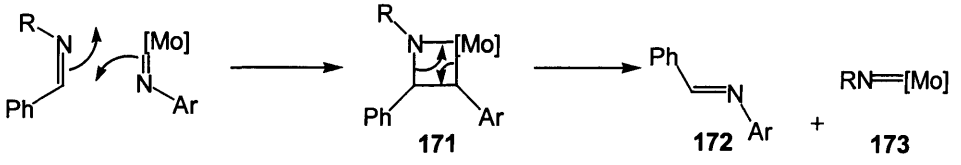
Still interested in testing the idea of forming a carbon-nitrogen double bond, we employed the principle of imide/imine metathesis to the following systems. Each imine was made from the corresponding aldehyde and 1,4-diaminobutane **169** with the use of Dean Stark equipment, for the azeotropic removal of water to give **170a-d** The *tert*-butyl imine had to be used immediately as it was very unstable compared to the rest of the imines synthesised. The imine structure was confirmed by the characteristic imine proton which occurs at 8.18 ppm.



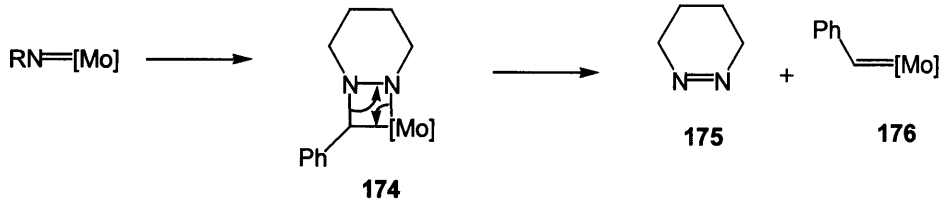
R =	tBu **170a** 51%	 **170b** 42%	 **170c** 53%	 **170d** 88%

These imines were reacted with the Grubbs catalyst **13b**, and heated at 60^0C for six hours. After this period the reaction mixture was monitored via mass spectrometry and NMR but the information portrayed only starting material to be present. Thus the reactions were then left overnight at 60^0C, but no further reaction took place.

The lack of success of these reactions may have been due to the type of catalyst used. In order to test the scope of these reactions, they can be repeated using the Schrock catalyst of the type reported by Cantrell and Meyer[48] and see whether the following reaction may occur. A [2+2] cycloadddition between the imine and the imide functionality of the catalyst occurs to form a four membered metallocycle intermediate **171**. This could then break down giving a new imine **172** and the catalyst attached to one end of the butane chain **173**. This could undergo a second [2+2] cycloaddition at the other end of the molecule to form the six membered heterocycle, attached to the metallocycle intermediate **174**, which undergoes a cycloreversion to give the tetrahydro-1,2-diazine **175** and the metal alkylidene complex. From the following reaction the original metal imide catalyst is not regenerated, but a metal alkylidene complex **176** which gives rise to the question whether or not the cycle is catalytic.

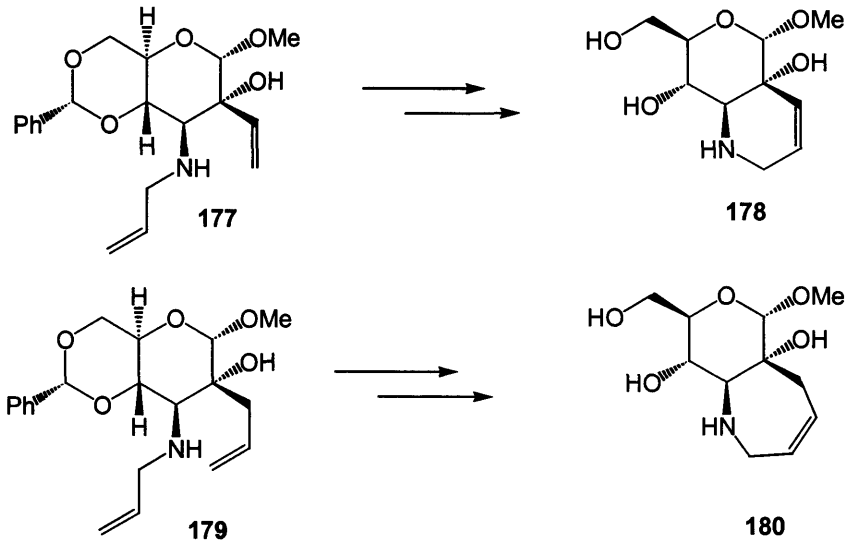


R = $(CH_2)_4NCHPh$

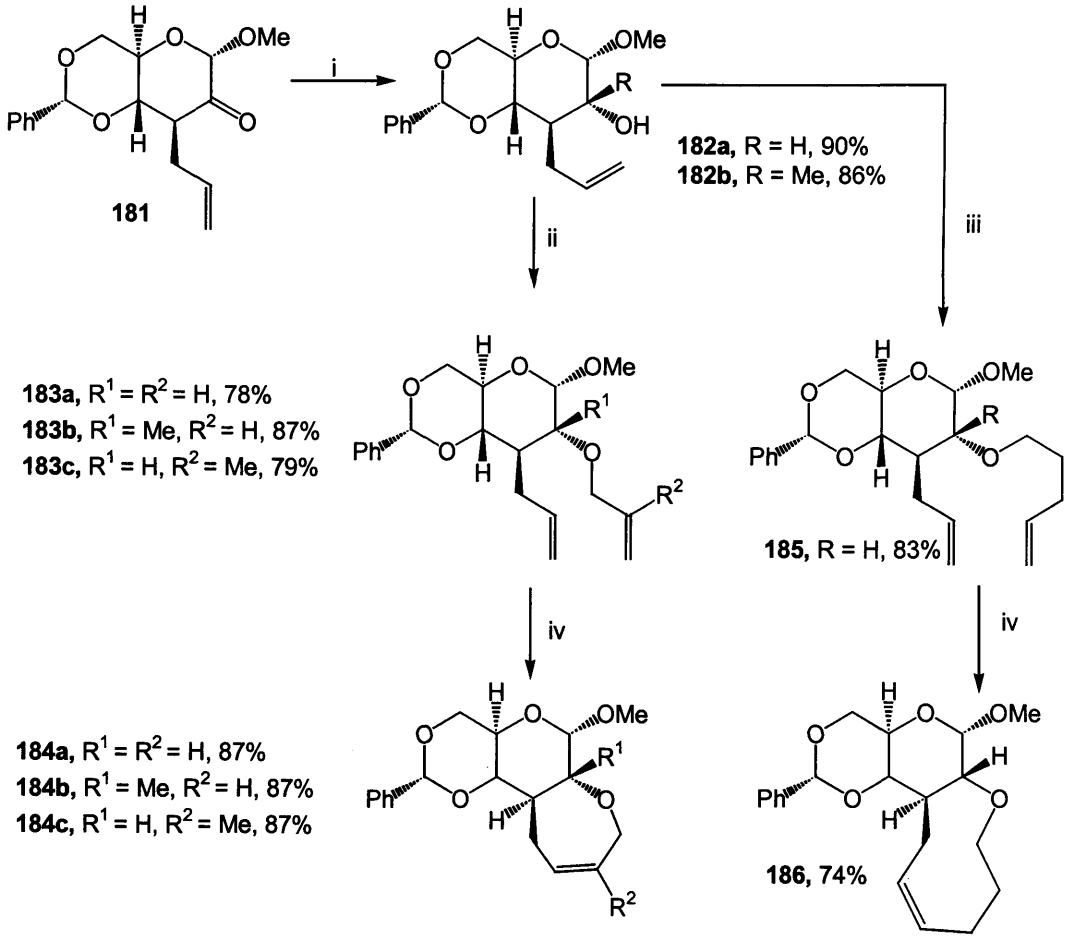


1.8.4 Studies on RCM Using Carbohydrate Precursors

To diversify the project we envisaged that the synthesis of dienes **177** and **179** would be possible using methods similar to our published procedure.[38] To probe the synthesis of these heterocyclic sugar derivatives which may have interesting properties as glycosidase inhibitors once deprotected to give **178** and **180**, it was decided to synthesise them to see whether they would undergo RCM.



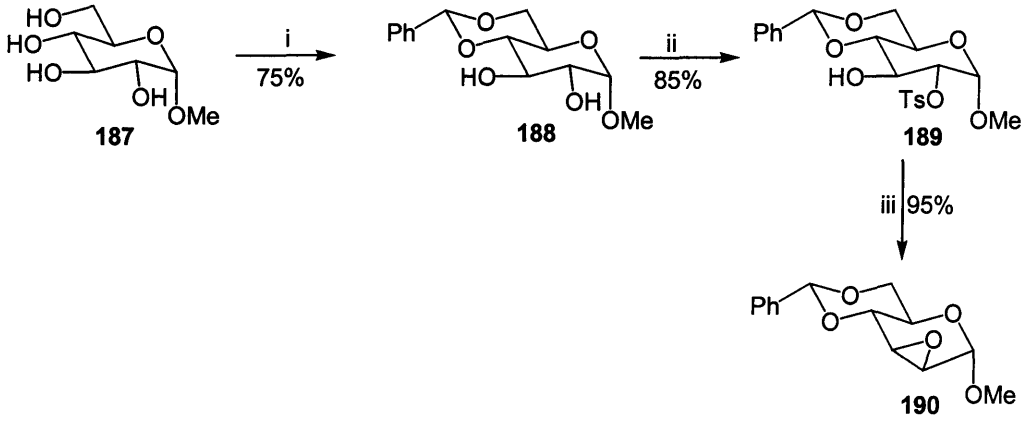
This synthesis can be seen as an extension to the work performed within the research group of our Indian collaborators, Professor S Ghosh, by Jagannath Panda.[37] They studied the synthesis of medium ring oxygen-containing heterocyclic annulated sugars, *via* RCM (Scheme 7).



Scheme 7, Reagents and Conditions: i) **182a** – $LiAlH_4$,THF, 0^0C, then 3 h, reflux; **182b** – MeLi, THF, 0^0C, then 6 h rt; ii) NaH, THF, HMPA, $H_2C{=}CR^2CH_2Br$, 2 h reflux; iii) NaH, THF, HMPA, $H_2C{=}CH(CH_2)_3Br$, 2 h, reflux; iv) **13b** (4 mol%),C_6H_6, 60^0C, 6-14h.

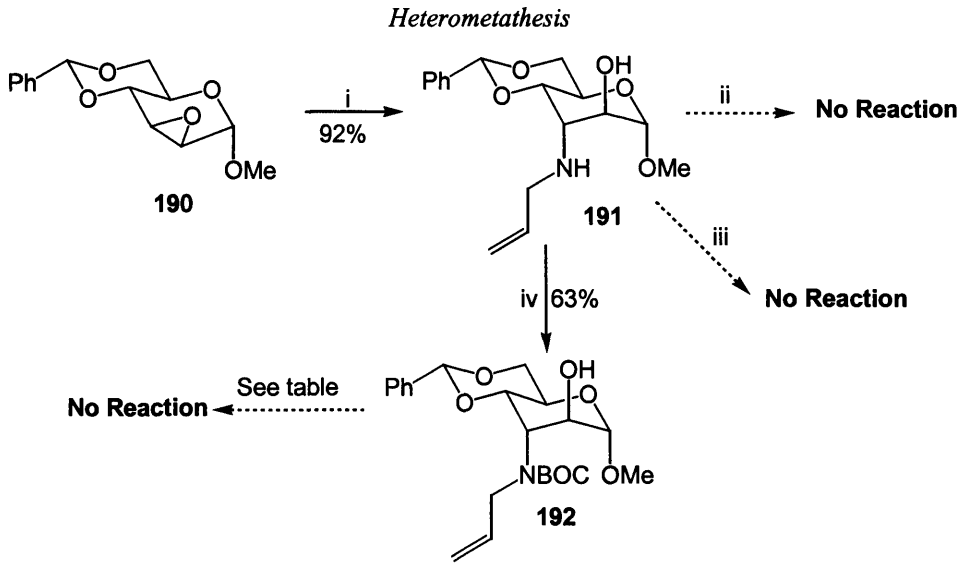
The addition of lithium aluminium hydride or methyl lithium to ketone **181** produced alcohols **182a** and **182b** in 90 and 86% yield respectively. Conversion to the ethers **183a-c** was accomplished in 78-87% yield and the three RCM reactions occurred to afford oxepine derivatives **184a-c**. Alcohol **182a** was converted into the ether **185** in 83% yield, which furnished the oxo-cyclononene **186** as product of RCM.

We opted to start with the cheap and readily available methyl-α-D-glucopyranoside **187** (Scheme 8). An acetal protection of the C-4 and C-6 hydroxyl groups with benzaldehyde dimethyl acetal effectively locks the sugar moiety into its pyranosidic form in a fairly rigid chair-chair conformation.[55] Reaction of the diol **188** with *p*-toluene sulfonyl chloride furnishes exclusively the C-2 tosyl ether **189.**[56] Deprotonation of the remaining hydroxyl group effects an intramolecular nucleophilic tosyloxy displacement producing the "up-epoxide" **190**[57] which served as a convenient precursor for our RCM studies.



Scheme 8, Reagents and Conditions; i) benzaldehyde dimethyl acetal, DMF, *p*-toluene sulfonic acid, 65°C, 3 h; ii) *p*-toluene sulfonyl chloride, CH_2Cl_2, DMAP, Et_3N, rt, 2.5 h; iii) NaH, DMF 0°C then 2.5 h, rt.

All the above products were white crystalline solids that were easily prepared and purified on a large scale by recystallisation. Treatment of the epoxide **190** with allylamine and lithium perchlorate which acted as a catalyst[58] (Scheme 9) gave the amino alcohol **191** *via* a *trans* diaxial opening and gave once again the characteristic allylic pattern in the NMR (as described earlier for compound **157**). The amino alcohol was subjected to Swern oxidation which was deemed unsuccessful since the signal for H-2 was still present in the NMR spectra.



Scheme 9, Reagents and Conditions; i) allylamine, $LiClO_4$, MeCN, 22 h, 90°C; ii) DMSO, $(COCl)_2$, CH_2Cl_2, -78°C, then Et_3N, 24 h; iii) p-toluene sulfonyl chloride, CH_2Cl_2, DMAP, Et_3N; iv) Et_3N, 30 mins, rt then BOC anhydride, 50°C, 80 h, CH_2Cl_2

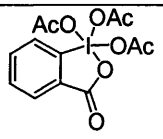
	Conditions employed for the oxidation of 191
1	DMSO, TFAA, Et_3N
2	PCC, Acetic anhydride
3	DMSO, Acetic anhydride
4	PDC, Acetic anhydride, DMF
5	TPAP, NMO, mol sieves, DCM
6	Dess-Martin periodinane 

Table 5: Conditions employed for the oxidation of 191

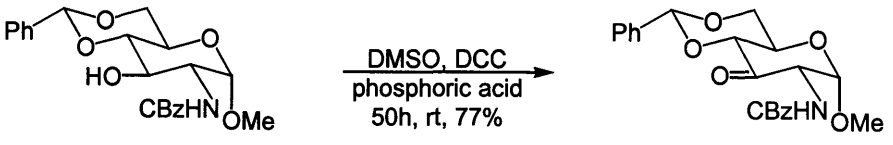
A number of other oxidation conditions detailed in Table 5 were implemented with limited success. In the case of PCC, PDC and TPAP a brown gum was recovered which could not be analysed since it was only sparingly soluble. The amine may have coordinated to the metal causing the molecule to decompose. Employment of DMSO and Dess-Martin periodinane starting material was recovered. Attempts were then made to protect the amino functionality. Firstly tosylation was employed which proved unsuccessful, since both the alcohol and the amine were tosylated, thus a *tert*-butoxycarbonyl (BOC) group was used producing **192**. This group was chosen since it can be cleaved relatively easily using 3M hydrochloric acid. Once protected the oxidation conditions that were previously tried were employed once again to no avail, either starting material was recovered, or the products formed possessed uninterpretable NMR. These inherently negative results led use to cease work in this area.

1.9 Conclusion

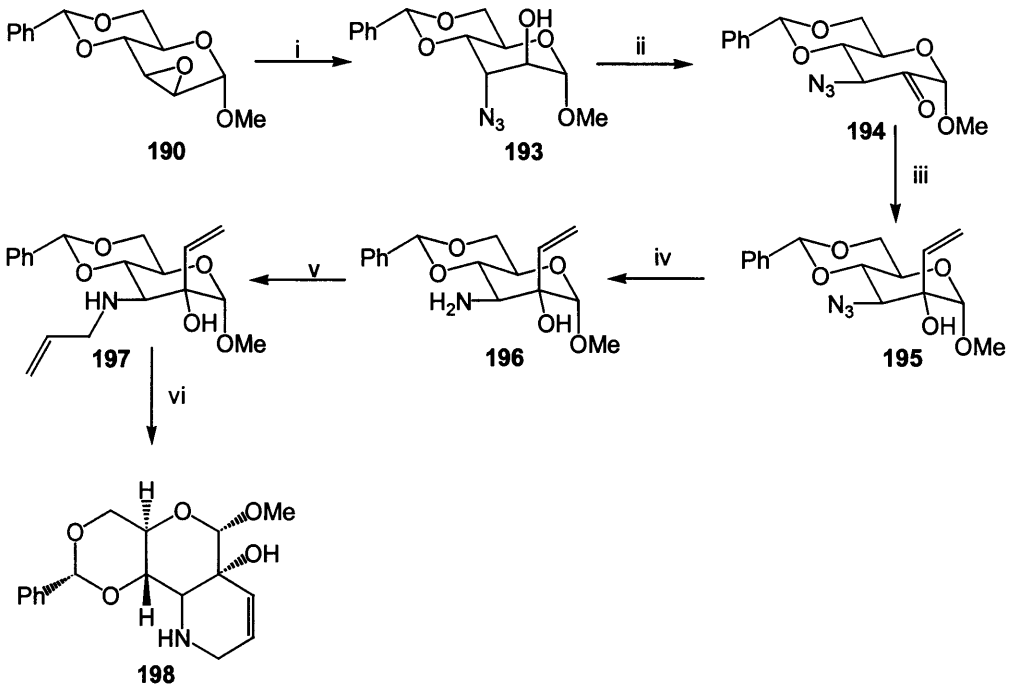
Ring closing metathesis exclusively involves the reaction between two olefins to form a cyclic structure with formal loss of ethane. Metathesis of other double bonds for example carbon-nitrogen is not known. In this chapter we have constructed a substrate with an olefin and an oxime and have studied its ring closure metathesis. Our results show that metathesis does indeed occur, but that the reaction takes place on the olefin of two separate molecules rather than the olefin and the oxime of the same molecule. It would appear that the oxime double bond is far less reactive to the ruthenium catalyst **13b** compared to the olefin.

In order to remove the possibility of the olefin metathesis a bis-imine was constructed and subjected to RCM conditions. No metathesis was observed with these substrates. It is known from the literature[59] that RCM is not a successful reaction when basic nitrogen's are present. A possible explanation maybe the lack of reactivity of the imine and oxime in metathesis and the coordination of the nitrogen to the catalyst which results in deactivation.

Studies on the preparation of azasugars using RCM were also studied. Difficulties were observed in preparation of the substrates in the metathesis reaction. We were able to successfully open the epoxide with allylamine, however subsequent oxidation of the alcohol was unsuccessful even on the boc protected form. Klemer *et al.*[60] were able to oxidise a similar CBz protected compound in 77% yield using the Pfitzner-Moffatt[61] reagents shown below. Our oxidation may have failed due to the stereochemistry of the initial alcohol.



An alternative reaction could incorporate the use of an azide nucleophile to open the epoxide ring (Scheme 10). This would produce a protected amine functionality **193**, which would then enable the oxidation of the secondary alcohol to take place. Reaction with vinyl grignard would afford the vinyl alcohol **195**. The azide functionality could then be reduced down to the amine **196** and reacted with allyl bromide to furnish the diolefins **197** which could undergo RCM. The drawback to this alternative reaction scheme is the number of steps involved.



Scheme 10;Reagents and Conditions; i) NaN_3, NH_4Cl, reflux, ii) DMSO, $(COCl)_2$, iii) vinyl grignard, iv) PPh_3 v) allyl grignard, vi) **13b**

1.10 References

1. Grubbs, R.H., Chang, S., *Tetrahedron*, **1998**, *54*, 4412; Schuster, M., Bleckert, S., *Angew.Chem.Int.Ed.Engl*, **1997**, *36*, 2036; Maier, M.E., *Angew.Chem.Int.Ed.Engl.*, **2000**, *39*, 2073
2. Anderson, A.W., Merckling, M.G., *Chem.Abstr.*,**1955**, *50*, 3008i
3. Natta, G., Dall'Asta, G., Mazzanti, G., *Angew.Chem.Int.Ed.Engl.*, **1964**, *3*, 723
4. Calderon, N., Chen, H.Y., Scott, K.W., *Tetrahedron Lett.*, **1967**, *34*, 3327
5. Mol, J.C., Moulijn, J.A., Boelhouwer, L., *Chem.Commun*, **1968**, 633
6. Herrison, J.L., Chauvin, Y., *Makromol.Chem*, **1970**, *141*, 161
7. Grubbs, R.H., Tumors, W., *Science*, **1989**, *243*, 907
8. Kress, J., Aguero, A., Osborn, J.A., *J.Mol.Catal.*, **1986**, *36*, 1; Ivin, K.J., Osborn, J.A., *J.Mol.Catal.*, **1988**, *46*, 351; Kress, J., Osborn, J.A., Ivin, K.J., *Chem.Commun.*, **1989**, 1234
9. Schaverian, C.J., Dewan, J.C., Schrock, R.R., Feldman, J., Cannitzo, L.F., Grubbs, R.H., *Macromolecules*, **1987**, *20*, 1172; Schrock, R.R., Krouse, S.A., Knoll, K., Feldman, J., Schaverian, C.J., Dewan, J.C., Liu, A.H., *J.Chem.Soc.*, **1988**, *110*, 1423
10. Schrock, R.R., Murdzek, J.S., Bazan, G.C., Robbins, J., DiMare, M., O'Regan, M., *J.Am.Chem.Soc.*, **1990**, *112*, 3875
11. Nguyen, S.T., Grubbs, R.H., Ziller, J.W., *J.Am.Chem.Soc.*, **1993**, *115*, 9858
12. Schwab. P., France, M.B., Ziller, J.W., Grubbs, R.H., *Angew.Chem.Int.Ed.Engl.*, **1995**, *34*, 2039
13. Grubbs, R.H., *J.Macromol.Sci-Pure Appl.Chem.*, **1994**, *A31*, 1829
14. Scholl, M., Ding, S., Lee, C.W., Grubbs, R.H., *Organic Lett*, **1999**, *1*, 953
15. *"Alkene Metathesis in Organic Synthesis"*, Ed. Fürstner, A., Springr-Velag, Berlin, **1998**
16. Tsuji, J., Hashiguch, S., *Tetrahedron.Lett.*, **1980**, *21*, 2955
17. Fu, G.C., Grubbs, R.H., *J.Am.Chem.Soc.*, **1992**, *114*, 5426; Fu, G.C., Grubbs, R.H., *J.Am.Chem.Soc.*, **1993**, *115*, 3800;; Miller, S.J., Kim, S.H., Chen, Z.R., Grubbs, R.H., *J.Am.Chem.Soc.*, **1995**, *117*, 2108
18. Kirkland, T.A., Grubbs, R.H., *J.Org.Chem.*, **1997**, *62*, 7310
19. Ackermann, L., El-Tom, D., Fűrstner, A., *Tetrahedron*, **2000**, 56, 2195
20. Crimmins, M.T., King, D.N., *J.Org.Chem.*, **1996**, *61*, 4192
21. Sturino, C.F., Wong, J.C.Y., *Tetrahedron Lett.*, **1998**, *39*, 9623
22. Rutjes, F.P.J.T., Kooistra, T.M., Hiemstra, H., Schoemaker, H.E., *Synlett*, **1998**, 192

23. Wenz,M., Groβbach, D., Beitzel, M., Bleckert, S., *Synthesis*, **1999**, *4*, 607
24. Dyatkin, A.B., *Tetrahedron Lett*, **1997**, *38*, 2065
25. Bassindale,M.J., Edwards, A.S., Harnley, P., Adams, H., Harrity, J.P.A., *Chem.Commun*, **2000**, *12*, 1035
26. Huwe, C.M., veider, J., Bleckert, S., *Angew.Chem.Int.Ed.Engl.*, **1996**, *35*, 2376.
27. Fujimura, O, Grubbs, R.H., *J.Org.Chem*, **1998**, *63*, 824; Fujimura, O, Grubbs, R.H., *J.Am.Chem.Soc.*, **1996**, *118*, 2499
28. La, D.S., Alexander, J.B., Cefalo, D.R., Hoveyda, A.H., Schrock, R.R., *J.Am.Chem.Soc.*, **1998**, *120*, 9720
29. Wallace, D.J., Cowden, C.J., Kennedy, D.J., Ashwood, M.S., Cottrell, I.F., Dolling, U.H., *Tetrahedron Lett.*, **2000**, *41*, 2027
30. Bertinato, P., Sorensen, E.J., Meng, D., Danishefsky, S.J., *J.Org.Chem.*, **1996**, *61*, 8000; Meng, D., Betinato, P., Balog, A., Su, D., Kamenecka, T., Sorensen, E.J., Danishefsky, S.J., *J.Am.Chem.Soc.*, **1997**, *119*, 10073
31. Kim, S.H., Figueroa, I., Fuchs, P.L., *Tetrahedron Lett.*, **1997**, *38*, 2601
32. Clark, J.S., Hamelin, O., *Angew.Chem.Int.Ed.Engl.*, **2000**, *39*, 372
33. Overkleeft, H.S., Pandit, U.R., *Tetrahedron Lett.*, **1996**, *37*, 547
34. Zielgler, F.E., Wang, Y., *J.Org.Chem.*, **1998**, *63*, 7920
35. Fürnster, A., Muller, T., *J.Org.Chem.*, **1998**, *63*, 424
36. Leeuwenburgh, M.A., Overkleeft, H.S., van der Marel, G.A., Van Boom, J.H., *Synlett*, **1997**, 1263
37. Holt, D.H., Barker, W.D., Jenkins, P.R., Panda, J., Ghosh, S., *J.Org.Chem*, **2000**, *65*, 482
38. Holt, D.H., Barker, W.D., Jenkins, P.R., Davies, D.L.,Garatt, S., Fawcett, J., Russell, D.R., Ghosh, S.,*Angew.Chem.Int.Ed.Engl.*, **1998**, *37*, 3298
39. Kafferer, P., Sarabia, F., Vasella, A., *Helv.Chim.Acta.*, **1999**, *82*, 645
40. Evans, P.A., Murthy, V.S., *J.Org.Chem.*, **1998**, *63*, 6768
41. Fu, G.C., Grubbs, R.H., *J.Am.Chem.Soc.*, **1992**, *114*, 7324; Fu, G.C., Nguyen, S.T., Grubbs, R.H., *J.Am.Chem.Soc.*, **1993**, *115*, 9856
42. Huwe, C.M., Bleckert, S., *Tetrahedron Lett*, **1995**, *36*, 1621
43. Huwe, C.M., Bleckert, S., *Synthesis*, **1997**, 61
44. Miller, J.F., Blackwell, H.E., Grubbs, R.H., *J.Am.Chem.Soc.*, **1996**, *118*, 9606.
45. Abell, A.D., Gardiner, J., Phillips, A.J., Robinson, W.T., *Tetrahedron Lett.*, **1998**, *39*, 9563
46. Arisawa, M., Takezawa, E., Nishida, A., Mov, M., Nakagawa, M., *Synlett*, **1997**, 1179

47. Osipov, S.N., Bruneau, C., Picquet, M., Kolomiets, A.F., Dixneuf, P.H., *Chem.Commun.*, **1998**, 2053
48. Cantrell, G.K., Meyer, T.Y., *J.Am.Chem.Soc.*, **1998**, *120*, 8035; Cantrell, G.K., Meyer, T.Y., *Organometallics*, **1997**, *16,* 5381
49. Rocklage, S.M., Schrock, R.R., *J.Am.Chem.Soc.*, **1980**,*102*, 7808; Rocklage, S.M., Schrock, R.R., *J.Am.Chem.Soc.*, **1982**, *104*, 3077
50. Myer, K.E., Walsh, P.J., Bergman., R.G., *J.Am.Chem.Soc.*, **1994**, *116*, 2669
51. McInnes, J.M., Mountford, P., *Chem.Commun.*, **1998**, 1669
52. La Chapelle, M., St-Jacques, M., *Tetrahedron.*, **1988**, *44*, 5033
53. Manske; I., *J.Chem.Soc.,* **1926**, 2348
54. *"Frontier Orbitals and Organic Chemical Reaction",* Fleming, I., London, Wiley, **1976**
55. Evans, M.E., *Carbohydr.Res.*, **1972**, *21*, 473
56. Hicks, D.R., Fraser-Reid, B., *Synthesis*, **1974**, 203
57. Pougny, J.R., Sinay, P., *J.Chem.Res.*, (M), **1982**, 186
58. Vega-Perez, M.; Candela, I.; Vega, M.; Iglesias-Guerra, F. *Carbohydr. Res.* **1995**, *279*, C5
59. Rutjes, F.P.J., Schoemaker, H.E., *Tetrahedron Lett.*, **1997**, *38*, 677
60. Klemer, A., Wilbers, H., *Liebigs Ann.Chem.*, **1985**, *12*, 2328
61. Pfitzer, K.E., Moffatt, J.G., *J.Am.Chem.Soc.*, **1963**, *85*, 3027

Chapter 2

The Synthesis of Aziridines

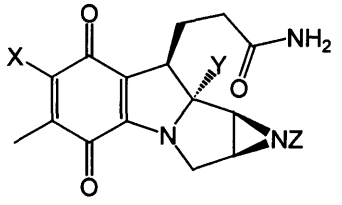
2.1 Introduction[1,2]

Aziridines are saturated three membered heterocycles containing one nitrogen atom. The first synthesis of these types of compounds dates back to 1888.[3] Like other three-membered rings such as epoxides and cyclopropanes, ring strain renders the aziridine susceptible to ring opening reaction. It is these reactions that dominate their chemistry and makes them useful synthetic intermediates.

A variety of synthesis to construct aziridines is available. One can start from enantiomerically pure sources such as amino acids and carbohydrates or from asymmetric transformation of C-C double bonds. These two general approaches are discussed.

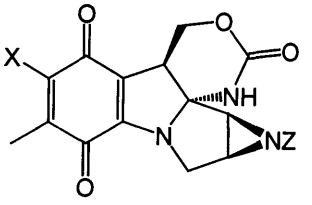
2.2 Biological Activity of Aziridines

A number of molecules possessing an aziridine ring have been shown to exhibit potent biological activity, which is associated with the reactivity of the strained heterocycle. Mitomycin A, B and C **199** along with Mitiromycin **200**, represent an important class of naturally occurring mitosanes, isolated from soil extracts of *Streptomyces verticillatus*. They exhibit both anti tumour (since they are able to cross-link with DNA) and antibiotic activity.[4]



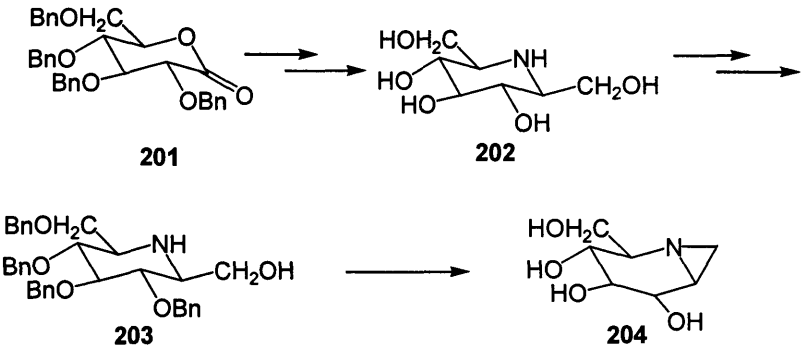
199

A. X = OMe Y = Me Z = H
B. X = OMe Y = H Z = Me
C. X = NH_2 Y = Me Z = H

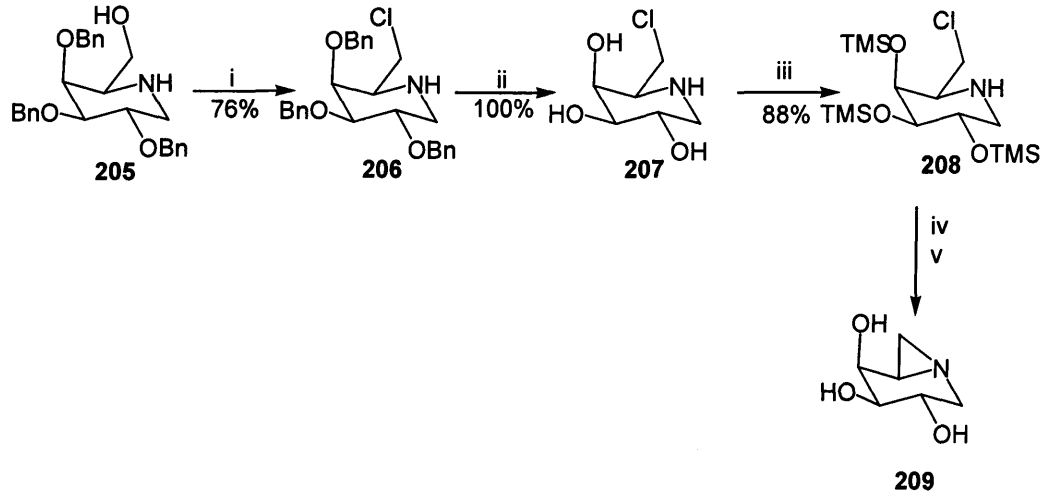


200

Whilst synthesising β-homonojirimycin **202** from the lactone **201,** Saavedra and co-workers was able to report the preparation of a 1-*N*-anhydro derivative **204**. Such aziridines are extremely interesting as potential active site-directed irreversible inhibitors of glycosidases.[5]

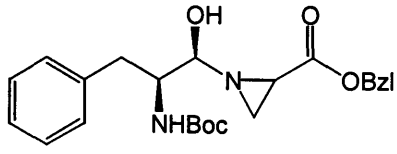


Ganem *et al.*[6] furnished the aziridinyltrol **209** from the known piperidine **205** in a number of steps (Scheme 11). Mesylation and displacement of **205** afforded the chloride derivative **206** in 76% yield. This was converted to the triol **207**and silylated to give **208.** Exposure to *n*-butyl lithium and deprotection produced **209** which displayed potent inhibition of green coffee bean α-galactosidase but had little effect on yeast α-glucosidase, jack bean α-mannosidase or bovine β-galactosidase.



Scheme 11; **Reagents and Conditions** i) MsCl, py, 20°C, LiCl, CH_2Cl_2-ether, reflux; ii) Li, NH_3; iii) TMSCl, py, H_2O; iv) *n*-butyllithium, 1 equiv, THF; v) K_2CO_3-CH_3OH, rt

The development of several competitive inhibitors and mechanism based inactivators of Substilisin BPN', which is a serine protease with a wide range of substrate specificity have been described by Zhong and co-workers.[7] Inhibition kinetics showed **210** to be a competitive inhibitor binding to the active site ~30 times more strongly than the corresponding Phe-Pro peptide.

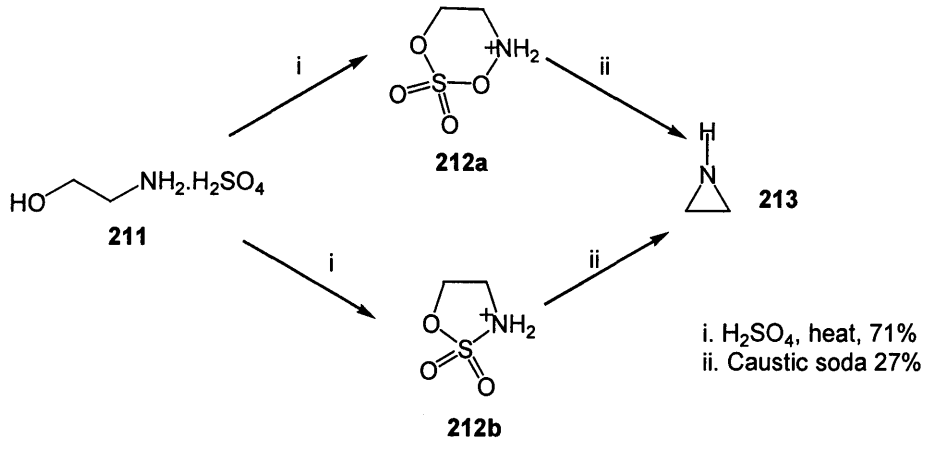


210

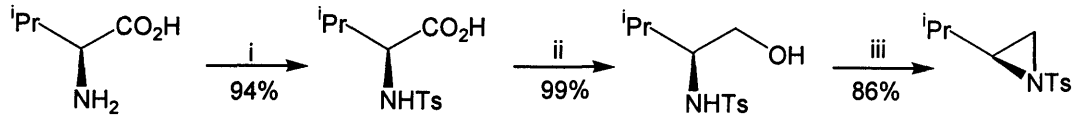
2.3 Synthesis from Amino Alcohols

An obvious synthesis of aziridines utilises 1,2-amino alcohols as precursors. The reaction can be readily achieved when the hydroxyl functional group is converted into a good leaving group. Intramolecular nucleophilic displacement reaction by either the amide anion or the amine lone pair yields the aziridine ring.

Using enantiopure amino alcohols, asymmetric production of enantiomerically pure aziridines is possible. Wenker[8] reported an example of this synthetic transformation in the preparation of achiral aziridines. The ethanolamine was converted to ethylene imine **213** *via* 'β-aminoethylsulphuric acid' **211**.



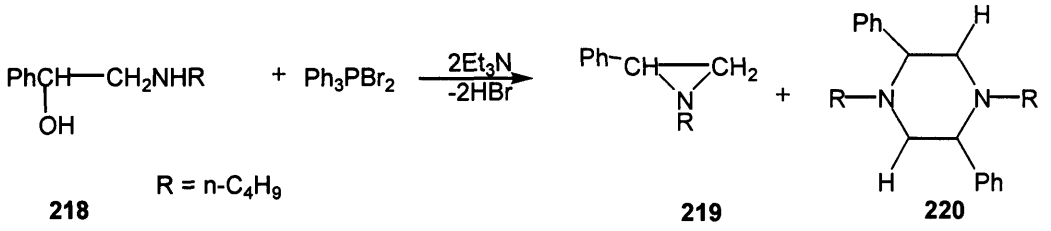
Enantiomerically pure 1,2-amino alcohols are frequently obtained via the reduction of enantiopure 2-amino acids (Scheme 12).



Scheme 12 **Reagents and Conditions** i) NaOH, 0°C, then TsCl, EtN^iPr_2, rt, 6h; ii) Et_2O, $LiAlH_4$, rt; iii) TsCl, DMAP, DCM, Et_3N, rt, 6h

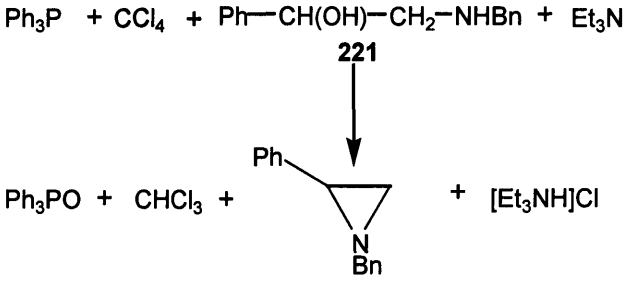
The efficiency of the aziridine formation has been hindered by the difficulty in isolating the intermediate amino alcohol due to the formation of water soluble metal complexes. Thus to overcome this problem the *N*-Ts amino alcohol **216** is furnished from reduction of the acid **215** with lithium aluminium hydride. Cyclisation then occurs in 86% yield producing **217**.[9]

There are a number of examples in the literature where triphenylphosphine has been employed where it can be exploited by being displaced as an oxyphosphonium species. Okada *et al.*[10] synthesised a variety of aziridines using triphenylphosphine dibromide in a one pot method under mild reaction conditions. Amino alcohol **218** was reacted with TPP dibromide in the presence of triethylamine in acetonitrile at room temperature to give the phenyl aziridine **219** in 60% yield as well as the piperazine **220** as a by-product.



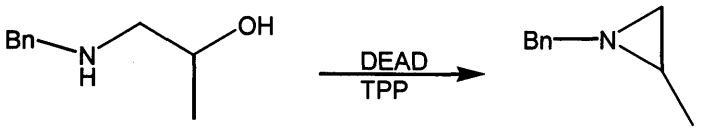
218 **219** **220**

Triphenylphosphine along with carbon tetrachloride has been used to produce aziridines from amino alcohols. Appel *et al.*[11] illustrated its use in converting amino alcohol **221** to the aziridine **222** in reasonable yield.



222

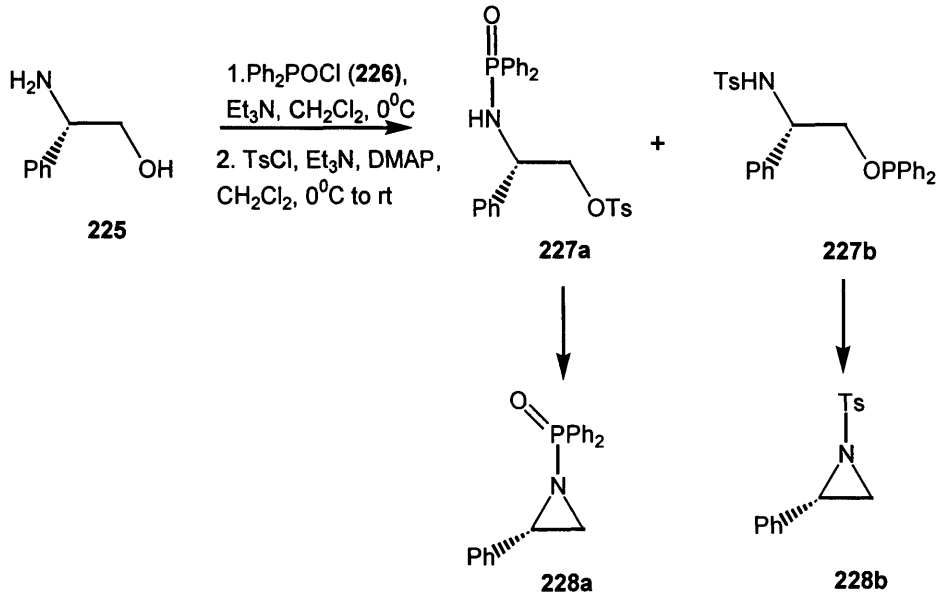
In contrast to the use of carbon tetrachloride Pfister[12] incorporated the use of DEAD thus performing a Mitsunubo type reaction on amino alcohol **223** producing the *N*-benzyl substituted aziridine **224** in 90% yield.



223 **224**

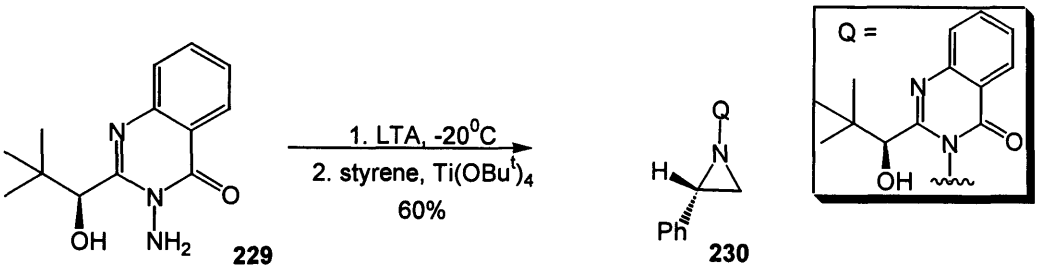
Other phosphorous reagents have been employed including the use of diphenylphosphinic chloride **226** which can be considered to be both a protecting group and nucleophile. Amino alcohol **225** was subjected to **226** and tosylation afforded two possible products **227a** and **227b**, where we see a migration of the phosphinoyl group

from nitrogen **227a** to oxygen **227b**.[13] Cyclisation using sodium hydride affords aziridines **228a** and **228b**.



2.4 Synthesis from Nitrene Addition to Alkenes

Aziridination of alkenes by direct nitrene addition resembles the peracid epoxidation of alkenes. Chiral quinazoline-4(3H)-ones have been shown to effect highly stereoselective aziridination of alkenes. The *tert*-leucine derived quinoxazoline **229** aziridinates styrene with complete diastereoselectivity in the presence of alkyl titanates. The active species an *N*-acetoxy is generated *in situ* by reaction of the precursor **229** with LTA.[14]

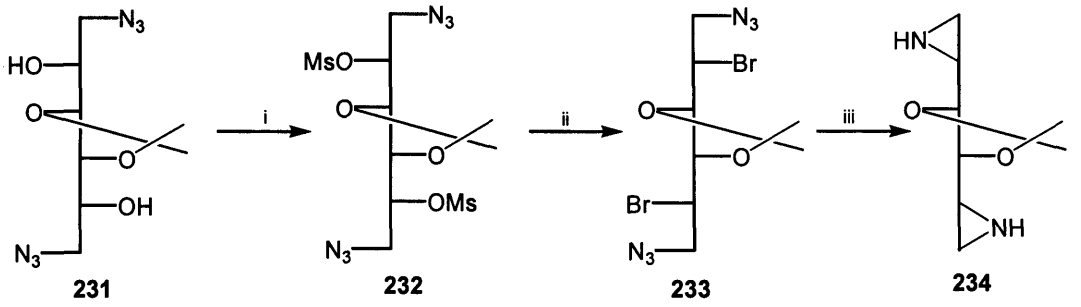


2.5 Synthesis from Carbohydrates

Sugar derivatives possess a number of stereochemical, functional and conformational attributes which make them desirable for a variety of synthetic purposes including the preparation of aziridines.

Duréault *et al.*[15] have taken the diol **231** and mesylated both the alcohol groups to furnish **232**. Nucelophilic substitution with inversion produced **233** in an overall yield of

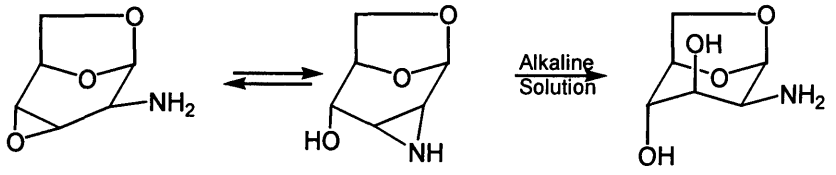
50%. The diazido dibromo compound **233** was then reduced using lithium aluminium hydride which enabled cyclisation to take place to give the bisaziridine **234** which provides a route to α-amino acids and aldehydes.



231 **232** **233** **234**

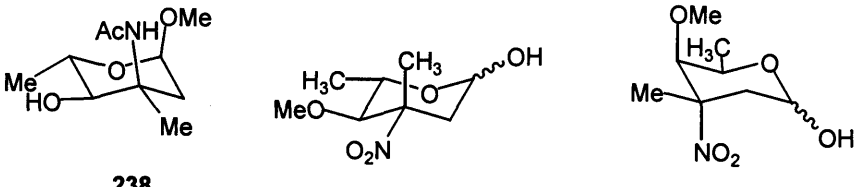
Scheme 13: **Reagents and Conditions;** i) MsCl, Py, 20°C, 20h; ii) $MgBr_2$, CH_2Cl_2-ether, 40°C, 20h; iii) $LiAlH_4$, THF, 20°C, 6h

Isomerisation of *trans*-aminoepoxides into *trans*-hydroxy aziridines has been described on numerous occasions for 1,6-anhydrohexopyranoses.[16] Thus conversion of the epimine **236** into **237** proceeds via the intermediary epoxide **235**. Further reaction of **237** synthesises 2-aminohexopyranose derivatives suitable for biological studies.



235 **236** **237**

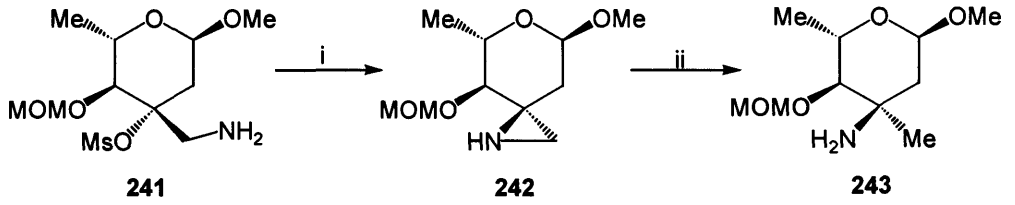
Spiroaziridines have served as useful intermediates in the synthesis of important branched chain amino sugars such as the antibiotics A35512B **238**[17], L-evernitrose **239**[18], and D-ribranitose **240**.[19]



238 **239** **240**

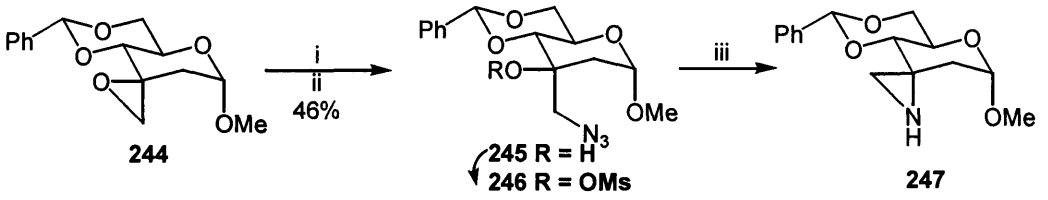
In an attempt to synthesise the novel branched chain amino sugar of antibiotic A35512B, Brimacombe *et al.*[17] produced the spiroaziridine **242** *via* cyclisation of the

mesylate **241**. Hydrogenation over Raney nickel gave **243** which serves as a convenient precursor for **238.**



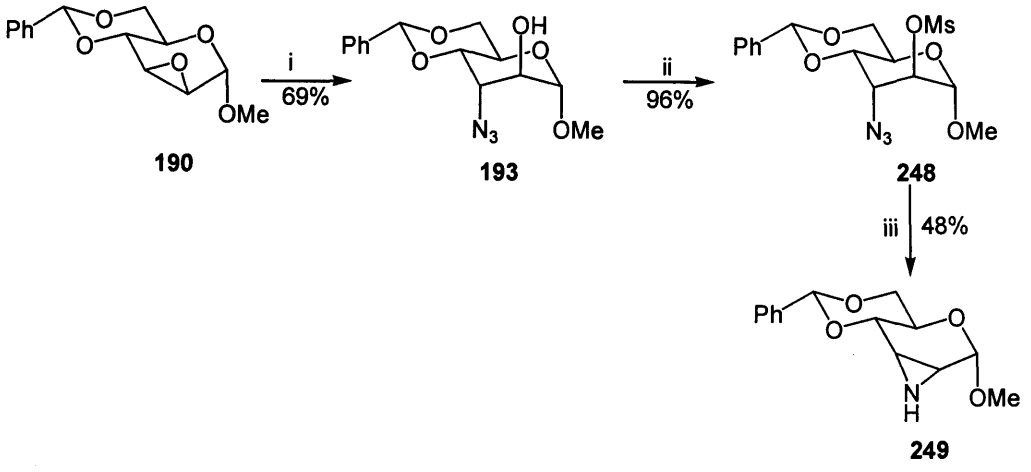
Scheme 14; Reagents and Conditions; i) $LiAlH_4$; ii) Raney nickel

Methyl-3,3-anhydro-4,6-O-benzyliene-2-deoxy-3-C-hydroxymethyl-α-D-arabinohexopyranoside **244** was reacted with sodium azide in DMF to give **245** which was mesylated upon treatment with methanesulphonyl chloride in pyridine producing **246**. Hydrogenation of **246** furnishes **247** the spiroaziridine.[20]



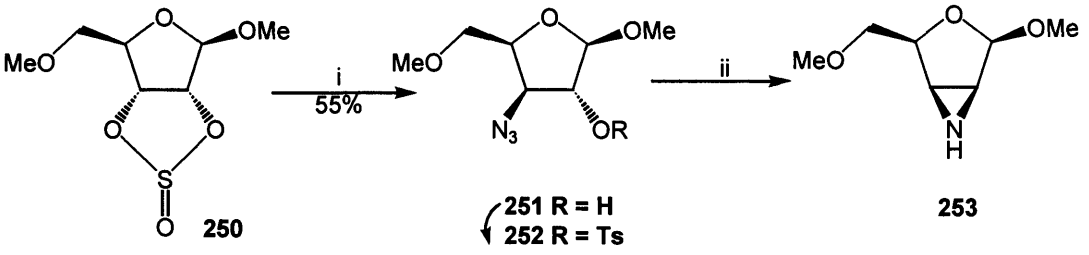
Scheme 15 ; Reagents and Conditions; i) NaN_3; ii) MsCl; iii) H_2, PtO_2

Guthrie and Murphy also incorporated the use of the azide ion in producing sugar aziridines of the type **193**. Once again the epoxide **190** is opened using sodium azide, mesylation takes place and cyclisation affords the aziridine **249** with inversion at the oxygen bearing carbon atom.[21]



Scheme 16; Reagents and Conditions; i, NaN_3, NH_4Cl,reflux, 22h, ii, MsCl, pyridine, 0^0C, then rt, iii, Raney nickel, NH_2NH_2

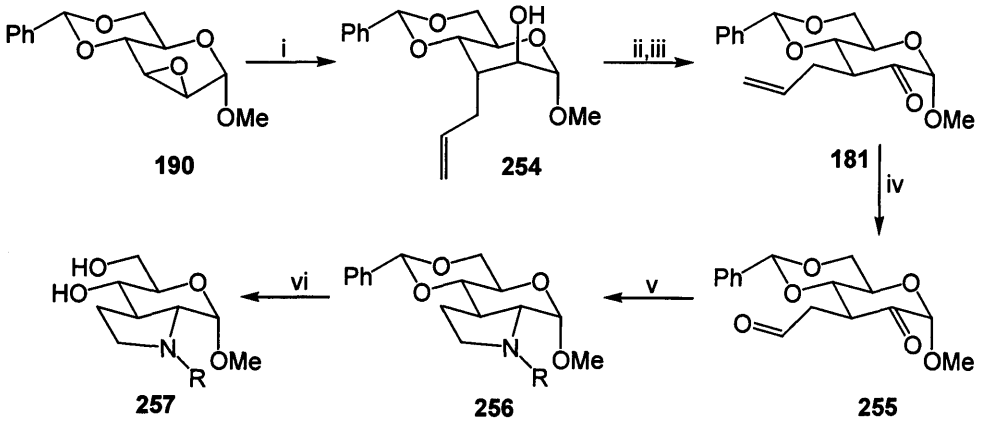
The first example of the application of the Staudinger[22] reaction in the preparation of aziridines in the furanoside series has been illustrated by Dubois *et al.*[23] They transformed the 2-3 cyclic sulphite **250** into the azido alcohol **251** Tosylation followed by treatment of triphenylphosphine afforded the furanose aziridine **253** in 60% yield, which is used as an intermediate in forming optically active amino acids.



Scheme 17; Reagents and Conditions; i) NaN_3, DMF, 155°C; ii) PPh_3, THF, 2h, then NaOH

2.6 Results and Discussion

Previous members of the Jenkins group had employed the following reaction Scheme 18 and synthesised a number of bicyclic sugars which displayed inhibition against β-galactosidase from bovine liver.[24]

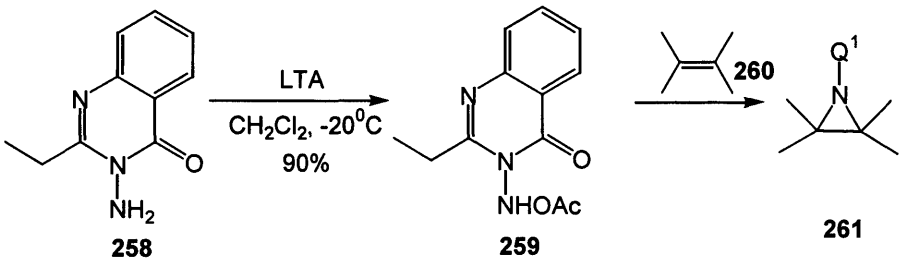


Scheme 18: Reagents and Conditions, i) allyl MgCl; ii) DMSO, $(COCl)_2$; iii) Et_3N; iv) O_3, DCM, thiourea; v) RNH_3Cl, $NaBH_3$, CN; vi) HCl, reflux

Ms Davies reacted the manno epoxide **190** with a grignard reagent to afford the *trans* allyl alcohol **254,** which was oxidised using swern conditions and epimerisation took place at C-3 using triethylamine. Ozonlysis of the alkene **181** afforded the 1,4 dicarbonyl compound **255**, which underwent reductive amination to produce the fused tricyclic structure **256**. The benzylidene group was cleaved under reflux using hydrochloric acid

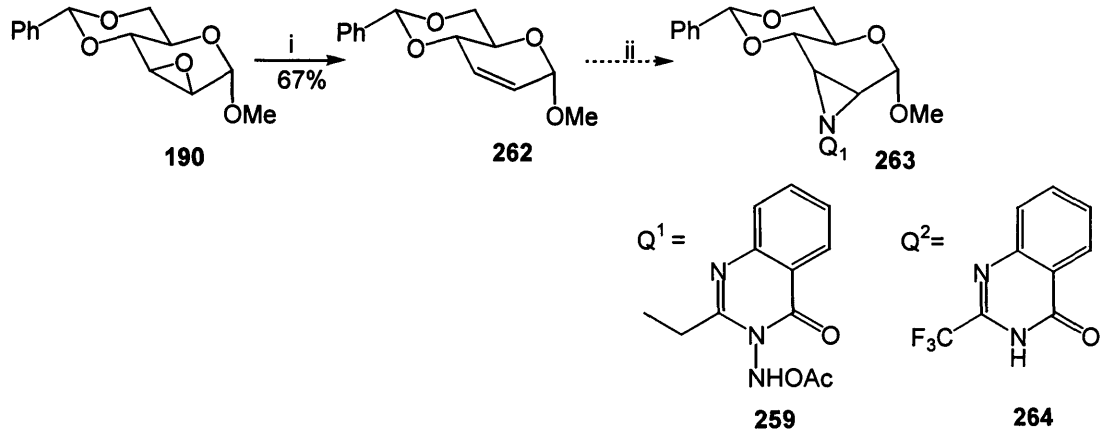
which furnished **257**. A variety of five and six membered rings were formed. The R-group corresponded to numerous functionalities. A number of these compounds displayed inhibition.[25]

From the results gained the decision was made to synthesise a smaller ring to see whether an aziridine ring fused to the sugar backbone would display inhibition. The Atkinson group (University of Leicester) had a wealth of experience of producing aziridines, incorporating the use of lead tetra acetate (LTA) and 3-aminoquinazoliones to afford these compounds.[26] An example of this is the oxidation of 3-amino-2-ethylquinazolinone **258** producing 3-acetoxyaminoquinazolinone **259** (Q^1NHOAc) which is able to bring about the aziridination of alkene **260** producing **261**.



258 **259** **261**

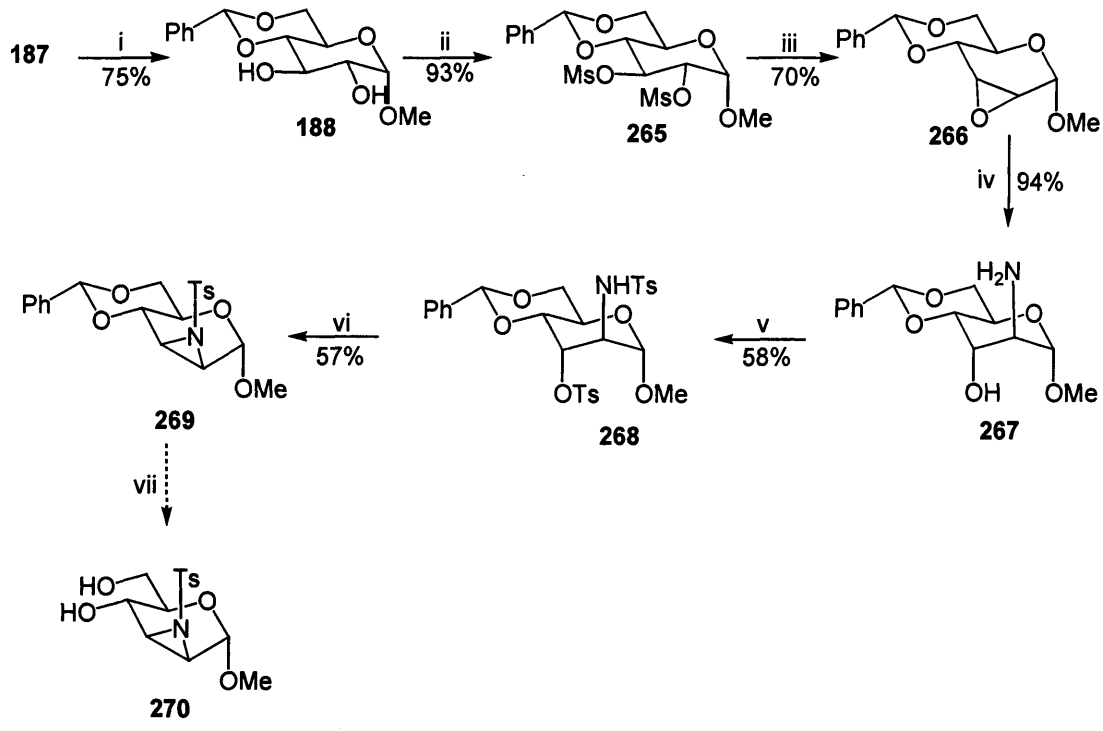
The synthesis of sugar alkene **262** was produced according to the method of Radatus *et al.*[27] The epoxide **190** was taken with zinc-copper couple and sodium iodide, to afford **262** in 67% yield (Scheme 19). The reaction proceeds by cleavage of the epoxide ring by NaI to produce the sodium alkoxide of the iodohydrin which can undergo a Boord reaction[28] to give the desired product.[29,30] The resulting alkene **262** was taken with **259** and LTA in DCM. The reaction was unsuccessful. The reaction mainly produced starting material **262** with another substance which could not be removed from the column. The reaction was repeated using quinazolinone **264** but only starting material was recovered. This may have been due to the bulky phenyl and methoxy group which could have hindered the approach of **264**, thus another method was sought.



190 262 263 259 264

Scheme 19; Reagents; i) Zn-Cu, NaI, DMF, DME; ii) $Pb(OAc)_4$, Q^1NH_2, DCM, TFA

Literature research led to the studies of Baker and Hullar.[31] They had taken the amino alcohol **267** and transformed it into the *N*-tosylaziridine **268** in a number of steps. This method was therefore followed (Scheme 20). We also decided to extend this methodology by deprotecting the *N*-tosylaziridine **269** to reveal the free diol derivative **270**.



188 265 266 267 268 269 270

Scheme 20: Reagents and Conditions, i) benzaldehyde dimethyl acetal, DMF, *p*-toluene sulfonic acid, 65°C, 3h; ii) MsCl, Et_3N, DCM, 0°C then rt for 19.5h; iii) Na, MeOH, refrigerate, 7d; iv) NH_3 (aq, conc.), 65h, 100°C; v) p-toluene sulfonyl chloride, pyridine, 50°C; vi) 1M NaOMe, rt, then CO_2; vii) various reagents and conditions-see table 6

Methyl 3-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside **267** was readily synthesised from the protected diol **188** (Scheme 20). Mesylation of **188** gave **265** in 93% yield which was taken with sodium metal in methanol and left in the refrigerator for seven

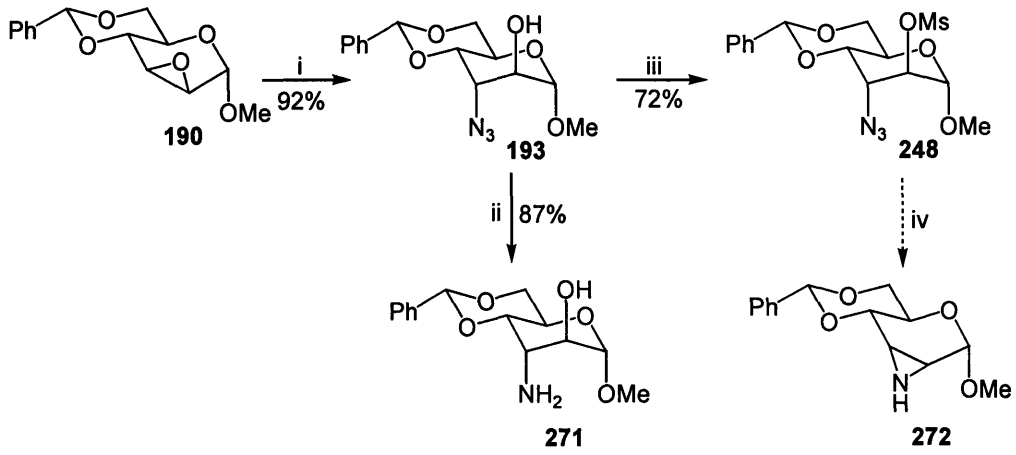
days to afford the allo epoxide **266**. Using the method of Robertson[32] **267** was synthesised in 94% yield using excess ammonia. Tosylation of the amino alcohol afforded **268**. Cyclisation of **268** in a solution of methanolic sodium methoxide at room temperature furnished the aziridine **269** in 57% yield. Ring closure was straight forward due to the correct orientation of the two substituents – *trans* diaxial. Hence the readily formed anion of the sulfonamide nitrogen is ideally positioned to effect displacement of the tosylate ion. A number of deprotection methods were employed to cleave the benzylidene group which would allow for the deprotection of the amino functionality (Table 6).

Entry	Conditions
1	H_2SO_4, $BaCO_3$, MeOH
2	60% acetic acid
3	Amberlite CG-120 Na+ form, 80% MeOH
4	PPTS, EtOH
5	I_2, MeOH
6	Na, NH_3
7	$FeCl_3$, DCM
8	20% $Pd(OH)_2$ on carbon, EtOH, cyclohexene

Table 6: Deprotection methods/conditions employed to remove the benzylidene group from **269**

All proved unsuccessful. Starting material was mainly recovered in the majority of cases, with the exception of entry 4, 6 and 7 which produced an orange sticky gum. The tosyl group may have made the aziridine ring more susceptible to ring opening. Ali *et al.*[33] have reported that under acidic conditions the free aziridine undergoes hydrolysis of the benzylidene group without rupture of the aziridine ring, but its *N*-substituted derivatives predominately undergo diaxial ring opening before hydrolysis of the benzylidene group. Thus the free aziridine was sought. In order for this to occur one used an azide ion to open the epoxide which can be looked on as a blocked amino group from which the free amino group can be obtained by mild reduction. The azide ion is a powerful nucleophile and when reacted in boiling aqueous 2-methoxy ethanol with the manno epoxide **190** in the presence of ammonium chloride produced the 3-azido-3-deoxy-α-D-altro derivative **193**. It had been reported that cyclic[34] and acyclic[35] vicinal azido-alcohols undergo cyclisation to furnish the aziridine when treated with tertiary phosphines. However when **193** was treated with triphenylphosphine only the 3-amino-3-deoxy altroside derivative **271** was

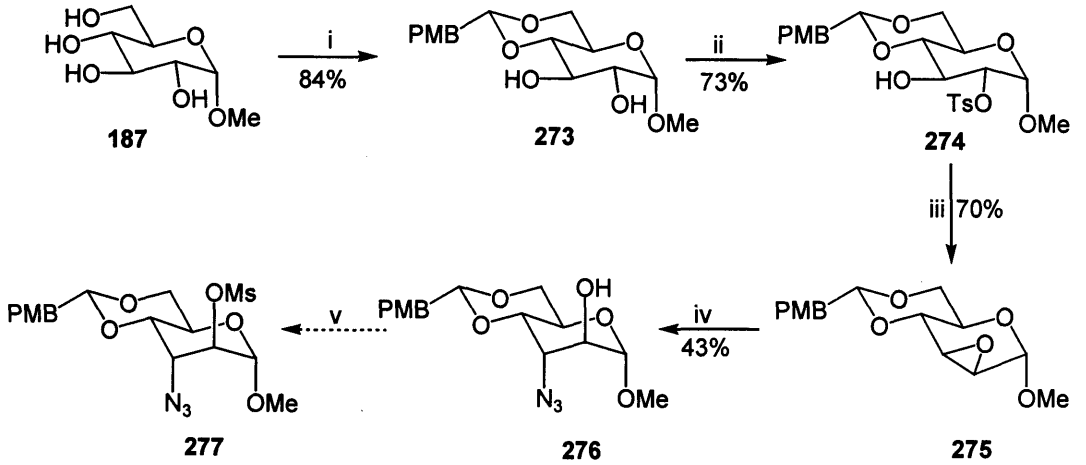
isolated. This was confirmed by IR which saw the disappearance of the azide peak at 2102 cm^{-1}.



Scheme 21; Reagents and Conditions; i) NaN_3, NH_4Cl,reflux, 22h, ii) PPh_3, iii) MsCl, pyridine, iv) Raney nickel, NH_2NH_2

Another method employed was to convert the alcohol into a better leaving group by converting it to the sulfonate ester **248** in the standard way. **248** was subjected to alkaline reduction using hydrazine hydrate and Raney nickel. Unfortunately this method failed after a number of attempts and changes to the reaction conditions, and only starting material was recovered.

Since the benzylidene proved difficult to remove, from the literature[36] it was discovered that the *p*-methoxy benzylidene group can be removed ten times more quickly than its original counterpart. Scheme 22 was followed with *p*-methoxy benzylidene protecting group. We successfully opened the epoxide **275** using sodium azide in 43% yield, but were unable to mesylate the alcohol. No identifiable substance was recovered.



Scheme 18, Reagents and Conditions; i) *p*-anisaldehyde dimethyl acetal, DMF, *p*-toluene sulfonic acid, 65°C, 3 h; ii) *p*-toluene sulfonyl chloride, CH_2Cl_2, DMAP, Et_3N, rt, 2.5 h; iii) NaH, DMF 0°C then 2.5 h, rt. iv) NaN_3, NH_4Cl, reflux, 22h, v) MsCl, pyridine, Et_3N, 0°C

2.7 Conclusion

The Mitomycin class of natural products containing an aziridine ring portray anticancer activity. A limited number of examples of carbohydrate aziridines have been synthesised as intermediates in the synthesis of diamino sugars[37], amino-halo sugars[38], and unsaturated sugars.[39] As part of a continuing study of aza annulated carbohydrates as novel glycosidase inhibitors, we carried out a study on the synthesis of a carbohydrate aziridine. We successfully synthesised the protected *N*-tosyl aziridine **269** in 57% yield. All attempts at deprotection of the *N*-tosyl aziridine **269** lead to decomposition. We also changed the protecting group of the sugar from a benzylidene group to a *p*-methoxybenzylidene, we were only able to synthesise the azido alcohol and so testing of an NH aziridine was not possible.

2.8 References

1. Tanner, D., *Angew.Chem.Int.Ed.Engl.*, **1994**, *33*, 599
2. Osborn, H.M.I., Sweeny, J., *Tetrahedron Asymm.*, **1997**, *8*, 1693
3. Gabriel, S., *Ber.Dtsch.Chem.Ges.*, **1888**, *21*, 1049
4. Kasai, M.,Kono, M., *Synlett*, **1992**, 778
5. Saavedra,K.L., Clare, A.E., Layman, R., *J.Am.Chem.Soc.*, **1990**, *112*, 3241
6. Tong, M.K., Ganem, B., *J.Am.Chem.Soc.*, **1988**, *110*, 312
7. Zhong, Z., Bibbs, J., Yuan, W., Wong,C., *J.Am.Chem.Soc.*, **1991**, *113*, 2259
8. Wenker, H., *J.Am.Chem.Soc.*, **1935**, *57*, 2328
9. Berry, M.B., Craig, D., *Synlett.*, **1992**, 41
10. Okada, I., Ichimura, K., Sudo, R., *Bull.Chem.Soc.Jpn.*, **1970**, *43*, 1185
11. Appel, R., Kleinstuck, R., *Chem.Ber.*, **1974**, *107*, 5
12. Pfister, J.R., *Synthesis.*, **1984**, 969
13. Osborn, H.M.I., Cantrill, A.A., Sweeny, J.B, Howson, W., *Tetrahedron Lett.*, **1994**, *35*, 3159
14. Atkinson, R.S., Gatrell, W.T., Ayscough, A.P., Rayner, T.M., *Chem.Commun.*, **1996**, 1935
15. Dureault, A., Greck, C., Depezay, J.C., *Tetrahedron Lett.*,**1986**, *27*, 4157
16. a) Černy, M., Elbert, T., Pácák, J., *Collect.Czech.Chem.Commun.*, **1974**, *39*, 1752; b) Černy, M., Černy, I., Pácák, J., *Collect.Czech.Chem.Commun.*, **1976**, *41*, 2942; c) Černy, I., Budésinsky, M., Trnka, T., Černy, M., *Carbohydr.Res.*, **1984**, *130*, 103
17. Brimacombe, J.S., Hanna, R., Tucker, L.C.N., *J.Chem.Soc.Perkin Trans I.*, **1983**, 2277
18. Brimacombe, J.S., Mengech, A.S., *J.Chem.Soc.Perkin Trans I.*, **1980**, 2054
19. Yoshimura, J., Yasumari, T., Kondo, T., Sato, K., *Carbohydr.Res.*, **1982**, *106*, C1
20. Brimacombe, J.S., Rahman, K.M.M., *Carbohydr.Res.*, **1983**, *113*, C6
21. Guthrie, R.D., Murphy, D., *J.Chem.Soc.*,**1963**, 5288
22. Staudinger, Meyer., *Helv.Chim.Acta.*, **1919**, *2*, 635
23. Dubois, L., Dodd, R.H., *Tetrahedron*, **1983**, *49*, 901
24. Davis, M., First Year Report
25. See Appendix 2
26. Atkinson, R., *Tetrahedron*, **1999**, *55*, 1519
27. Radatus, B.K., Clarke, I.S., *Synthesis.*, **1980**, 47
28. Dykstra, H.B., Lewis, J.F., Boord, C.E., *J.Am.Chem.Soc.*, **1930**, *52*, 3396
29. Cornforth, J.W., Cornforth, R.H., Matthew, K.K., *J.Am.Chem.Soc.*, **1959**, *81*, 112

30. Cristol, S.J., Rademacher, L.E., *J.Am.Chem.Soc.*, **1959**, *81*, 1600
31. Baker Baker, B.R., Hullar, T.L., *J.Org.Chem.*, **1965**, *30*, 4049
32. Myers, K.E., Robertson., *J.Chem.Soc.*, **1943**, *65*, 8
33. Ali, Y., Richardson, C., Gibbs, C.F., Hough, L., *Carbohydr.Res.*, **1968**, *7*, 255
34. Ittah, Y., Sasson, Y., Shahak, I., Tsaroom, S., Blum, J., *J.Org.Chem.*, **1978**, *43*, 4271
35. Chakraborty, T.K., Gangakhedkar, K.K., *Tetrahedron Lett.*, **1991**,*32*, 1897
36. Greene T.W., Wuts, P.G.M., *Protective Groups in Organic Synthesis*, 3rd Ed, John Wiley and Sons, **1999**, 224
37. Guthrie, R.D., Murphy, D., *J.Chem.Soc.*, **1965**, 3828
38. Buss, D.H., Hough, L., Richardson, A.C., *J.Chem.Soc.*, **1965**, 2736
39. Guthrie, R.D., King, D., *Carbohydr.Res.*, **1966**, *3*, 128

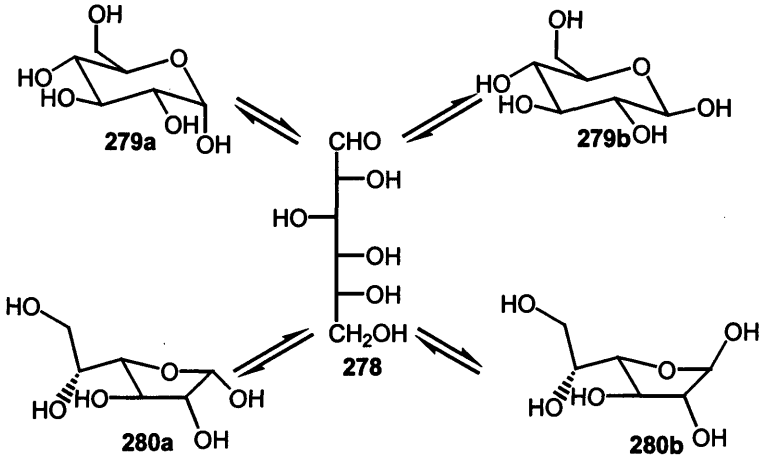
Chapter 3
Novel Glycosidase Inhibitors

3.1 Review of Carbohydrates[1]

Carbohydrates form a very large group of compounds of enormous theoretical and practical importance. As a result of many new developments this field is now broad in scope and rich in variety, touching on almost all aspects of chemistry and biology.[2] The term carbohydrate refers to the broad class of polyhydroxylated aldehydes and ketones capable of existing in cyclic or acyclic forms. They are relatively cheap and are an endless source of chiral carbon compounds; they are available in a variety of acyclic and cyclic forms, oxidation states and chain lengths. Due to these attributes carbohydrates find themselves at the forefront of chemical modification. These very features ensure a measure of regio- and stereocontrol in bond-forming reactions that are not easily matched by other classes of organic compounds.

The study of carbohydrates and their derivatives has increased our knowledge of chemistry, especially with regards to molecular shape and conformations in chemical reactions. Carbohydrates are involved in complex biochemical pathways where they provide recognition motifs that control enzyme mechanisms. They also offer a basis for the recognition of the enzymatic defects of several genetic disorders.[3]

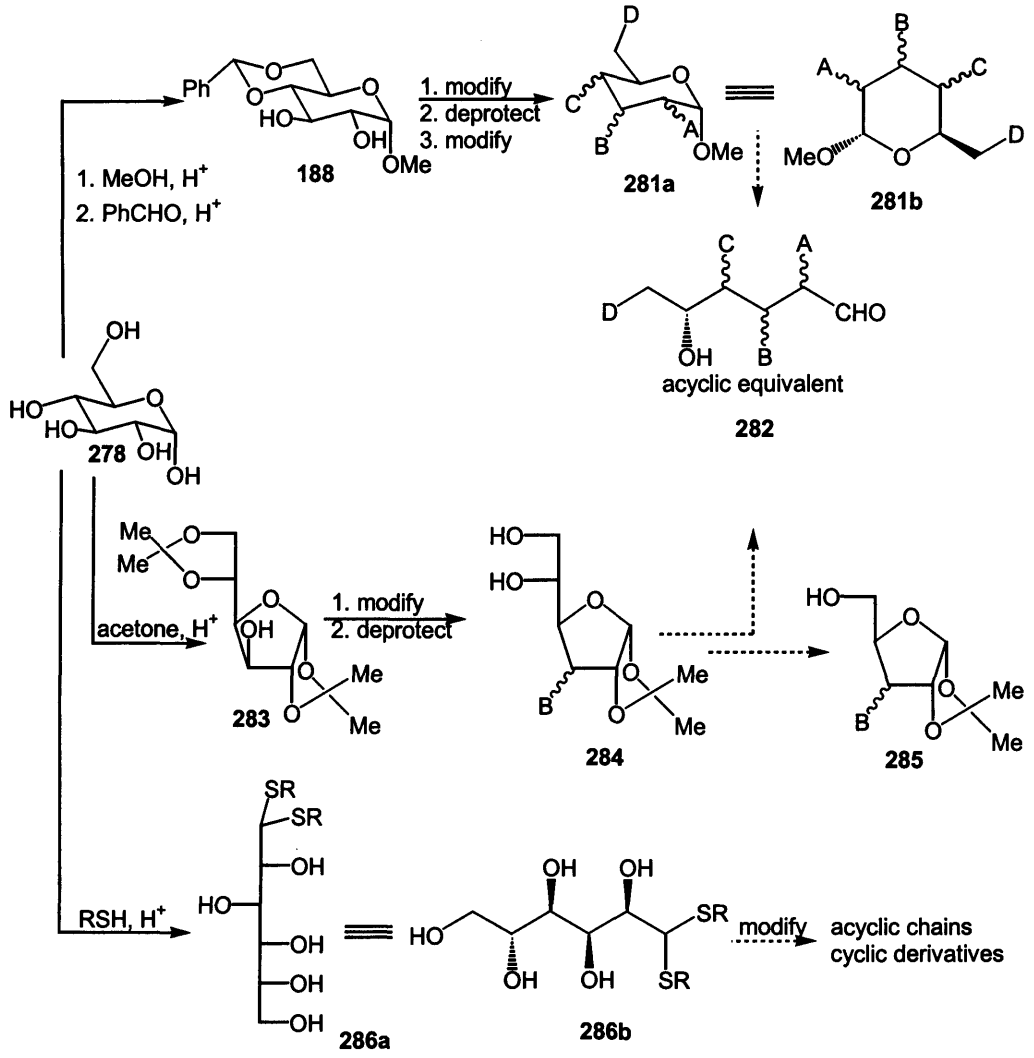
Carbohydrates are useful starting materials for the synthesis of target molecules containing multiple centres of chirality and functionality. D-glucose **276** is the most abundant monosaccharide found in nature and exists in solution as a mixture of isomers (Scheme 23). Nucleophilic attack of the oxygen atom at C-5 on the carbonyl carbon of the acyclic species **276** generates a hemiacetal ring and new asymmetric carbon at C-1, giving rise to diastereoisomeric hemiacetals **277a** and **277b**, which are named α and β anomers respectively. Cyclisation involving C-4 rather than C-5 results in a five membered ring resembling a furan, and is therefore designated as a furanose **278a** and **278b**.[4]



Scheme 23. Cyclic forms of glucose

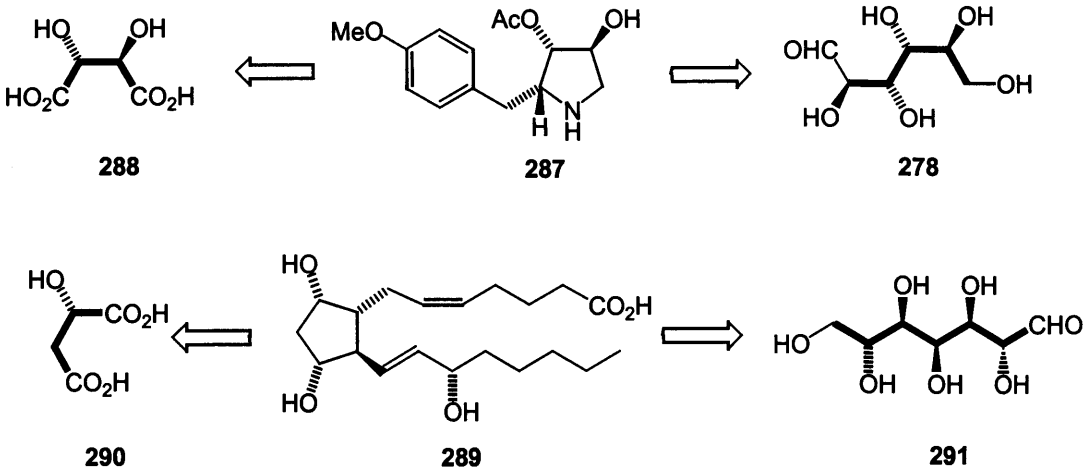
The open chain D-glucose **278** has been used as a chiral, polyhydroxylated starting material in many synthetic sequences. Conversion to a pyranose ring is readily achieved *via* reaction sequentially with methanol and benzaldehyde to produce the benzylidene derivative **188.**[5] Modification and deprotection leads to the pyranose structure **281a** more commonly represented as structure **281b.** This can also be converted into an acyclic equivalent **282.** In these well described reaction sequences enantiomerically pure intermediates are produced for heterocyclic synthesis using either **281b** or **282.** The versatility of D-glucose **278** is further illustrated by its conversion into 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose **283**[6] which is another significant compound from carbohydrates. The furanose ring is maintained in the subsequent reaction sequences leading to **284** and **285** or ring opened acyclic structure such as **282.**

D-glucose **278** can also be used directly in its acyclic form by conversion to the dialkyl dithiolacetal[7] **286a** (equivalent to structure **286b**) which can be considered for synthetic purposes. Several examples of the use of this sequence in the synthesis of enantiomerically pure acyclic compounds have been reported.[6]



Scheme 24. Key transformations used in synthetic routes starting from carbohydrates

The combination of practicality and innovation seems to be present when considering carbohydrate derived precursors in comparison to other chiral precursors such as amino acids,[8] terpenes[9] and hydroxy acids.[10] Access to some natural products from a carbohydrate and a different chiral precursor are portrayed. Anisomycin **287**[11] can be derived either from (*R,R*)-Tartaric acid **288**[12] or from D-glucose **278**. Retrosynthetic analysis of **289** leads to either (*S*)-Malic acid **290**[13] or the carbohydrate precursor **291** D-Glycero-D-gluco-heptose.[14] It is interesting to note that the carbohydrate precursor provides a greater segment (highlighted in bold) of the carbon skeleton of the target molecule, compared to the other precursors shown.



Scheme 25. Access to natural products from amino acids and carbohydrates

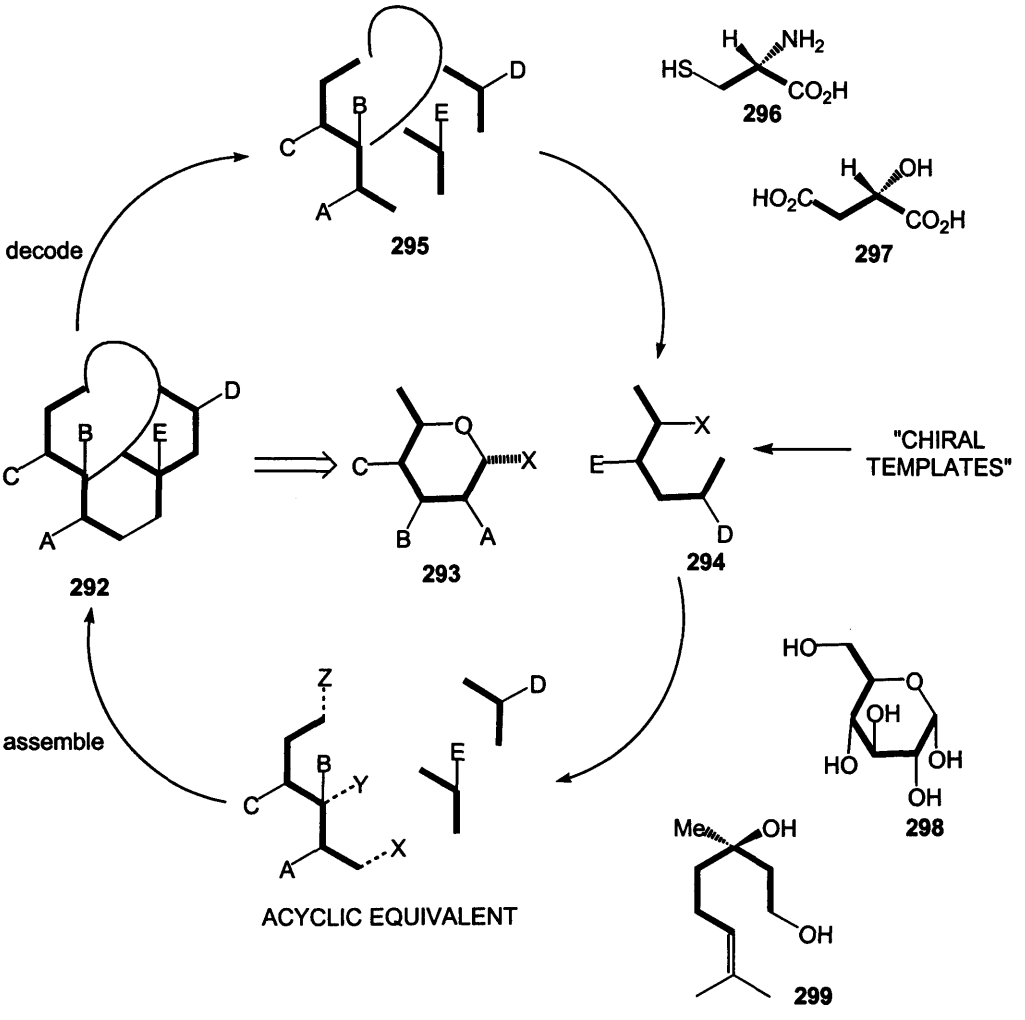
It is therefore possible to utilise carbohydrates in the construction of polyfunctional acyclic, heterocyclic and carbocyclic compounds containing one or more centres of chirality. In order to reach such targets one employs the concept of 'chiral templates', which involves a number of key aspects;

- The generation of chirons which represent enantiomerically pure synthons
- Locating elements of symmetry/similarity, chirality and functionality
- Decoding of the above information
- Transposing the information onto the carbon framework of suitable synthetic precursors.

3.1.1 The Chiron Approach

A mention should be made of the difference between 'synthon' and 'chiron'[1] to enable better understanding of the Chiron approach. Corey[15] first introduced the term

synthon to describe an idealised fragment usually a carbanion, a carbonium ion or a synthetic equivalent, produced by bond disconnection during retrosynthetic analysis. In the synthon based approach, the strategy is based on bond disconnections relating to logical, but not necessarily the most strategic sites, which take into account stereochemical features. With the need to synthesise enantiomerically pure molecules the synthon approach must make additional provisions for stereochemical control. The 'chiron' approach differs in that strategic bond disconnections proceed by locating segments containing a number of chiral centres. The target molecule **292** is scrutinised to locate the ensemble of stereochemical, functional and topological features present in the carbon framework (Scheme 26). This information is then decoded as shown in **295** and transposed onto a carbon framework of suitable synthetic precursors ('chirons') **293** and **294,** which are obtained from chiral starting materials (chiral templates). Chiral starting materials such as **298** and **299** resemble chirons **293** and **294** (synthetic equivalents) and can be assembled into the target molecule **292.** Thus the combination of natural chirality and conformational bias in carbohydrates provides an ideal carbon framework for achieving a high level of regio- and stereocontrol in the target molecule.



Scheme 26. Chiron Recognition

There are simple guidelines that one can follow in order to locate carbohydrate type symmetry in a given molecular target.[1]

- We have become familiar to the structure of the carbohydrate molecule in terms of acyclic or cyclic five and six membered ring structures, and the substitution pattern of hydroxyl groups. The carbohydrate framework can be used as a flexible platform for a variety of C-C bond forming reactions since hydroxyl groups can be systemically replaced by various other functionalities with stereocontrol. The carbohydrate structure can therefore be seen as an assembly of functional groups and substituents on a carbon backbone which can be made to adopt the topology of many targets and segments contain therein. A number of shapes not normally associated with the carbohydrate structure but which converge with the framework of the carbohydrate starting material are illustrated (Figure 1).

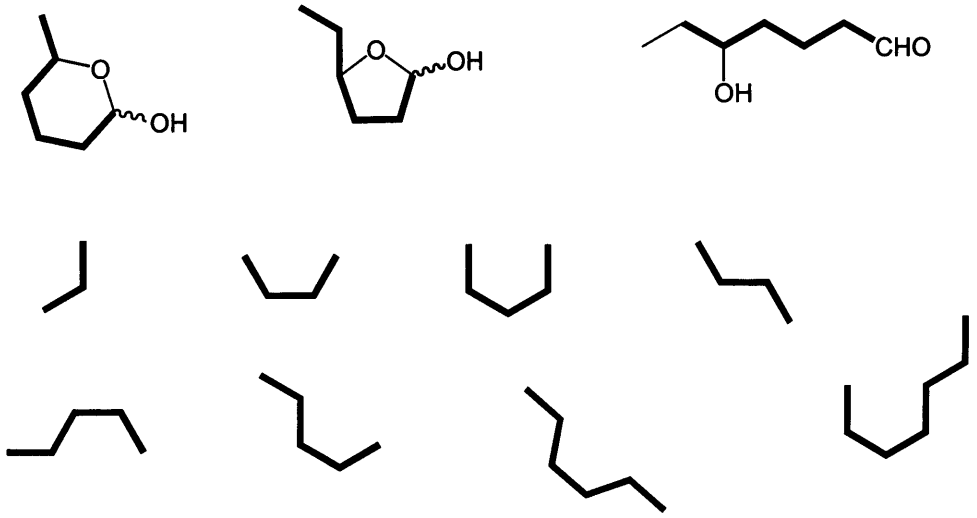
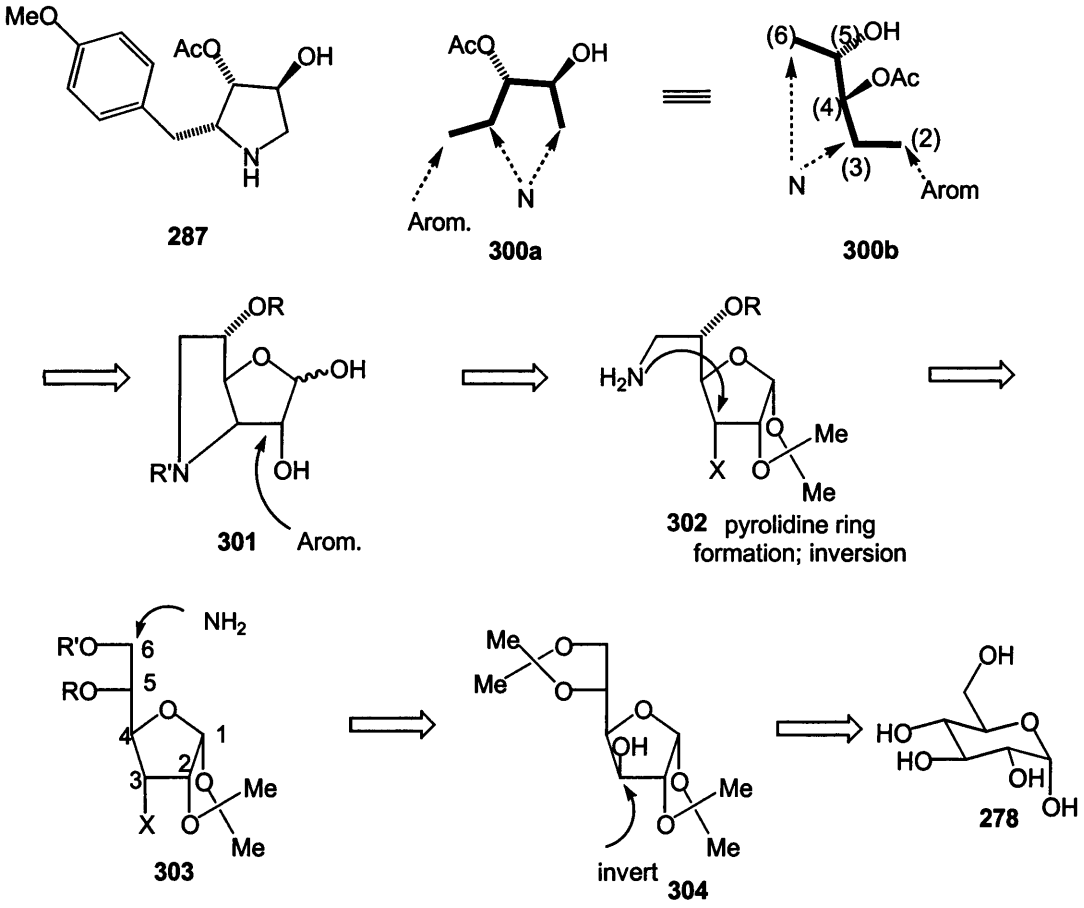


Figure 1. Carbon chains in molecules where carbohydrates may be useful starting materials

- A strategic bond disconnection is vital in order to achieve a viable chiron. The hydroxyl-aldehyde or ketone connection is important in locating the carbohydrate portion in the target. One can use the spatial relationships between carbon atoms bearing an oxygen and a given carbon atom located four or five atoms away along the chain or ring. Possible functional overlap of the carbon framework of a suitable carbohydrate with a segment of the target molecule can then be found and substituents can then be introduced by manipulation of the hydroxyl groups.
- The use of the scaffold process, which involves the manipulation of the carbohydrate structure founded on constructing a segment of the target, based on a rigid entity, such

as a ring. Once the required level of chirality and functionality is reached, the original carbohydrate portion of the molecule is unveiled to show the target. An example of this synthesis is anisomycin.[11] The retrosynthetic analysis shown below (Scheme 27) portrays how D-glucose **278** was used as a "scaffold" onto which **302** was created, spanning C-3 and C-6 of a D-glucofuranose structure **303**. The pyrrolidine ring **301** is formed by intramolecular cyclisation from a primary amine with inversion of configuration at a ring carbon. The original carbohydrate section becomes an extension of the new heterocyclic ring.



Scheme 27. RSA of Anisomycin

3.1.2 Summary

From the abundant examples present in the literature, it is clear that the use of carbohydrates in producing chiral fragments for further synthesis is an extremely powerful method. To be effective it's imperative that the starting materials are readily available and cheap and the synthetic steps to attain the target are not too numerous and are high yielding. The reactions performed need to be selective and afford the appropriate product which should be easily purified. The overall operation must also be repeatable on a

reasonably large scale. On the whole carbohydrate synthesis meets the majority of these criteria, although there is a need to improve efficiency in almost every area.

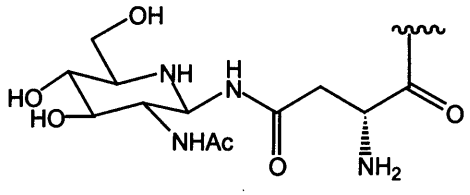
3.2 Carbohydrates in Biology

Glycobiology concentrates on the nature and role of carbohydrates in biological events. There are a number of examples of carbohydrates acting as signals for biological communication with and between cells. These include cell growth, inflammation and metastasis. Acquiring a certain understanding of the language and syntax of oligosaccharide signalling mechanisms is at the heart of glycobiology, and this promises to change the way many serious diseases are treated. Learning how to alter or block those signals could prove useful in treating diabetes,[16] HIV,[17] metastic cancer[18] and lysosomal storage diseases.[19]

Molecules in which carbohydrates are covalently bound to proteins and lipids have been termed glycoconjugates. These groups of compounds are enormous in number are variable in structure and have in a number of biological functions.

3.2.1 Glycoproteins

The vast majority of cell surface and secreted proteins are glycosylated, whereby the carbohydrate moiety is covalently linked to the protein in two ways. In *N*-linked glycoprotein's **303**, the glycan usually has an *N*-acetylglucosamine residue at its reducing termini, which is linked to an amide group of an aspargine residue in the protein chain.[20] Conversely, in *O*-linked glycoproteins **304** the glycan has an *N*-acetylgalactosamine residue at the reducing end, which is linked via a hydroxyl group of serine or threonine.[21]



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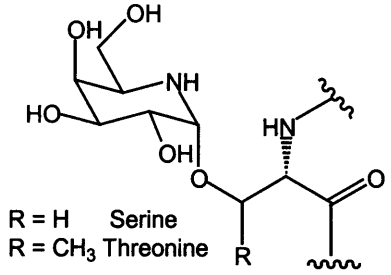
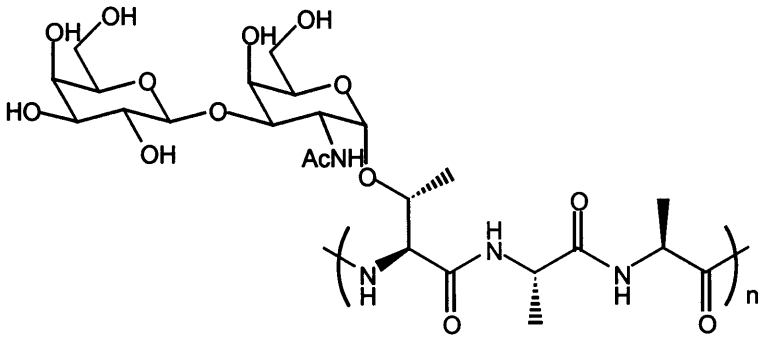


306

It has been shown that the carbohydrate moiety may participate directly in recognition events,[22] and also modify the properties of the protein. For example, they increase solubility and protect the peptide backbone against protein degrading enzymes

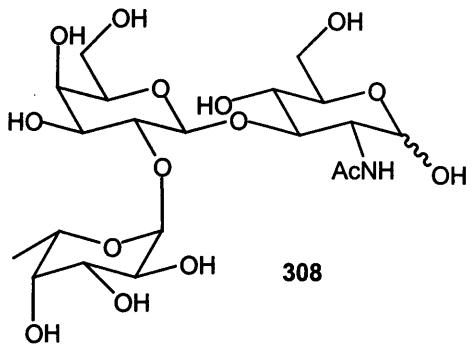
called peptidases.[23] Circulating in the blood of Antartic fish are antifreeze glycoproteins (AFGPs) which enable them to avoid freezing in their icy environment where temperatures frequently reach – 1.9^{0}C. AFGPs contain proteins with the repeat structure [Gal(β-1→ 3)GalNAc(α-1→ 0)]Thr-Ala-Ala, **307** where the hydroxyl groups bind to and inhibit the growth of ice crystals.[24]



307

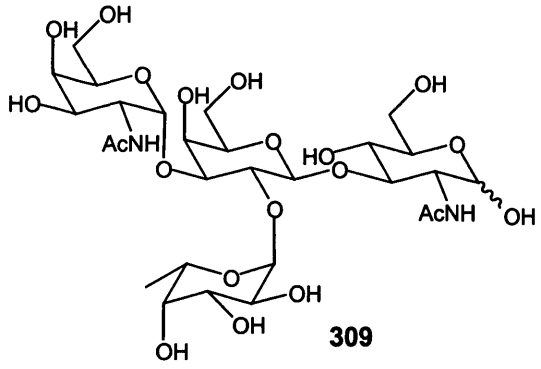
Another role of glycoproteins is in reproduction. For fertilisation in mammals to occur the sperm has to bind to the outer coat of the egg which comprises a variety of *N*- and *O*-linked glycoproteins. The *O*-linked glycoproteins are responsible for the binding event, and recognition is thought to be mediated by a β-(1-4)-galatosyltransferase, an enzyme found to be present on all mammalian sperm heads.[25]

The discovery of the ABO blood group system was based on the observation that humans could be divided into different groups according to the presence or absence of serum constituents that would unite red cells isolated from other humans.[26] It is now known that the agglutinating serum constituents are antibodies and that their antigens are oligosaccharides whose structures are genetically polymophoric.[27] The discovery of these antigens on the surface of leukocytes[28] led them to be classified as blood group antigens. The A, B, and H antigens are formed by action of glycosyltransferases on different saccharide precursors. The H antigen **306**, is found in O type individuals and is a precursor to the A and B antigen.



308

The A antigen **309** is found in A type individuals, it produces an enzyme that adds an *N*-acetylgalactosamine to the terminal residue of the H antigen. Individuals who possess the B antigen **310** produce an enzyme which adds a galactose residue to the terminus of the H antigen **308**.



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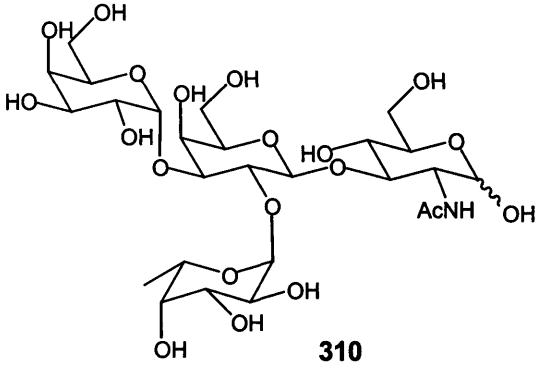
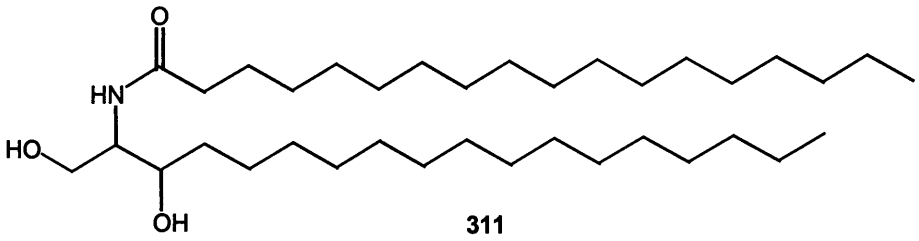


310

3.2.2 Glycolipids[29]

Like glycoproteins, glycolipids are commonly found in the plasma membrane of all vertebrate cells. They are amphiphilic molecules possessing a hydrophilic head group (sugar) and apolar hydrophobic hydrocarbon chain (lipid portion). Generally glycolipids are important factors of membrane stabilisation and cell surface rigidisation. They also play a number of roles in biological processes such as cell-cell communication. According to the structure of the membrane anchor they can be divided into two groups; glycoglycerolipids that are abundant in bacteria and plants and glycosphingolipids, which are found in animals.

Glycosphingolipids **312** are anchored to the membrane via ceramide **311** which consists of sphingosine that is substituted at its amino group by fatty acids of various chain lengths. The carbohydrate portion is linked to the primary hydroxyl group of ceramide *via* glycosidic linkages. These lipids are abundant in the nervous system and can be found in high quantities in the brain (cerebroside).[30]



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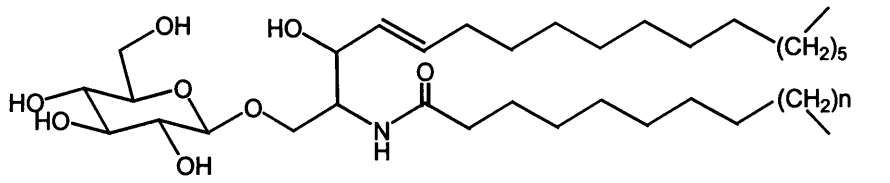


312 n = 6, 8, 10,...

The degradation of glycolipids needs to be regulated in the cell, if a glycosyl hydrolase which catalyses this process is lacking due to a genetic deficiency then glycolipids build up in the lysosomes of the cell and can cause serious diseases such as Gauchers and Tay-Sachs disease. The former results from a genetic mutation which disables β-glucocerebroside, a β-glucosidase which hydrolyses glucocerebroside to glucose and ceramide as part of the normal degradation pathway for membrane glycolipids,[31] Tays-Sachs disease results from mutations in the expression of β-hexosaminidase.[32]

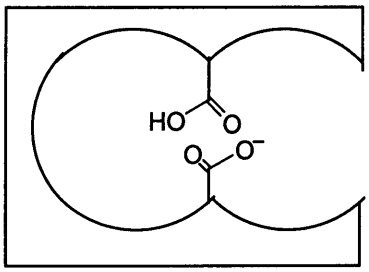
As more is learnt about the structures and specificities of carbohydrates on cell surfaces, the potential for the design of highly selective, powerful inhibitors of cell interactions becomes apparent. The potential for carbohydrate-based therapeutics to be routinely employed for the prevention and treatment of inflammations, infections, the consequences of heart attacks and perhaps even cancer remains promising.

3.3 Glycosidases

There are two types of carbohydrate processing enzymes known as glycosidases and glycosyltransferase which are powerful catalysts for the synthesis of sugars, they trim, assemble and shape carbohydrates into bioactive glycoprotein and glycolipid conjugates.[33] Essentially the overall process involves cleavage of the glycoside bond linking a sugars anomeric carbon with an oligo- or polysaccharide or a nucleoside diphosphate group. Each linkage, be it α or β is hydrolysed by the corresponding glycosidase. The liberated glycosyl group may then be transferred to water (by glycosidases) or to another nucleophilic acceptor (by transferases). Cells are constantly recycling certain metabolic

intermediates and adjusting concentration of key compounds as needed, it is for this reason that the enzymes which catalyse glycosyl group transfer reactions are vital for the normal growth and development of all cells.

Glycosidases, in particular, play many fundamental roles in biochemistry and metabolism. They act upon a variety of substrates known as glycosides, which are sugar derivatives in which the hydroxyl group at C-1 has been replaced by an OR group, where R can be a second sugar unit or a multitude of other functional groups. Exoglycosidases **313** are capable of removing sugars one at a time from the non reducing end of an oligo- or polysaccharide. They are involved in the breakdown of glycogen and starch, the processing of eukaryotic glycoproteins and the biosynthesis and modification of glycospingolipids. Endoglycosidases **314** are involved in the catabolism and clearance of aged glycoproteins and catalyse the alteration of bacterial and plant cell walls and also the hydrolysis of structural polysaccharides like chitin and cellulose.[34,35]



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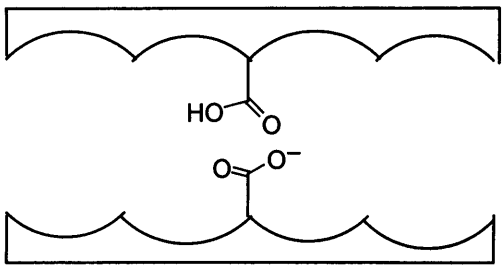


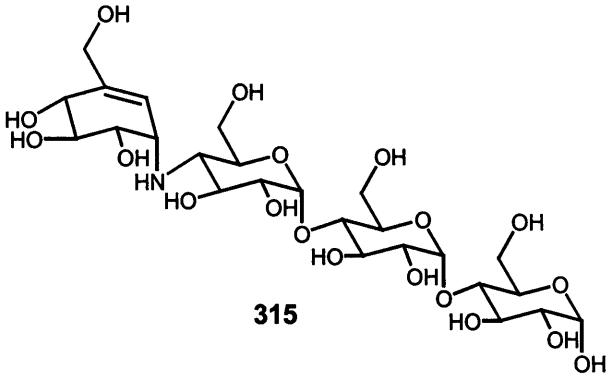
314

3.3.1 Biological effects of Glycosidases

Glycosidases are necessary in the functioning of most organisms and their dysfunction leads to specific diseases and disorders. In humans lactose intolerance is among the most common genetically based syndromes and arises from a reduction in the levels of lactose.[36] In the disease mannosidosis a mannosidase is absent and sugar chains containing mannose builds up in the cells, causing toxicity. Swainsonine has been shown to induce a similar condition in cultures of normal cells and is being used to study the pathology of the disease.

Starch is digested by a group of α-glucosidases (amylases) released in pancreatic juices and from the walls of the duodenum. This leads to a sharp rise in blood sugar levels which is normally regulated by insulin in a negative feedback mechanism. Patients with diabetes mellitus, the dysfunction of the insulin mechanism can be treated by α-glucosidase inhibitors which can regulate the activity of intestinal sugar production.

Acarbose[37] **315** which is used in the USA reversibly inhibits the function of pancreatic α-amylase and membrane bound intestinal α-glucoside hydroylase. Miglitol[38] **316** and voglibose[39] **317** have cleared clinical trials.



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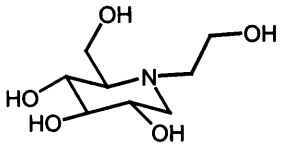

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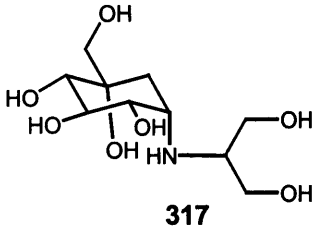
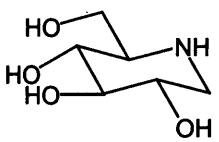
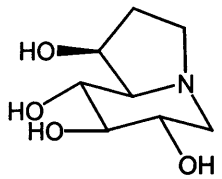


317

Glycoproteins from the surface of eukaryotic cell membranes and are involved in intracellular protein processing and transport.[40,41] The *N*-glycoprotein is normally biosynthesised in the Golgi-apparatus and the endoplasmic reticulum as a complete unit which is then processed by a group of "trimming enzymes". Glucosidase I removes the terminal α-1,2-linked glucose units and glucosidase II removes the two α-1,3-linked glucose residues.[42,43] Alteration of glycoproteins by inhibition of one or more of these trimming enzymes interferes with their intracellular processing and in the case of human immunodeficiency virus (HIV) can thereby retard viral replication. Inhibitors such as 1-deoxynorjirimycin **318** and Castanospermine **319** have shown *in vitro* activity against HIV, affecting infectivity at concentrations not cytotoxic to lymphocytes.[44] These inhibitors are still in clinical trials, because of side effects resulting from their inhibition of glycosidases other than those targeted. Diarrhoea and flatulence are a common result of ingesting α-glycosidase inhibitors.[45]



318



319

Inhibiting glycosidase enzymes which are involved in the carbohydrate processing of glycoproteins have been valuable in the treatment of cancer.[46] Healthy cells exhibit characteristic oligosaccharide structures, while tumour cells display abnormal glycosylation due to an altered expression of glycosyltransferases[47] and it has been known that the levels of glycosidases are increased in the sera of many patients and in the interstitial fluid of the tumour itself. It is believed that these complex structures provide signal stimuli for rapid proliferation and metastasis of tumour cells. A potential strategy incorporating the use of glycosidase is to take advantage of the different growth rates of normal cells and cancer cells to inhibit the assembly of complex oligosaccharides. An example of this is seen in the indolizidine alkaloid swainsonine an inhibitor of Golgi α-mannosidase II, which reportedly reduced tumour cell metastasis, enhances cellular immune responses and slows tumour cell growth in mice. Patients with advanced malignancies using swainsonine saw significant reduction of tumour mass and it seems to be a promising drug therapy for those suffering from breast, liver and other maglignancies.[48]

3.3.2 Classification of Glycosidases

Numerous groups[49] have classified glycoside hydrolases based either on the substrate specificity, similarities between the amino acid sequences or on how the glycoside attacks i.e. 'exo' or 'endo'. With regards to substrate specificity *O*-glycosides are given the code EC 3.2.1.x (recommended by the International Union of Biochemistry and Molecular Biology IUBMB) where x represents the substrate specificity and in some cases the molecular mechanism or type of linkage.

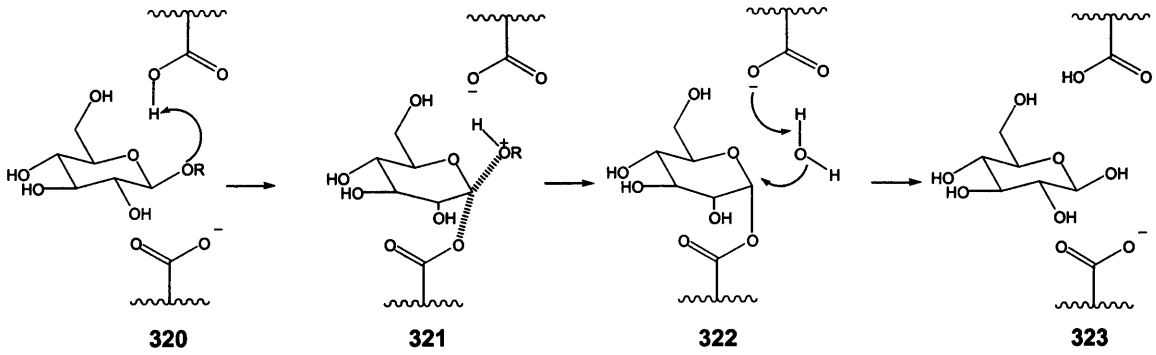
Henrissat has made a noteworthy contribution to the classification of glycoside hydrolases into families which contain amino acid sequence similarities. Enzymes belonging to the same family usually have the same arrangement of catalytic amino acid residues at the active site. Since sequence and structure are related, useful structural and mechanistic information can be determined based upon amino acid sequence alone.[50]

3.3.3 Mechanism of Glycoside Hydrolysis

Enzymatic hydrolysis of glycosidic bonds proceeds by two distinct mechanisms, which results in one of two stereochemical outcomes; net retention or net inversion of the anomeric configuration, hence glycosidases are classified as either retaining or inverting.

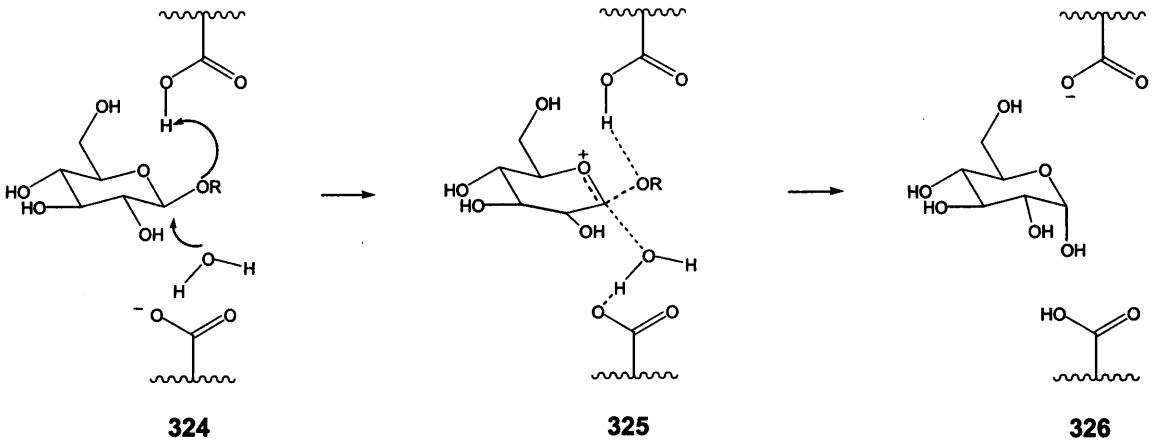
All natural glycosidase examined have two carboxyl residues in their active sites that affords catalysis. The first rational insight into the basic mechanisms for these processes was given by Koshland.[51]

The catalysis of retaining glycosidases occurs by protonation of the glycosidic oxygen by one of the carboxylic acids **320**. The other acid residue acts as a nucleophile, i.e. forms a glycosyl-enzyme ester intermediate and the aglycon departs **321**. A water molecule is deprotonated **322** and attacks the anomeric carbon which generates a product with the same stereochemistry as the substrate **323**. A covalent oxocarbenium ion-like intermediate **322** is involved which is a carboxylic ester of the catalytic nucleophiles. The distance between the two catalytic carboxyls is 4.5-5.5Å allowing a close approach of the nucleophile to the anomeric carbon.[52]



Scheme 28. Mechanism of retaining glycosidases

The reaction pathway in inverting enzymes consists of the glycosidic oxygen being protonated by one of the acids **324** and the aglycone departs with the attack of the water molecule which has been activated by the second carboxylic acid residue **325**. The product **326** is generated with an inversion of stereochemistry at the anomeric carbon. In this pathway the two acid residues are 9-9.5 Å apart presumably to create enough space for the participating water molecule.[52]



Scheme 29. Mechanism of inverting glycosidases

3.3.4 Active site

The topology of the active site of a glycosidase is dependant on the structure of its substrate. Exo enzymes which selectively cleave one glycosyl residue from the non-reducing end of a conjugate or oligosaccharide contain a pocket-shaped active site; this prevents the entry of more than one glycosyl residue. In regards to the endo-glycosidases that cleave oligo- or polysaccharides in the middle their active site forms either a groove or a tunnel which allows the enzyme to proceed along the chain hydrolysing successive residues. These features are illustrated by the breakdown of cellulose by bacteria[53]

1) Endocelluloses cleaves bonds within the cellulose chain, which decrease the degree of polymerisation and create new chain ends hence a groove active site is present
2) The tunnel active site allows cellobiohydrolases access to chain ends which in turn release cellobiose gradually from chains
3) Exoglycosidases (pocket) convert cellobiose to glucose

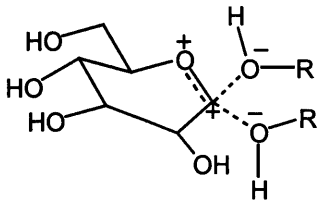
3.4 Glycosidase Inhibitors

Glycosidase inhibitors can be divided into three classes based on their binding behaviour which is deciphered from their kinetics: competitive, non-competitive and uncompetitive. Competitive inhibitors bind to the free enzyme at its active site, the uncompetitive inhibitors bind to the enzyme–substrate complex and the non-competitive inhibitors bind to both. Competitive inhibitors can be classified further depending on whether they resemble the substrate or ground state of the hydrolysis or the transition state.

An approach to synthesising potent inhibitors is to create compounds that mimic the transition state of the enzyme-catalysed reaction. According to the hypothesis articulated by Pauling,[54] the enzyme, in order to effectively catalyse the reaction should bind the transition state more tightly than the ground state, therefore lowering the energy barrier between the states. Hence ground state inhibitors are expected to be uncharged and possess a chair like conformation in analogy to the substrate. Jenks[55] elaborated on the hypothesis stating, the transition state of a reaction is stabilised by interactions with residues in the enzyme active site, which lowers the activation energy of the reaction. These observations led to inhibitors being synthesised on the understanding that any stable molecule that mimics the overall shape and charge of the transition state should bind

tightly to the enzyme, and potentially act as a potent inhibitor. One can screen huge libraries of compounds in the hope that some will portray inhibitory properties. This approach is very random and needless to say non-productive. The use of designing compounds according to the transition state theory can lead to a greater understanding of the mechanisms of enzymatic hydrolysis, since systematic derivatisation on the compounds can provide an abundance of information.

Consideration of the mechanism of glycoside hydrolysis discussed previously suggests that an ideal transition state analogue has a flattened (half chair) conformation and a (partial) positive charge at or adjacent to the anomeric centre (cationic intermediate). This distortion of the substrate has been suggested to be an important component of catalysis by glycosidases and is considered to be the hypothetical reactive intermediate with substantial oxocarbenium ion character **327**.



327

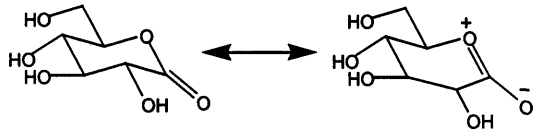
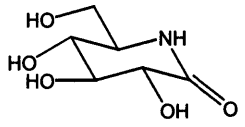
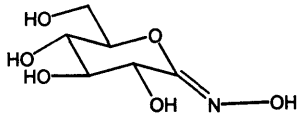
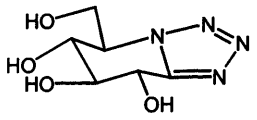
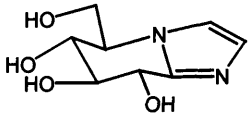
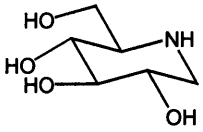
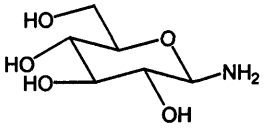
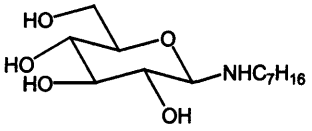
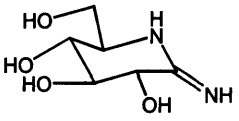
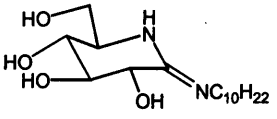
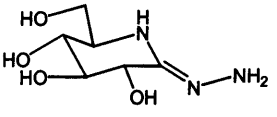
The positive charge on the anomeric carbon is shared with the endocyclic oxygen, which requires double bond character to be present in the ring between carbon and the endocyclic oxygen. Support for this structure has been proved by studies of secondary α-deuterium effects on reaction rate, which confirmed a change in hybridisation at C-1 from sp^3 to sp^2 in the rate limiting step. Thus one can use this approach to synthesise potent inhibitors.[56]

3.4.1 Transition State Analogues

Enzymes promote reactions by lowering the activation energy, which is the result of the strong binding to the transition state (TS) structure, thus the TS binding governs the substrate specificity. Transition state analogues are reversibly bound in the active site and compete with the substrate by mimicking the shape and /or charge of the transition state. Analogues which have been synthesised fall into three broad categories; a) possessing a flattened anomeric centre, b) a positive charge or c) containing both a and b.

The lactone **328**,[57] lactam **329**,[33] the lactone oxime **330**,[58] the tetrazole **331**,[59] and the imidazole **332**[60] possess a planar anomeric centre but are uncharged (at a pH of *ca.* 4-7); 1-deoxynojirimycin **318**,[61]isofagomine **333**[62] and the glucosylamines **334**[63] and **335**[63]

contain a basic atom adjacent to the anomeric centre, but are not really flattened;[64] and the amidines **336**[65]and **337**,[66] the amidrazone **338**,[65,67] and the lactam oxime **339**[68] and **340**[69] comprise both features.

		Type	Reference
328		A	57
3279		A	33
330		A	58
331		A	59
332		A/C	60
318		B	61
333		B	62
334		B	63
335		B	63
336		C	65
337		C	66
338		C	65,67

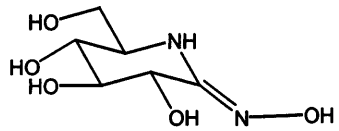
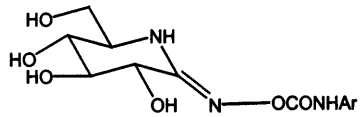
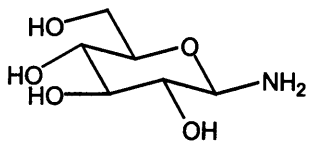
339		C	68
340		C	69

Table 7 Transition State Analogues Table reproduced from Heightman T[70]

3.4.2 Features of Basic Sugar Analogues important for Glycosidase Inhibition

Studies have shown that there are a number of generalisations that can be made about the inhibition characteristics, structural features required for strong inhibition:
Position of the basic centre; Almost all glycosidase inhibitors contain a basic centre, which is usually an -NH- group. This group can be protonated forming stabilising interactions with a carboxylic acid residue in the enzyme active site. Nojirimycin **342** was isolated from mircoorganisms (*Streptomyces*) by Ishida *et al.*[71] and is a potent competitive inhibitor of α- and β-glucosidases from various sources. 1-Deoxynojirimycin **318** was subsequently obtained by Inouye *et al.*[72] by reduction of **342**.[73] These are examples of 5-azasugars in which the endocyclic oxygen has been replaced by a nitrogen and they inhibit α- and β-glucosidases up to 10^6-fold better than the corresponding hexoses.[74] An explanation for this has been attributed to the formation of an ion pair consisting of the protonated inhibitor and a carboxylate group. As ionic interactions are weakened in aqueous solution, this large enhancement of inhibition by presence of the –NH- group suggests that the access of water to this part of the active site is restricted. This has been found to be true.[74]

Glycosylamines **334** have been synthesised to resemble the intermediate carbocation which contains more charge on the anomeric carbon than the ring oxygen **341**.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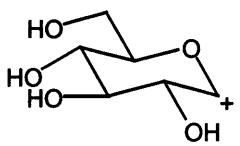
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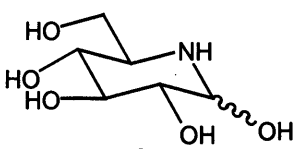
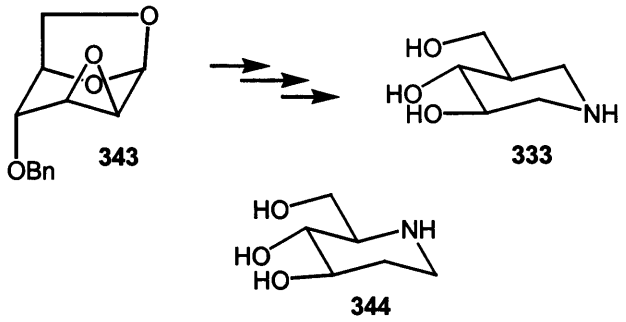

342

They have their basic centre in the form of an exocyclic nitrogen, and are found to be weaker inhibitors than the 5-azasugars.[75] The reason for this is thought to be due to the carboxylic acid residues in the active site being in a position to stabilise a glycosyl cation-like transition state, where the positive charge is distributed within the ring. The catalytic

residues cannot stabilise a positive charge in the exocyclic position due to their restricted mobility.

1994 saw Jespersen *et al.*[76] synthesise the first synthetic azasugar isofagomine **333** which contains a nitrogen in the pseudo-anomeric position from 1,6:2,3-dianhydro-4-*O*-benzyl-β-D-mannopyranose **343**.[77] It bears a close resemblance to the natural product fagomine **344**. Isofagomine **333** has been recognised as a potential drug in the treatment of type II diabetes.[76]



Isofagomine **333** is a potent inhibitor of β-glucosidase. It has been shown to inhibit β-glucosidase from sweet almonds 50 times better than the 5-azasugar **344** (K_i 0.1 μM compared to 47 μM with 1-deoxynojirimycin **318** at pH 6.8) but inhibited yeast α-glucosidase three times less effectively than **318**. There have been several explanations offered for the inhibitory profile of the isofagomines compared to that of the related 1-deoxynojirimycins. The increase in inhibition was caused by the position of the – NH- group even though isofagomine lacks an OH group on C-2.[78]

It has also been proposed that the potent inhibition observed is a result of the strong electrostatic interaction between the protonated form of the piperidine and the active site carboxylate. The location of the active site carboxylate has been put forward as an explanation on how α- and β-glycosidases differentiate between isofagomine and 1-deoxynojirimycin.[79]

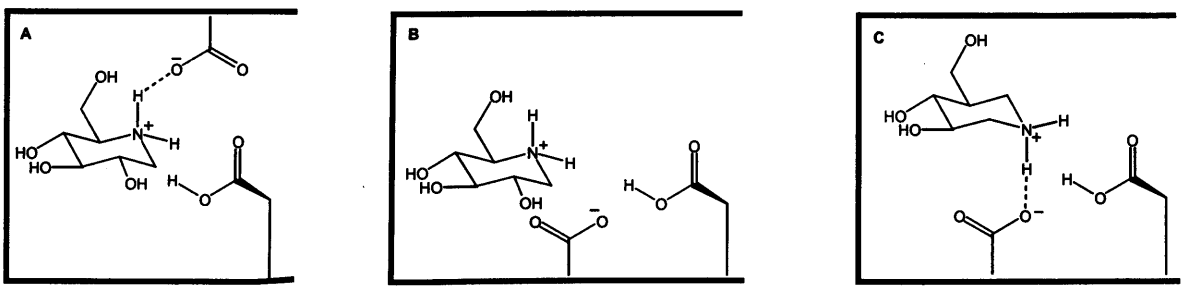
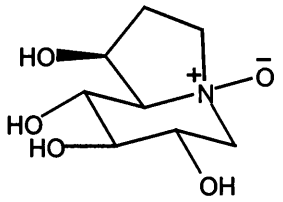


Figure 2.–Proposed favoured binding interaction of deoxynojirimycin in an α-glucosidase (a) but not in a β-glucosidase (b). Favoured binding interaction of isofagomine in β-glucosidase (c)

The carboxylate in (c) is located beneath the sugar ring, thus contact between isofagomine can occur, whereas no favourable interaction can be made with deoxynojirimycin (b). The α-glucosidase has the catalytic carboxylate situated above the sugar ring, hence an association can occur with deoxynojirimycin and not the protonated isofagomine.

Basicity: Strong inhibition by the formation of an ion-pair with glycon analogues requires that these are of sufficient basicity to either accept a proton from water or from an acidic group of the enzyme.[80] Ion-pair formation has been shown to make an important contribution to the binding energy illustrated by the 500-fold increase in Ki for castanospermine **319** by its conversion to the *N*-oxide in the inhibition of β-glucosidases from almonds.[33]



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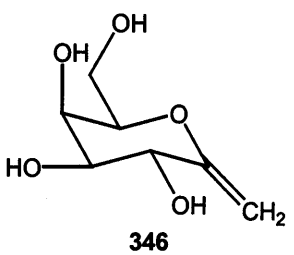
Relatively strong inhibitors generally have pKa values between 4.0-9.0.[77] If an inhibitor is not sufficiently basic it will not accept a proton and hence will not bind very well.

Geometry and charge distribution at the anomeric position: The question has been put forward enquiring which factors are more important for inhibition for a given type of glycosidase: charge or shape. As stated previously the glyconolactones **328** were believed to be effective inhibitors of glycosidases since their half chair conformation and planar geometry at C-1 is similar to the glycosyl oxocarbenium ion. Geometric factors are not the only contributing factor to the binding energy as portrayed by 2,6-anhydro-1-deoxy-D-galacto-hept-1-enitol **346**.[81] This compound was found to be a poor inhibitor of β-galactosidase from *E.coli*, even though it mimicked the overall shape of the lactones **328**.



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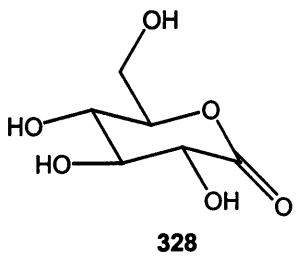

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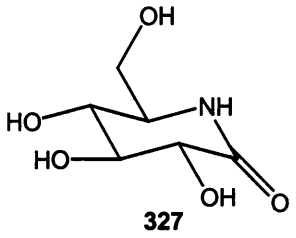
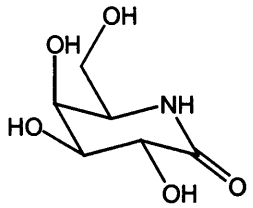


327

It seems it is the charge distribution of the glyconolactones that is significant in the binding process, with the carbonyl group being able to form stabilising interactions with residues in the enzyme active site. The glycono-1,5-lactones suffered a major

disadvantage. Under mildly basic conditions they underwent hydrolytic ring opening and under acidic conditions, conversion to inactive 1,4-lactones. For these reasons more stable analogues were designed.

Lactams such as **327**[72] were the first lactone analogues to be designed *via* oxidation of **342**. These derivatives combined the sp^2 hybridisation of the lactones with the positive charge of the azasugar. The lactams were found to be weaker inhibitors than the corresponding lactones. Studies with D-galactono-1,5-lactam **347** portrayed it to be a strong inhibitor of the β-specific enzyme whereas D-galactono-1,5-lactone **348** isomerised to the inactive 1,4 species.



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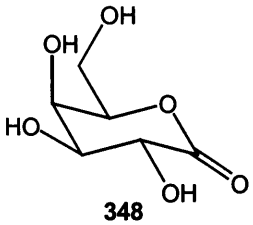

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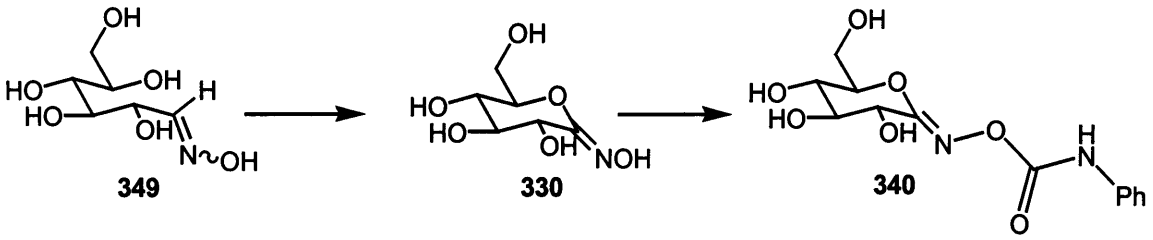
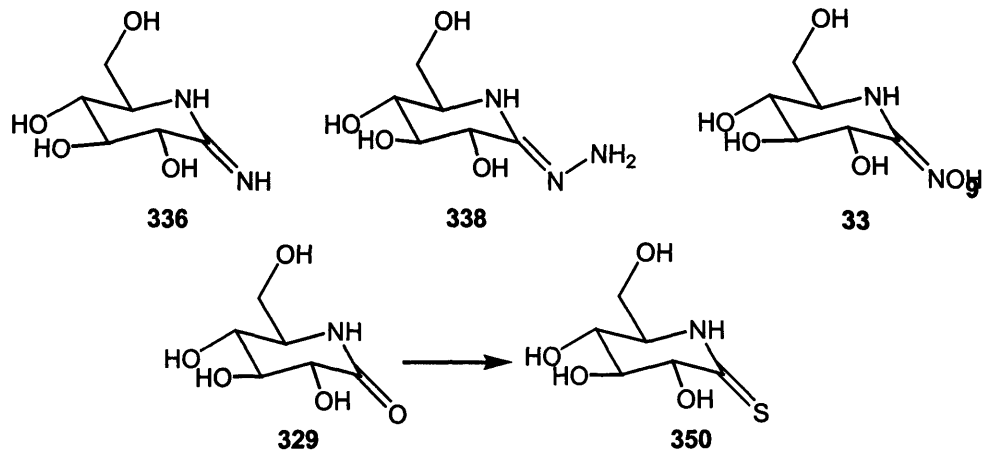
Beer and Vassella[58] synthesised and studied the hydroximolactone **328**, which was prepared by oxidation of the hydroxyl oxime **349**. The extra hydroxyl group provides the option of introducing a variety of substituents which can potentially mimic the aglycon.



349 **330** **340**

The unsubstituted hydroximolatone **330** inhibited β-glucosidase from sweet almonds ten times more weakly than **328** and weakly inhibited α-glucosidase from brewers yeast as well. On the other hand, the introduction of a substituent – phenylurethane on the exocyclic OH to give **340** gave stronger inhibition, reflecting the preference for the almond enzyme for aromatic glycosides.[82]

Other types of lactone analogues were synthesised by the Ganem group.[67] They produced the glyconoamindine **336**, the hydrazone **338** and the hydroximolactam **339**. The lactam **329** was activated to the thiolactam **350** allowing the introduction of nitrogen nucleophiles.



336 **338** **33**

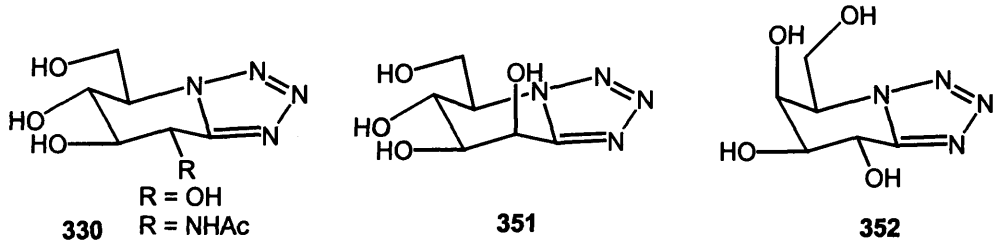
329 **350**

All three lactone analogues **336, 338, 339** showed mircomolar inhibition of several α and β glycosidases, Table 8.

Enzyme (Source)	336[8]	338[6]	339
α-glucosidase (Brewers Yeast)	-	-	2.9
β-glucosidase (Almonds pH 6.8)	10	8.4	14
β-glucosidase (Agrobacterium Faecalis)	-	-	0.6
β-galactosidase (Bovine Liver)	-	1.9	-
α-mannosidase (Jack Beans)	9.0	3.1	-

Table 8. K_i values [μM] of the amidine **336**, amidrazone **338**, and the lactamoxime **339**.

The Vasella[79] group synthesised a number of tetrazoles **330, 351** and **35** as neu2tral, stable azasugars. The six membered ring contained a half chair conformation due to its fusion with the aromatic ring.



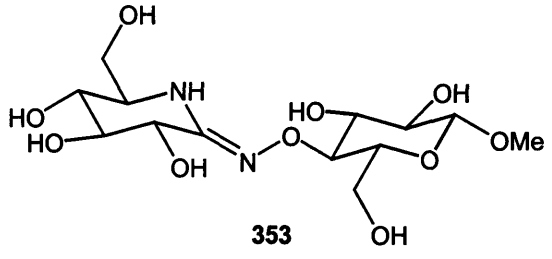
R = OH
330 R = NHAc **351** **352**

In order to evaluate the selectivity of these compounds, the gluco- and manno-tetrazoles **330** and **351** were tested by Withers *et al.*[83] against three glucosidases and three mannosidases. It was discovered that each compound inhibited both types of enzyme to some extent, but the 'correct' tetrazole was considerably more effective at inhibiting the corresponding glycosidase, thus β-glucosidases were inhibited more strongly by **330** than **352** and the mannosidase more strongly by **351** than **330**.

Mimicry of the Aglycon Site: Most inhibitors designed as transition state analogues mimic only the glycon part of the substrate. Since the natural substrates for representative glycosidases are di- or oligosaccharides, imitation of the aglycon part is believed to be important in creating an increase in potency and specificity. The aglycon R is part of the transition state and its interactions with the corresponding part of the active site makes a substantial contribution to catalysis.

Simple alkyl derivatives of D-glucosylamine and D-galactosylamine were the first inhibitors prepared for the study on aglycon site interactions. Large increases in inhibitory potency were observed for *N*-benzyl-D-glucosylamine with β-glucosidases from almonds,[84] and *N*-heptyl-D-galactosylamine against galactosidase from *E.coli*.[63] Various substituents were attached to 1-deoxynojirimycin type inhibitors which caused with few exceptions a relatively small increase of inhibitory potency compared to that of the glycosylamines. In some cases substitution was found to be detrimental, the reason being the position of the substituent differed from that of the aglycon.

Hydroximolactam **339** and hydroximolactone **330** both contain an oxime functionality which is oriented more closely towards the position occupied by the aglycon of a β-glycoside that of an α-glycoside. Therefore, an oxime substituent should imitate the aglycon of a β-glycoside more closely than an α-glycoside. This observation was put to the test. The cellobioside analogues **353** and **354** were synthesised and both were found to inhibit the β-glucosidase from *C.saccharolyticum* as strongly as the unsubstituted hydroximolactam **339**. Binding to yeast α-glucosidase is weak, showing that the second pyranoside ring can not be accommodated in the active site of this glycosidase.



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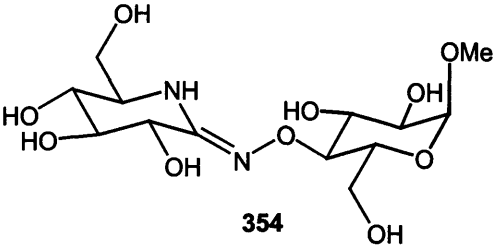

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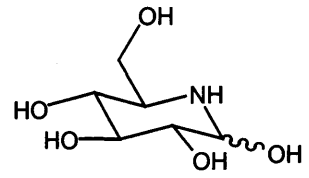
3.4.3 Basic Sugar analogues as Glycosidase Inhibitors

Several polyhydroxylated piperidine, pyrrolidine and indolizine alkaloids have been identified as naturally occurring glycosidase inhibitors in plants and microorganisms.

3.4.3.1 Piperidines

Nojirimycin **342** was the first amino-sugar inhibitor to be described by Ishida *et al.*[71] isolated from bacteria (*streptomyces*). The polyhydroxylated piperidine corresponds to glucose in the pyranose configuration and is a potent competitive inhibitor of α- and β-glucosidases, from various sources.

Examples of piperidine analogues of mannose and galactose, are nojirimycin B **355** and β-galactostatin **356**, they inhibit α-mannosidase[85] and β-galactosidase[86] respectively. Since these iminosugars with a hydroxyl group at C-1 are fairly unstable, they are usually stored as bisulphite adducts. Removal of the anomeric hydroxyl groups by catalytic hydrogenation with a platinum catalyst or $NaBH_4$ gives a more chemically stable class of compounds such as **318, 357** and **358**(1-deoxy derivatives) with powerful and specific glycosidase inhibitory properties.[87]



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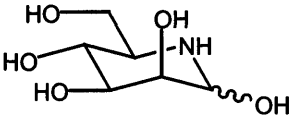

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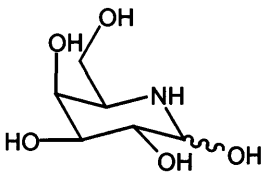

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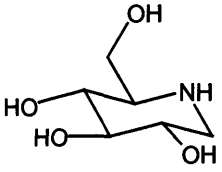

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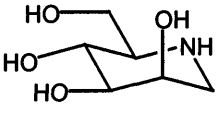

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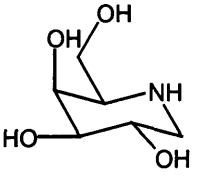

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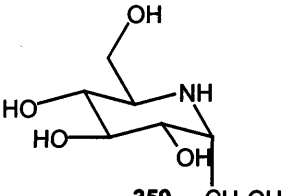

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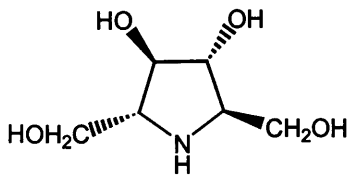
1-Deoxynojirimycin (DNJ) **318** was first prepared by chemical synthesis from L-sorbofuranose[88] and by the reduction of nojirimycin **342**.[72] It has also been isolated from the roots of Mulberry trees and called moranoline.[89] It is more stable than **342** and has become the model compound in this area of research. It has shown inhibition of α- and β-glucosidase up to 106 fold better than the corresponding hexose. Homonojirimycin (HNJ)[90] **359** extracted from *Omphaela diandra* is the first naturally occurring DNJ derivative with a carbon substituent which inhibits glycoprocessing mannosidase I and mammalian α-L-fucosidase.

3.4.3.2 Pyrrolidines

Polyhydroxylated pyrrolidines **360-364** are compounds that resemble sugars in the furanose configuration **365-367** and are potent inhibitors of the corresponding glycosidase.

Naturally occurring 2,5-dihydroxymethyl-3,4 dihydroxypyrrolidines (DMDP) **360** is an analogue of β-D-fructofuranose **365**. It is a powerful inhibitor of invertase, almond α-glucosidase and fungal β-xylosidase, but it does not inhibit the corresponding mammalian enzyme surase. Interestingly DMDP lacks the equivalent of the anomeric hydroxyl group, as do all the other natural furanose analogues except the analogue relating to D-arabinose **363**.



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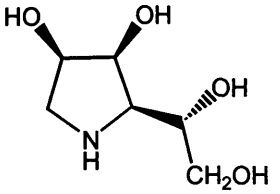

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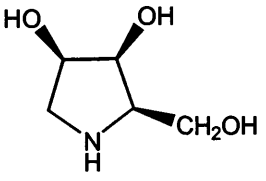

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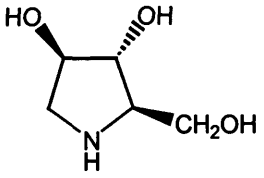

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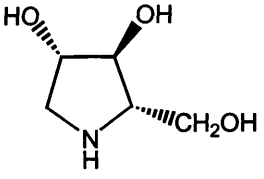

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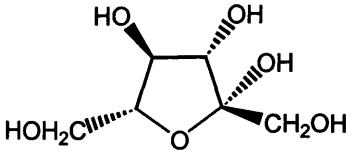

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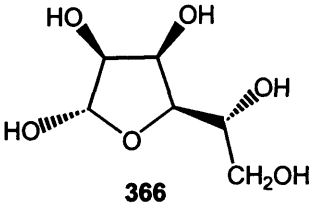

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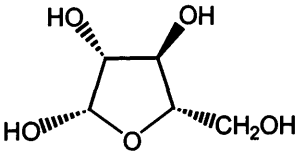

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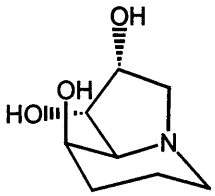
1,4-Dideoxy-1,4-imino-L-arabinitol[91] **364** inhibits α-glucosidase from yeast as well as α-L-arabinosidase.[92] In contrast the D- enantiomer **363** is more potent against α-glucosidase from yeast as well as inhibiting α-mannosidase from jack bean and β-glucosidase emulsion. The analogues of mannofuranose **366**, D-mannitol **361** was shown to be a competitive inhibitor of jack bean and human lysosomal from α-D-mannosidase by Fleet *et al.*[91] The same group also synthesised 1,4-dideoxy-1,4-imino-D-lyxitol **362**, which is related to the iminomannitol[92] **361** by a one carbon degradation of the side chain. Only moderate inhibition was exhibited against α-mannosidase by the iminolyxitol **364**, however it did show stronger inhibition against α-galactosidase from green coffee beans. This is the first report of inhibition of galactosidase activity by either a polyhydroxylated piperidine or pyrrolidine.

3.4.3.3 Indolizidines

The bicyclic polyhydroxy heterocycles fall into three groups; pyrrolizines which have two five membered rings, the indolizidines and the nortropanes which consist of one five membered ring and one six membered ring fused together. The structural relationship between these bicyclic alkaloids and the monosaccharides is not obvious but in each case the configuration of hydroxyl group on the ring can be said to be similar.



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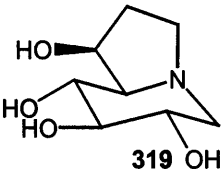

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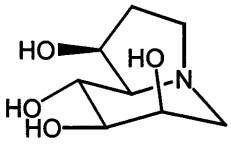

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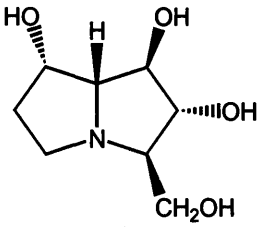
Castonospermine **319**, isolated from the seeds of *C.austrate*[93] may be regarded as a bicyclic derivative of DNJ **318**, with an ethylene bridge between the hydroxyl methyl group and the ring nitrogen. The hydroxylation pattern of the piperidine moiety resembles the pyranose form or glucose. It is a potent inhibitor of lysosomal α- and β-glucosidases[94] and processing α-glucosidase I and II.[95] Several epimers of castonospermine have been isolated, included is the manno analogue, 6-epicastonospermine **369** which is a good inhibitor of human neutral α-mannosidase.[96]

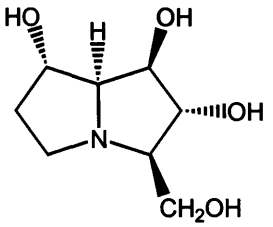
Swainsonine **368** which is the causative agent in swainsona toxicosis inhibits lysosomal α-mannosidase. This inhibition has been attributed to the similarity between the stereochemistry of the hydroxyl groups in the alkaloid and in mannose in the furanose configuration.[97]

3.4.3.4 Pyrrolizidines

Two fused pyrrolidines make up the pyrrolizidines **370** and **371**. There are several naturally occurring pyrrolizidines which have been extracted from *C.australe*.[98] and can be regarded as the ring contracted form of castanospermine **319**. Alexine **370** is the first pyrrolizidine with a carbon branch at C-3 to be isolated. It is a weak inhibitor of mouse disaccharidases but strongly inhibits fungal α-glucosidase.[99]



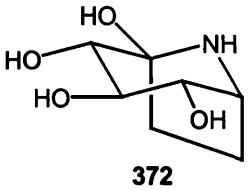
370



371

3.4.3.5 Nortropanes

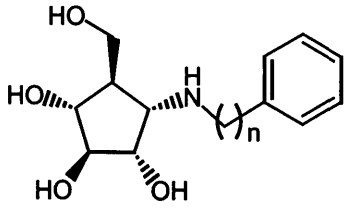
Nortropanes are a new class of azasugar mimetics. Calystegines **372** contain a nortropane ring, two to four hydroxyl groups which vary in position and stereochemistry and an aminoketal functionality, which generates a tertiary hydroxyl group at the bicyclic bridgehead.



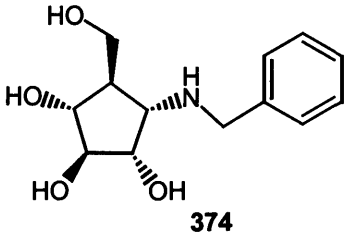
372

3.4.3.6 Aminocyclopentitols

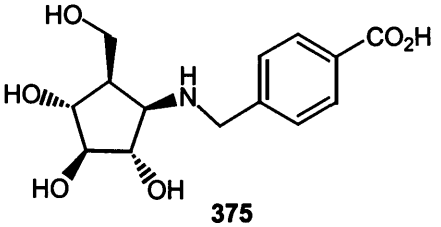
Analogues of β-D-glucose, galactose, and α-D-galactose possessing alkyl substituents as aglycon mimics on the amine function **373-375** were prepared and tested for inhibition by Dickson *et al.*[100] They not only incorporate the entire stereochemical pattern of glycopyranosides but the amino group imitates the protonated form of the leaving oxygen atom in either the α- or β- orientation.



373a n = 1
373b n = 2
373c n = 3



374



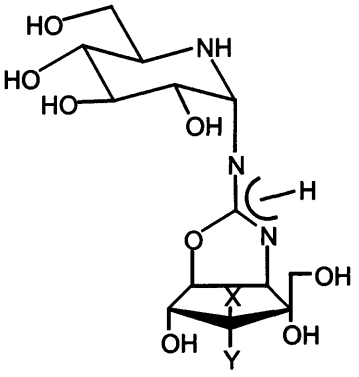
375

This group is able to interact favourably with the enzymes catalytic acidic groups by electrostatic and hydrogen bonding interactions. The strongest inhibitors were found to

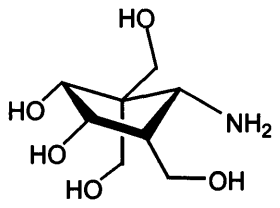
be the *N*-benzyl-β-D-galacto inhibitor **374** with β-galactosidase from bovine liver and the *N*-phenylpropyl-β-D-gluco inhibitor **373c**, with β-glucosidase from almonds.

3.4.4 Carba sugars

Carbo-sugars can be thought of as hydroxymethyl branched-chain cyclitols, which are similar in topology to normal sugars particularly in the arrangement of the hydroxyl and hydroxymethyl groups, but they have the oxygen atom of the pyranose ring replaced by methylene. A potent trehalase inhibitor named trehazolin **376b** has been isolated from the culture broth of *Amycolatopsis trehalostatica*.[101] Modification of the aminocyclitol part of **376b** has shown **377** to moderately inhibit β-glucoside.



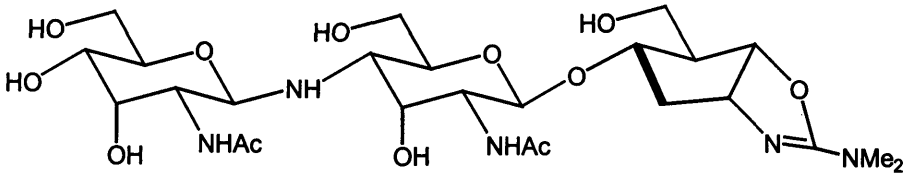
376a X = H; Y = OH (Trehalostatin)
376b X = OH; Y = H (Trehazolin)



377

3.4.5 Allosamidins

A potent chitinase inhibitor allosamidin isolated from *Streptomyces Sp.No1713* was reported by Suzuki and collaborators.[102] Its structure was shown to be that of a pseudotrisaccharide containing two β-linked *N*-acetyl-2-amino-2-deoxy-D-allopyranoside units, which in then linked to an aminocyclopentitol moiety **378**.

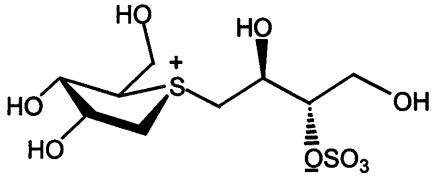


378

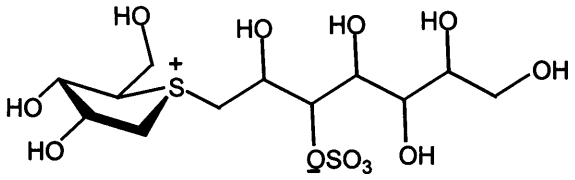
Since allosamidin inhibits chitinase which in turns disrupts the chitin biosynthesis, it has been recognised as a potential compound which can selectively control pest organisms.[103]

3.4.6 Sulphur containing inhibitors

A novel type of glucosidase inhibitor, Salacinol **379** has recently been isolated and synthesised.[104] Compound **379** has an interesting structure comprising of an intramolecular salt between a tetrahydrothiophene sulfonium ion and a sulphate tether with an erythntol chain. Kotalanol **380** is another natural compound which inhibits α-glucosidases. Due to there powerful affinity, they have been used as a treatment of diabetes in traditional Indian medicine.[104]

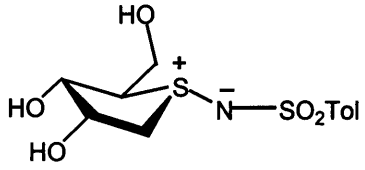


379

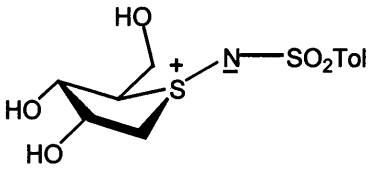


380

Wong and co-workers[105] investigated two diastereomeric compounds **381a** and **381b** which contain trivalent sulphur as glycosidase inhibitors. They were tested against a number of enzymes but only one of the diastereoisomers weakly inhibited β-glucosidase.

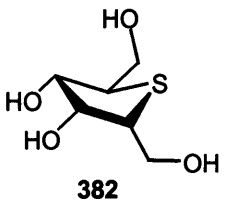


381a

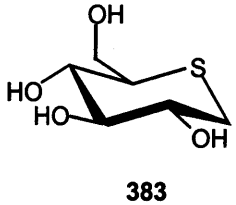


381b

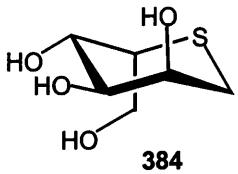
A series of 1,4-, 1,5-, and 1,6-thioalditols were studied as glycosidase inhibitors **382-385**. All displayed weak inhibition.[106] The strongest inhibitor was the mannose analogue **384**. A potent thioglycosidase was **385** which is the glucosamine derivative. It inhibited β-glucosidases with a K_i value of 166μM.[107]



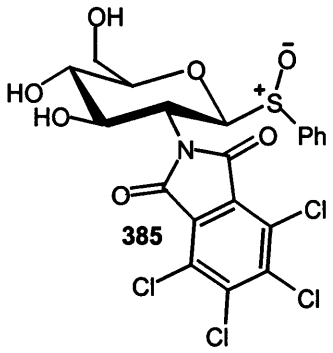
382



383



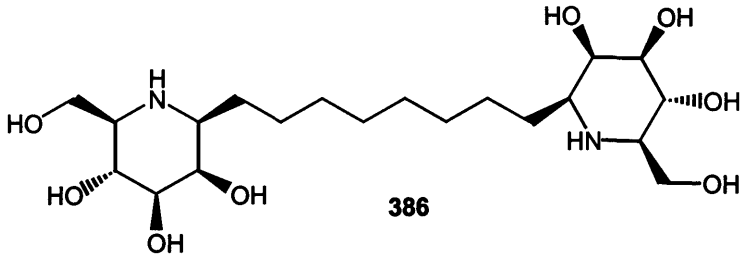
384



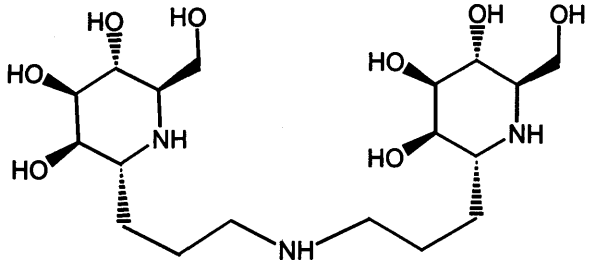
385

3.4.7 Bis-Sugars

Proteins are able to recognise clusters of sugar residues as structural motifs rather than individual monosaccharide units.[108] This observation led to Johns and Johnson synthesising a number of novel scaffolded bis-1,1'-C-linked azasugars.[109] The bis-azasugar **386** has a simple tether containing eight methylene units, while **387** possess a more complex scaffold. Compound **387** was screened against several common glycosidases, and was found to be active against amyloglucosidase giving an IC_{50} value of 20μM.

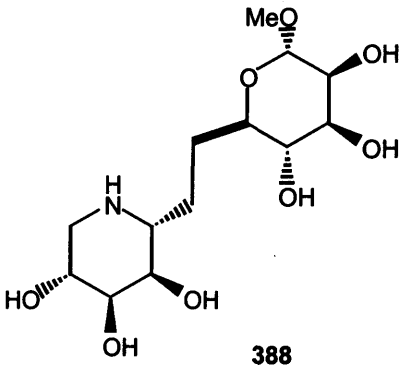


386

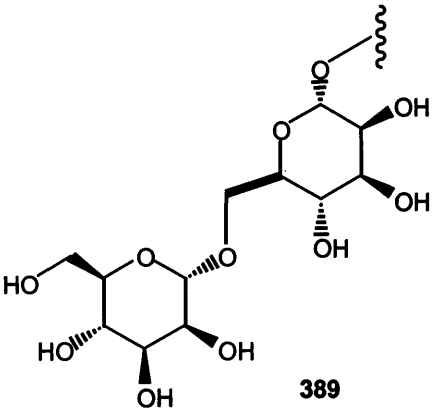


387

Wightman *et al.*[110] have synthesised **388** which is related to the sequence of α-D-Man(1→ 6)-α-D-Man **389**, which is hydrolysed by Golgi α-mannosidase II. Unfortunately no kinetic data was recorded for this compound.



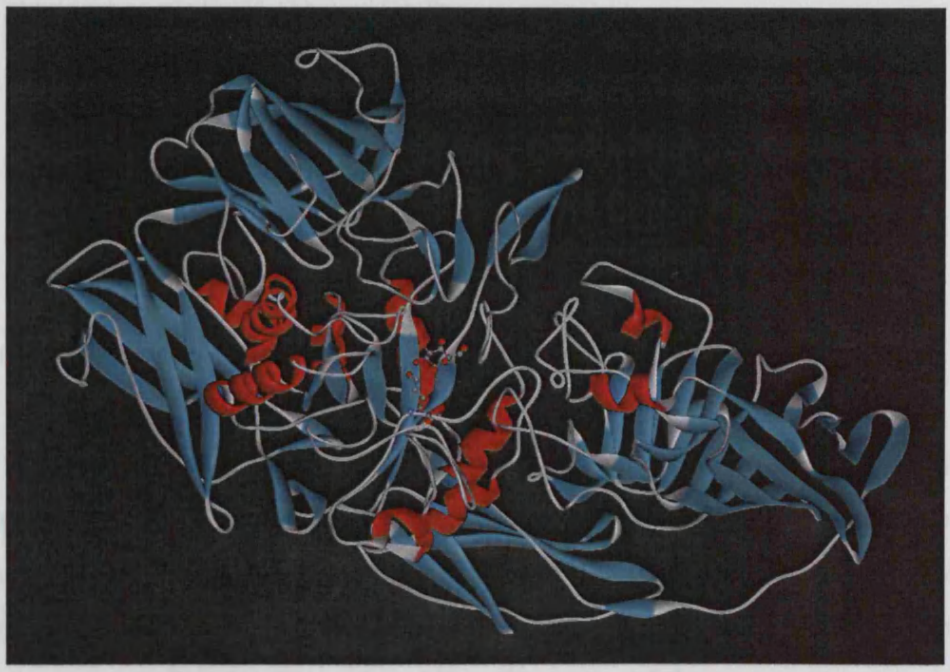
388



389

3.5 β-Galactosidase[111]

The enzyme β-galactosidase (EC 3.2.1.23) is well known and extensively studied. It catalyses the hydrolysis of milk sugar (lactose) into two monosaccharides namely D-galactose and D-glucose. Its activity is present in numerous species of yeast, protozoa and various fungi. Its amino acid sequence places it in family two, belonging to the GH-A clan together with many other retaining exo- and endo β-glycosidases. It was one of the first glycosidases to be crystallised from *E-Coli* and has a molecular weight of 540,000 daltons. The enzyme is tetrameric and composed of four identical subunits a monomer unit is shown (Picture 1). The enzyme is believed to have three activities that result in the complete breakdown of the disaccharide lactose. Firstly the β-gal. cleaves the lactose into galactose and glucose. Secondly, the enzyme acts as a transglycosylase, converting lactose into allolactose and thirdly, it hydrolyses allolactose into galactose and glucose.[111]



Picture 1. X-ray crystal structure showing a monomer of β-galactosidase

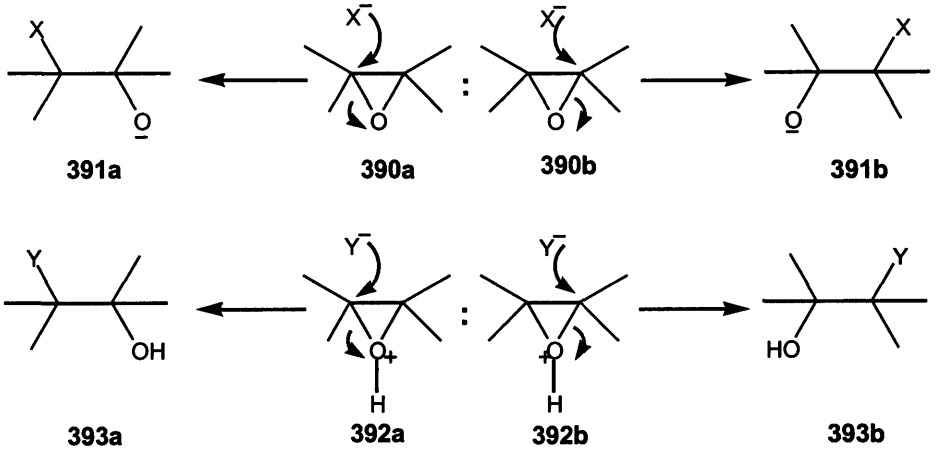
The enzyme has multiple physiological functions in mammalian organs. As stated earlier it is involved in the hydrolysis and absorption of dietary lactose and is a key enzyme in the degradation of glycolipids, glycoproteins and mucopolysaccharides. In the plant kingdom, high enzymatic activity is found in seeds and leaves where the enzyme is probably involved in the catabolism of galactolipids, such as β-D-galactosyl diacylglycerol.

Amino sugars and polyhydroxylated azaheterocycles have been shown to be an important class of compounds, many of which display significant biological activity. Many have portrayed potential applications in the treatment of cancer, HIV and diabetes.

As part of the groups' general interest in carbohydrate annulation and the synthesis of galactosidase inhibitors, we report the synthesis and biological activity of a series of novel amino sugar derivatives.

3.6 Results and Discussion

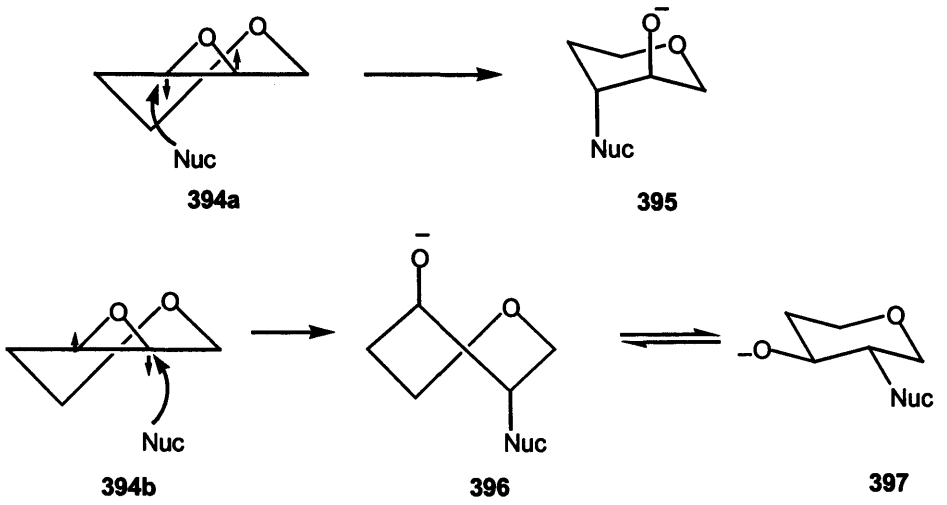
Sugars are readily converted into α and β epoxides at the C-2 and C-3 position of the carbohydrate. Peat[112] has described the stereochemistry of ring opening by nucleophilic reagents. Epoxides can be opened under basic conditions where nucleophilic attack **390a/390b** at either carbon leads to two regio isomers **391a** and **391b**



Scheme 30. Ring opening under basic and acidic conditions

Under acid catalysed conditions, protonation of the oxirane oxygen occurs first, to generate an oxonium ion **392a** and **392b**, which assists cleavage of the carbon-oxygen bond by facilitating the movement of electrons, and this is followed by nucleophilic attack at the carbon atom. In both mechanisms, two products are possible, depending on which carbon atom is attacked by the nucleophile. The ratio of the two products is dependent upon the structure of the epoxide, the reagent, and the conditions.

Mills[113] in 1953 suggested the applicability of the by Fűrnst and Plattner[114] rule to sugar epoxides. He found that steroid epoxides cleave to give predominately the di-axial isomer. *Trans* diaxial opening of epoxide **394a** gives the product **3915** in the chair conformation (small arrows indicate the direction of movement of the oxiran-carbon atoms). Less favourable *trans* diaxial opening of the epoxide **394b**, first places the substituents in an unstable twist boat conformation **396** which then ring flips into the more stable chair placing the substituents diequatorial **397**.



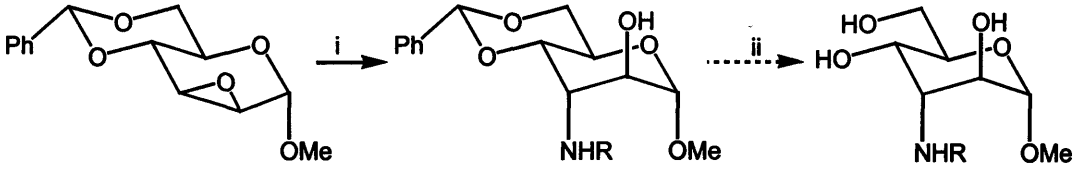
394a **395**

394b **396** **397**

These findings were considered when we employed the manno and allo anhydro sugars as starting points in synthesising novel glycosidase inhibitors

3.6.1 Ring opening reactions of the protected manno epoxide

Methyl-2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside **190** was synthesised in a three steps from methyl-α-D-glucopyranoside **187**.



190 **398a-d** **399**

a - R = H - 62%
b - R = $(CH_2)_2NH_2$ - 28%
c - R = $(CH_2)_3NH_2$ - 49%
d - R = $(CH_2)_4NH_2$ - 57%

Scheme 31:**Reagents and Conditions;** i) H_2NR, MeCN, $LiClO_4$, 90^{0}C; ii) H_2SO_4, MeOH, H_2O, $BaCO_3$, reflux

The manno epoxide **190** was reacted with a number of amines following the method devised by Vega-Pérez *et al.*[115], which incorporated the use of lithium perchlorate as a catalyst, which is able to coordinate to the oxygen atom of the epoxide. The reaction occurred by the addition of the amine to a solution of the starting oxirane and the catalyst in acetonitrile at 90^{0}C. Reaction times were in the region of days which proved extremely time consuming, thus another method was sought.

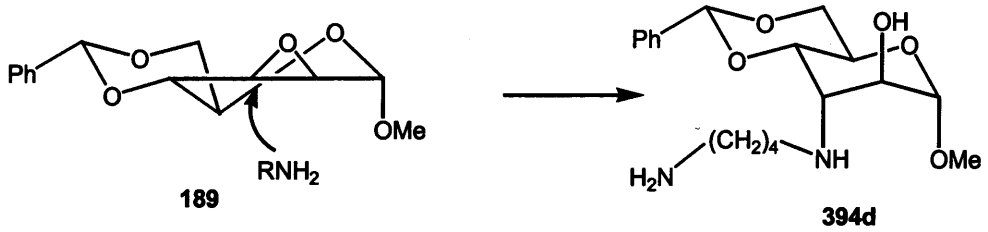
Investigations led to the procedure of Myers *et al.*[116] that took the neat amine and epoxide **190** in a sealed flask and applied heat for 24 hours. The products which were formed solely had the amino and the hydroxyl group *trans* diaxial producing the altro

configured sugar. The epoxide ring located on the pyranose ring is made rigid by being attached to a *trans* fused benzylidene group, thus it is not able to ring flip and adopt the other conformation. It is not viable for the nucleophile to attack at the C-2 position as this would lead to a twist boat which is thermodynamically unstable, as previously discussed.

The amino alcohols **398a-d** were subjected to column chromatography with the exception of methyl-3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside **394a** (solid) and were analysed using various methods.

3.6.1.1 Analysis of Methyl-3-(4-amino-butylamino)-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (398d)

From previous discussions we know that the oxirane ring located on the pyranose ring is made rigid by being attached to a trans-fused benzylidene group **190**. Axial attack of the nucleophile at C-3 in **190** produces the alto configured product where the amino group and hydroxyl group are both axial (Fűrst and Plattner rule) **398d.**



From the cosy NMR (Figure 4) we can see a coupling between proton 2 and proton 3. H-3 is also confirmed by displaying a coupling to H-4. The ^{13}C was assigned using ^{1}H-^{13}C correlation data (NOSEY) and from it we see a downward shift of the C-3 signal to 59.09 ppm in comparison to the starting material **190** from 50.90 ppm. The C-2 signal shifts downfield also but to 69.53 ppm from 54.20 ppm in **190**. The mass spectrum confirmed the product had been synthesised by indicating a MH^+ peak at 353. Compounds **398a-c** also showed the same characteristic peaks as detailed for **398d.**

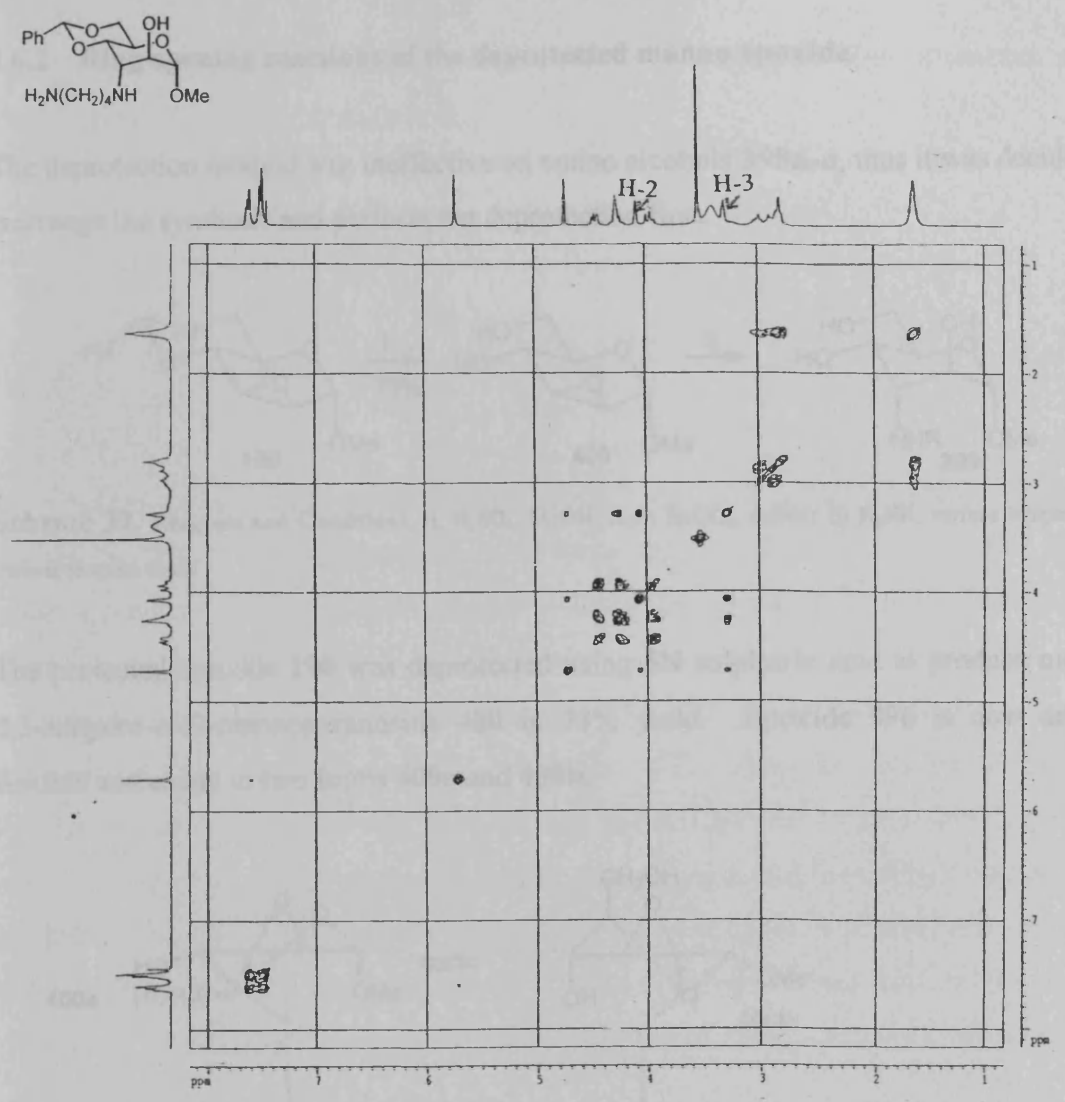
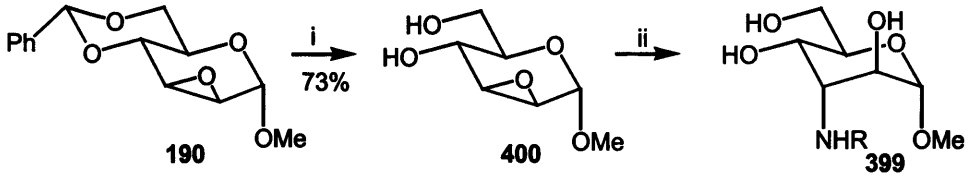


Figure 3. COSY spectrum of Methyl-3-(4-amino-butylamino)-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside **398d**

The methyl-4,6-*O*-benzylidene-3-amino sugars **398a-d** were then reacted with 5N sulphuric acid in order to cleave the benzylidene group. This method adopted from Jones *et al.*[117] proved unsuccessful and resulted in compounds which could not be identified by NMR or mass spectra, seventy percentage starting material was recovered. Other conditions such as 1M HCl were tried but were still futile. Recently a present member of the Jenkins group has successfully deprotected similar compounds using 80% aq AcOH.[118]

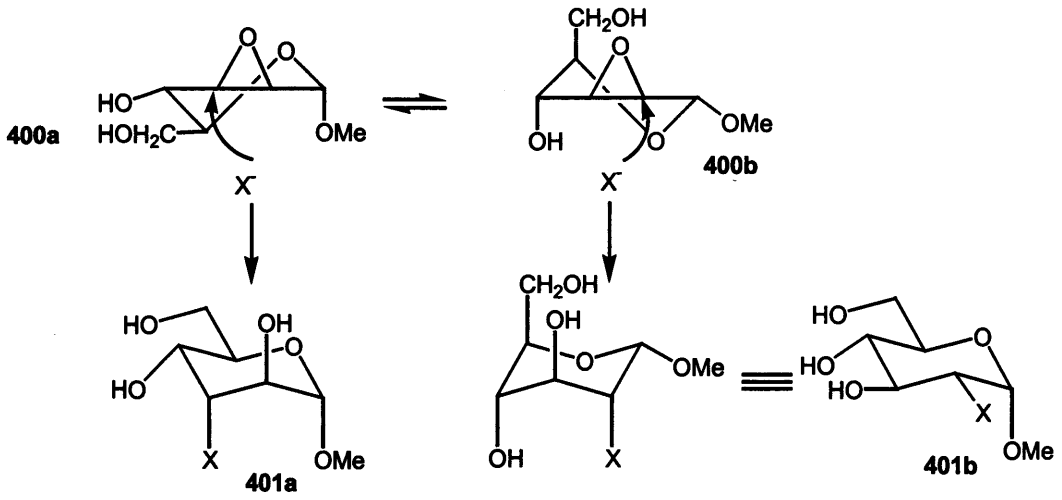
3.6.2 Ring opening reactions of the deprotected manno epoxide

The deprotection method was ineffective on amino alcohols **398a-d**, thus it was decided to rearrange the synthesis and perform the deprotection first.



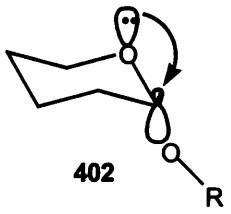
Scheme 32. **Reagents and Conditions**; i), H_2SO_4, MeOH, H_2O, $BaCO_3$, reflux; ii) H_2NR, various temperatures, various reaction times

The protected epoxide **190** was deprotected using 5N sulphuric acid to produce methyl-2,3-anhydro-α-D-mannopyranoside **400** in 73% yield. Epoxide **396** is now deemed flexible and exists in two forms **400a** and **400b**.

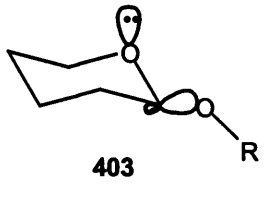


Each carbon-oxygen bond of the anhydro ring is equally accessible and both the altro **401a** and gluco **401b** orientations are achievable. Axial attack on both conformers would produce the substituents *trans* di-axial to each other, however, in the case of **400b** the resulting methoxy group is now pseudoequatorial, thus losing its role in stabilising the anomeric effect. The anomeric effect[119] arises due to the position of the electronegative substituent preferring to sit axial, even though the conformational analysis of cyclohexane derivatives is that the equatorial position is the favoured orientation of a large substituent. The axial (α) conformer is stabilised by delocalisation of an electron pair of the oxygen atom to the antiperiplanar C-O anti-bonding orbital[120] **402.** This shortens the C-O bond

between position one and six and gives it double bond character. This interaction is not present in the equatorial (β) anomer **403**.

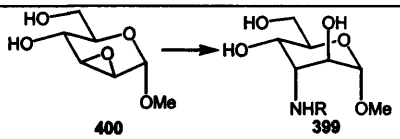


402



403

A previous member of the Jenkins group[121] had synthesised a number of diamine derivatives **399b-d** and attempted to purify them using an ion exchange column, but no suitable solvent system could be found to remove the amino alcohols from the column. Thus we resynthesised **399b-d** alongside synthesising **399g-w** by reacting the manno epoxide **400** with neat amines in a Youngs tube. The reactions subjected to various temperatures, and reaction times varied from 19-72 hours as detailed in Table 9. Purification of the products was the next step and this proved troublesome due to their extreme polar nature. An appropriate solvent system was found involving 2M ammonia in methanol mixed with either dichloromethane or ethyl acetate. The ammonia served the purpose of basifying the silica gel and the mixture of solvents eased removal of the amino compounds. Once this solvent system was established purification was straightforward.



400 **399**

	Product R=	**Yield (%)**	**Reaction Time (h)**	**Temp (^{0}C)**	**NMR Solvent**
399a	H	86	19	100	D_2O
399b	$H_2N(CH_2)_2$	40	24	120	D_2O
399c	$H_2N(CH_2)_3$	74	20	100	D_2O
399d	$H_2N(CH_2)_4$	87	24	120	D_2O
399e	$H_2N(CH_2)_5$	44	19.5	120	D_2O
399f	$H_2N(CH_2)_6$	30	24	110	D_2O
399g	$H_2N(CH_2)_7$	31	19	110	D_2O
395h	Allyl	75	19	110	D_2O
399i	Butyl	92	24	100	C_6D_6
399j	$HO(CH_2)_4$	92	24	100	C_6D_6

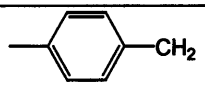
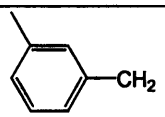
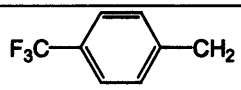
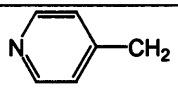
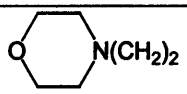
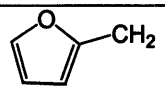
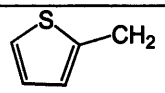
399k	Bn	68	24	222	$CDCl_3$
399l	$Ph(CH_2)_3$	94	72	125	$CDCl_3$
399m	$Ph(CH_2)_4$	77	24	100	D_2O
399n	*p*-$OMeC_6H_4CH_2$	92	31	100	MeOH
399o		96	24	100	C_6D_6
399p		85	24	100	MeOH
399q	H_2NPhCH_2	92	24	100	C_6D_6
399r		60	18	100	D_2O
399s		86	24	100	C_6D_6
399t		86	24	100	D_2O
399u		92	24	100	$CDCl_3$
399v		98	24	100	$CDCl_3$
399w		72	24	100	$CDCl_3$

Table 9 – Reaction times and temperatures for **399a-w**

The majority of the products were oils with the exception of methyl–3-(2-morpholin-4-yl-ethylamino)-3-deoxy-α-D-altropyranoside **395u**, which was recrystallised from a mixture of dichloromethane and methanol, and the resulting crystals were analysed by X-ray-diffraction which confirmed *trans*-diaxial ring opening of the starting epoxide **400**. Attack took place at C-3 in preference to C-2 and the pyranose ring adopted the $^4C_1$ conformation.

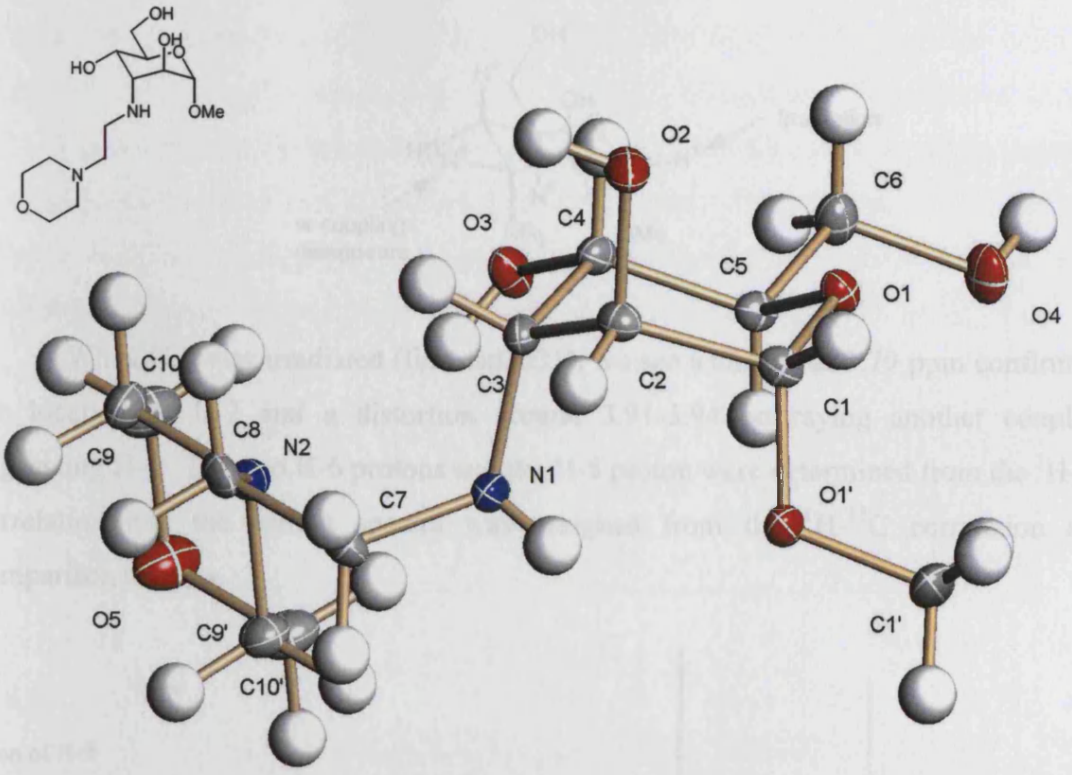
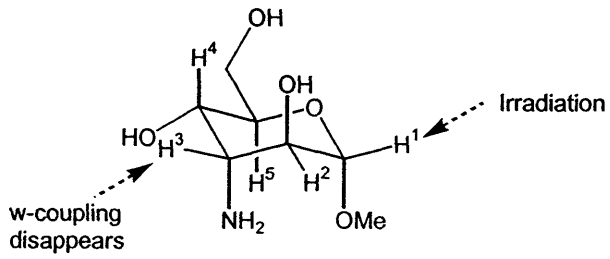


Figure 4. Methyl–3-(2-morpholin-4-yl-ethylamino)-3-deoxy-α-D-altropyranoside **399u**

The NMR of the products was performed in a number of solvents. In the case of the diamines deuterated water was used since they did not dissolve in any other solvent. Where $CDCl_3$ gave poor separation of the proton peaks, solvent induced diamagnetic shift was achieved with C_6D_6. This solvent gave improved clarity, separation and attainable measurement of *J* values.

3.6.2.1 Analysis of Methyl-3-amino-3-deoxy-altropyranoside (399a) and Methyl-3-allylamino-3-deoxy-α-D-altropyranoside (399i)

The first amino sugar derivative synthesised was **399a**, the ^1H NMR which was taken in D_2O (line cad2011) was complicated (Figure 5). We managed to perform homocoupling experiments on the sample, a copy of the results are included. The doublet at 4.67 ppm, we know as H-1 (the conventional carbohydrate numbering system is used). The anomeric proton always appears further downfield than the rest of the protons. When this signal was irradiated (line cad2021) we see the small *w* coupling on H-3 disappear (3.10 ppm) and a change at 3.79 ppm indicating a coupling therefore H-2.



When H-3 was irradiated (line cad2031), we see a change at 3.79 ppm confirming the location of H-2 and a distortion around 3.91-3.94 portraying another coupling suggesting H-4. The two H-6 protons and the H-5 proton were determined from the ^{1}H-^{1}H correlation and the carbon spectra was assigned from the ^{1}H-^{13}C correlation and comparison to **399a.**

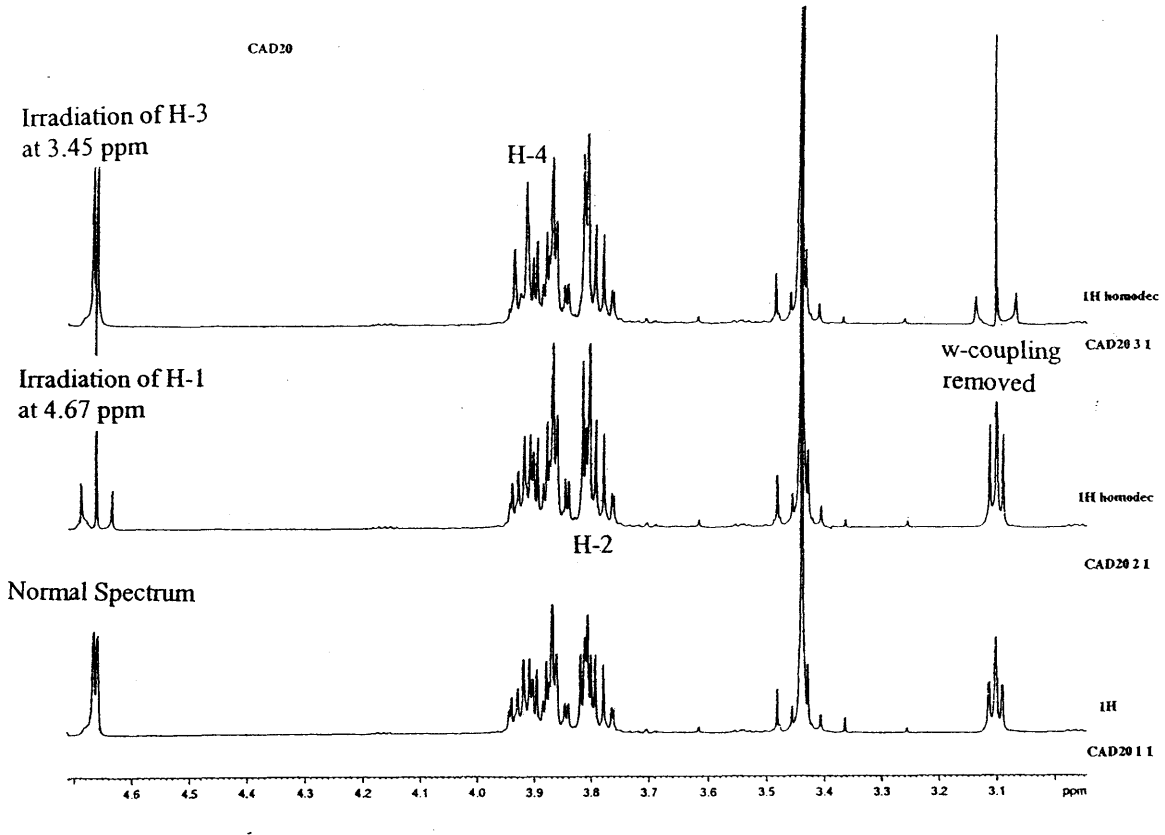


Figure 5 Homocoupling results for **399a**

The proton NMR of **399h**was performed in C_6D_6 and we can see how C_6D_6 has given the NMR improved clarity, and each proton has been assigned with the aid of the COSY NMR (Figure 6). The COSY shows coupling between H-3 and H-2 and also between H-3 and H-4, confirming H-3 is up field of the OMe peak. Coupling was detected between H-4 and H-5 and between H-5 and H-6. The *J* values that were obtained confirm this to be true $J_{3,4}$ was 5 Hz. This value can be regarded as an average value

pertaining to the hydrogens adopting both the axial and equatorial position, thus when in solution the amino alcohol adopts both the $^4C_1$ and $^1C_4$ orientation. The presence of the allyl group is indicated by the characteristic ddd for CH_2CH=CH_2 and the dd for each of the CH_2CH=C*H*H (*trans*) and CH_2CH=CH*H* (*cis*) protons. The ^{13}C sees an up field shift of the C-3 signal from 50.28 to 59.92 ppm and a downfield shift of the C-2 signal from 56.10 to 68.12 ppm indicating the presence of a polar group on C-2.

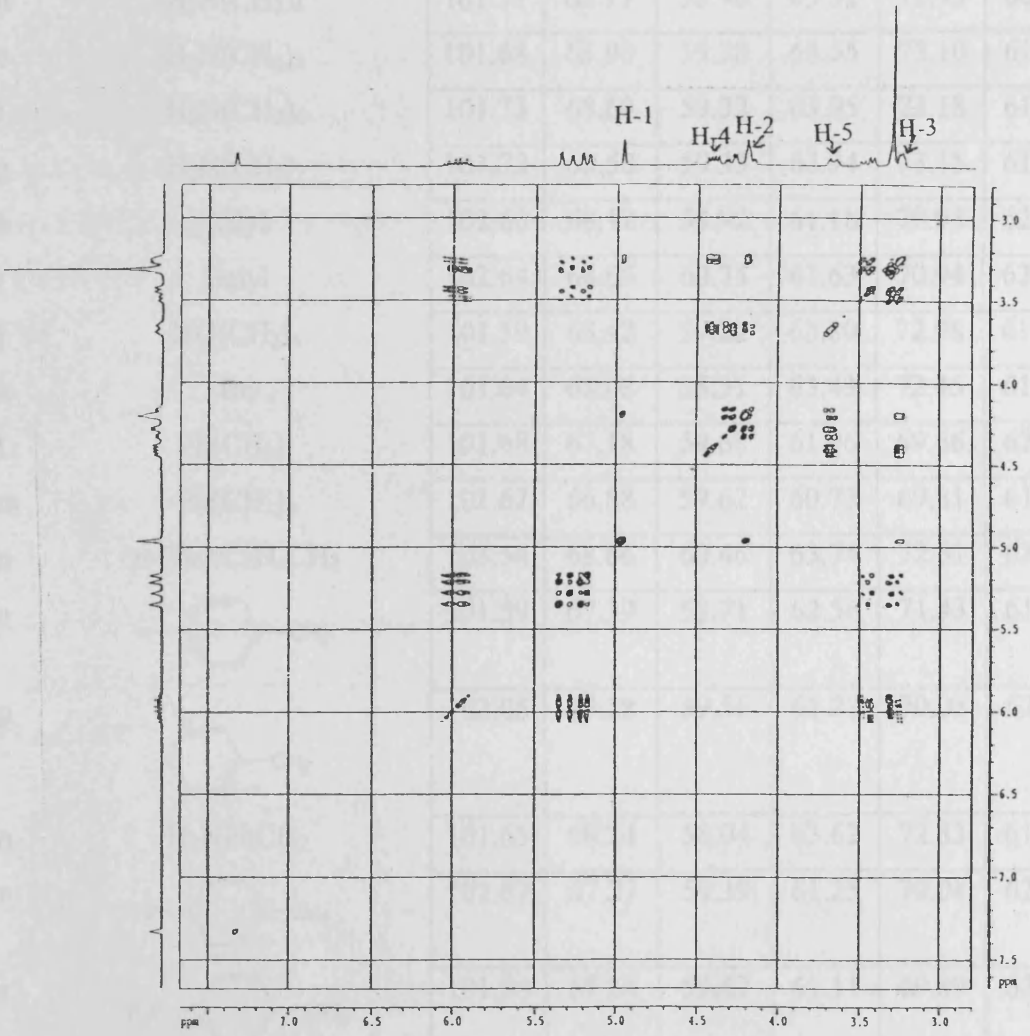
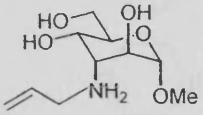


Figure 6. The COSY spectrum of Methyl-3-allylamino-3-deoxy-α-D-altropyranoside **395h.**



The accurate mass gave an ion relating to MH^+ and also a peak at 202 which indicates the loss of the methoxy group. Table 10 shows the ^{13}C shifts of **399a-399w,** which were assigned using ^1H-^{13}C correlation alongside comparison to **400**.

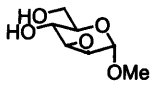
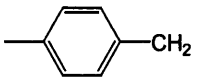
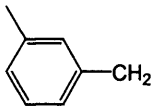
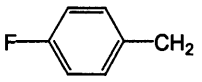
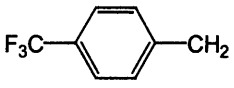
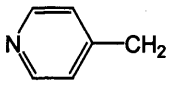
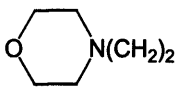
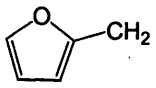
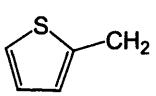
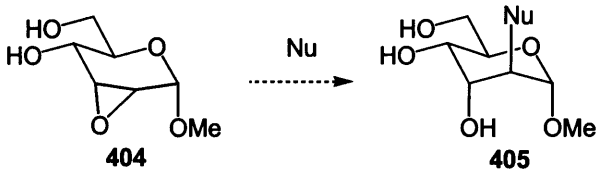
	R =	C-1	C-2	C-3	C-4	C-5	C-6
400		96.45	56.10	50.28	62.96	69.13	63.25
399a	H	101.27	70.22	53.18	64.74	70.46	61.37
399b	$H_2N(CH_2)_2$	101.65	63.83	59.36	68.46	72.76	61.18
399c	$H_2N(CH_2)_3$	101.72	68.45	59.39	63.87	72.86	61.20
399d	$H_2N(CH_2)_4$	101.31	68.17	58.90	63.52	72.73	60.74
399e	$H_2N(CH_2)_5$	101.68	63.90	59.28	68.55	73.10	61.12
399f	$H_2N(CH_2)_6$	101.73	68.60	59.32	63.95	73.18	61.17
399g	$H_2N(CH_2)_7$	101.72	68.58	59.33	63.94	73.15	61.18
399h	Allyl	102.60	68.12	59.92	61.18	70.93	62.74
399i	Butyl	102.64	68.05	60.75	61.63	70.94	62.78
399j	$HO(CH_2)_4$	101.59	68.42	59.22	63.80	72.98	61.05
399k	Bn	101.64	68.08	58.31	63.43	72.45	61.24
399l	$Ph(CH_2)_3$	101.68	67.18	59.68	61.06	69.66	62.34
399m	$Ph(CH_2)_4$	101.62	66.88	59.62	60.73	69.81	61.79
399n	*p*-$OMeC_6H_4CH_2$	103.64	68.86	60.46	63.74	72.61	63.86
399o		101.59	67.39	58.71	62.56	71.43	61.42
399p		102.05	67.28	59.56	61.23	70.08	62.25
399q	H_2NPhCH_2	101.65	68.24	58.04	63.62	72.83	61.17
399r		102.07	67.27	59.39	61.25	70.04	62.29
399s		101.96	67.24	59.62	61.11	69.89	62.07
399t		101.63	68.32	58.51	63.74	72.61	61.19
399u		102.09	68.28	59.74	62.28	70.16	62.62
399v		100.56	66.37	58.22	59.89	68.80	60.77
399w		101.49	67.89	59.85	61.79	70.73	62.63

Table 10. Comparison of ^{13}C shifts for **399a-w** and **400**

From the values one can see a trend in the data. A downfield shift of C-3 is observed from 50.28 ppm in the starting material **400** to 53.18-60.75 ppm. An average downfield shift of 10 ppm of the C-2 signal is also observed. This indicates the presence of a polar group namely the OH group attached to C-2, thus confirming *trans* attack of the amino group.

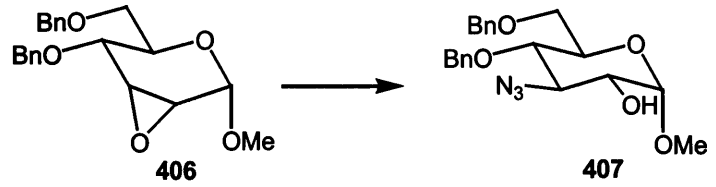
3.6.3 Ring opening reactions of the deprotected 'allo' Epoxide

The opening of the allo epoxide **266** was studied. In chapter two we opened the benzylidene protected down epoxide and we observed *trans* di-axial opening where ammonia attacked at C-2 to give **267**. In this chapter we wish to study the opening of the deprotected allo epoxide as a means of synthesising potential glycosidase inhibitors. We anticipated that we would observe *trans* diaxial opening at C-2 **405** as in the protected case **267**.

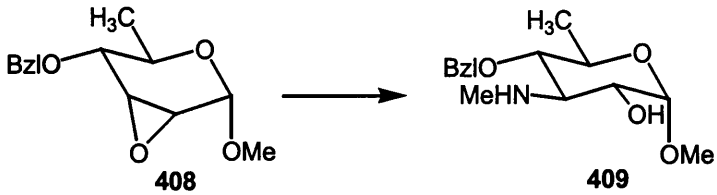


404 **405**

However literature precedence indicated the following examples where an allo epoxide was opened to give attack at C-3 and the diequatorial products as shown by examples **407**[122] and **409**[123]

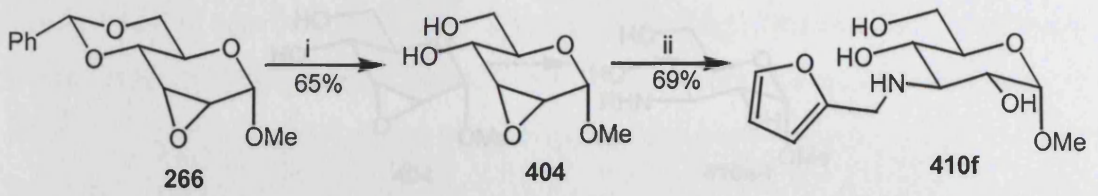


406 **407**



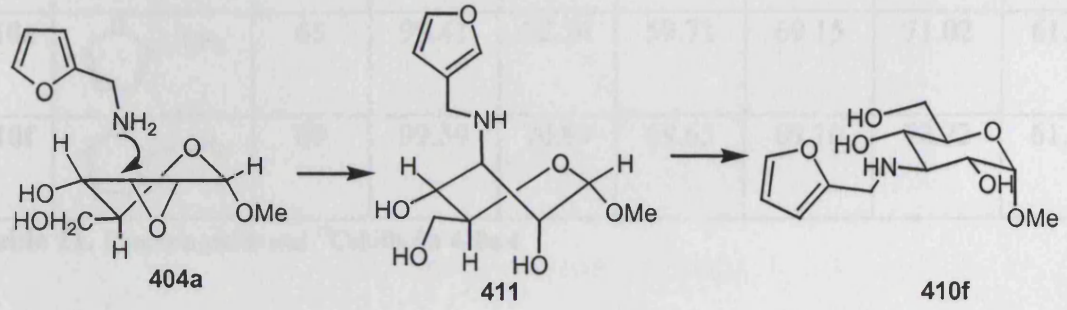
408 **409**

The protected allo epoxide **266** was deprotected to give epoxide **404** with acidified methanol and this was reacted with furfurylamine and the X-ray structure of the product **410f** was determined.



Scheme 33. **Reagents and Conditions**; i) H_2SO_4, reflux, MeOH, H_2O then $BaCO_3$; ii) furfurylamine, 24 h, 100^{0}C

The X-ray structure (figure 7) indicates that the amine has attacked at C-3 and we have obtained the diequatorial amino alcohol. This can be explained by nucleophilic attack at C-3 in the half chair conformation **404a** which leads to the boat conformation **411** which ring flips to the diequatorial amino alcohol **410f**.



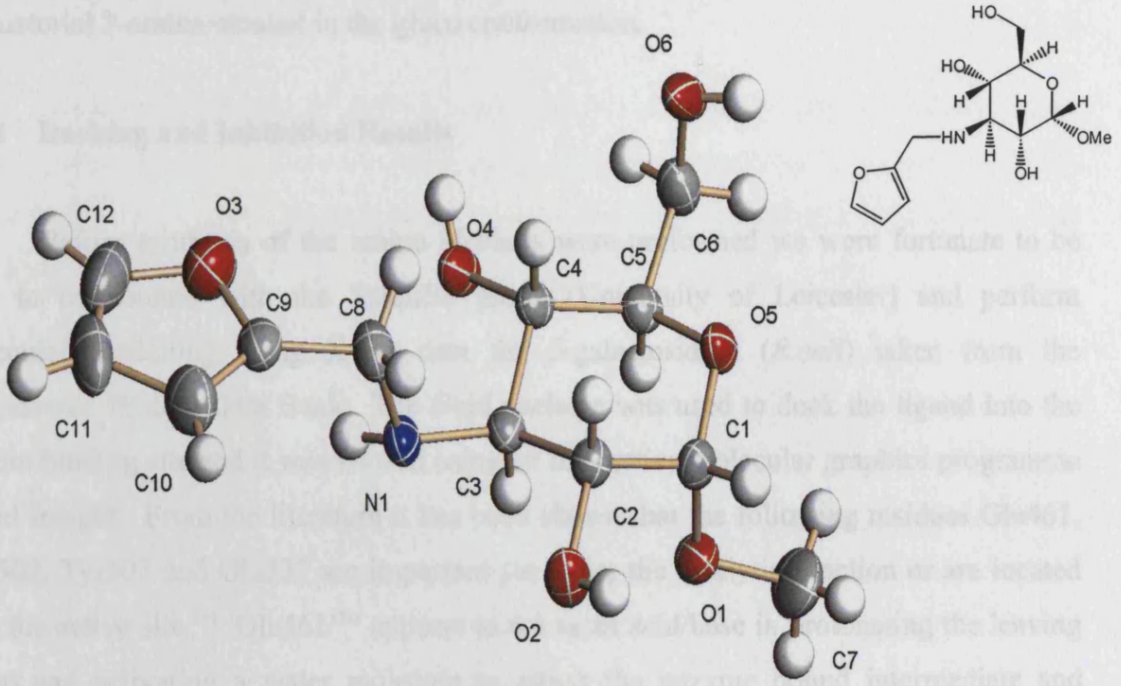
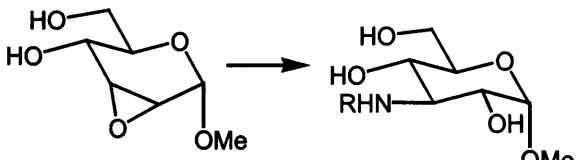


Figure 7. Methyl–3-(furan-2-ylmethyl)-amino-3-deoxy-α-D-glucopyranoside **410f**

A reduced number of amino alcohols were efficiently purified using the allo epoxide and we have also tabulated the ^{13}C shifts of C1-C6 of the sugar (Table 11).



404 410a-f

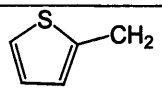
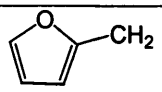
	Product R=	Yield (%)	C1	C2	C3	C4	C5	C6
410a	Allyl	22	101.14	70.46	58.62	64.81	69.21	62.99
410b	Butyl	21	101.19	70.37	59.62	64.94	69.28	63.11
410c	$HO(CH_2)_4$	66	99.45	70.47	60.81	68.64	72.33	61.06
410d	$F_3CC_6H_4CH_2$	38	99.81	71.89	61.24	69.84	72.77	61.63
410e		65	99.41	72.24	59.71	69.15	71.02	61.06
410f		69	99.39	70.99	59.65	69.10	72.22	61.00

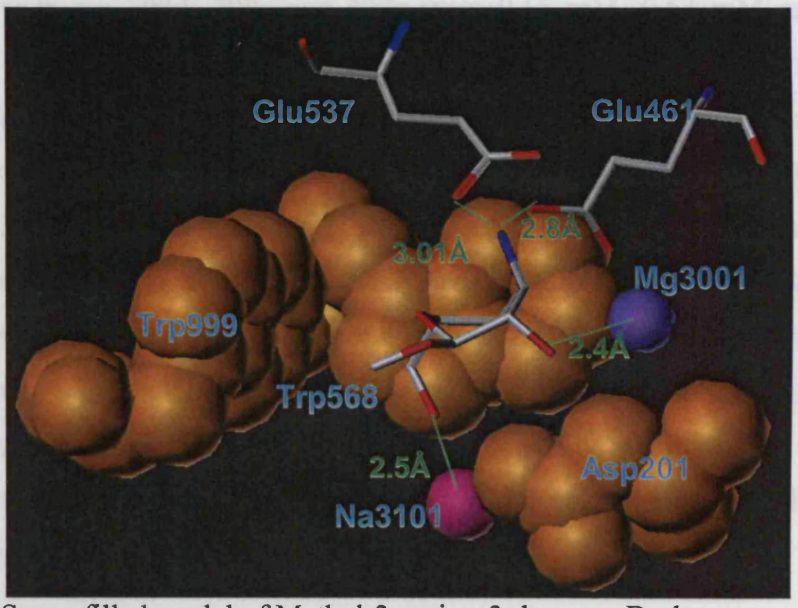
Table 11. Reaction yields and ^{13}Cshifts for **410a-f**

From the ^{13}C shifts we can extrapolate from **410f** that the other compounds have given the diequatorial 3-amino alcohol in the gluco conformation.

3.6.4 Docking and Inhibition Results

Before synthesis of the amino alcohols were performed we were fortunate to be able to collaborate with the Sutcliffe group (University of Leicester) and perform molecular modelling using X-ray data for β-galactosidase (*E.coli*) taken from the Brookhaven Protein Data Bank. The Gold package was used to dock the ligand into the protein binding site and it was viewed using an interactive molecular graphics programme called Insight. From the literature it has been shown that the following residues Glu461, Met502, Tyr503 and Glu537 are important for either the catalytic function or are located near the active site.[124] Glu461[124a] appears to act as an acid/base in protonating the leaving group and activating a water molecule to attack the enzyme bound intermediate and Glu537[124b] is the active site nucleophile that attacks the anomeric centre forming a covalent bond with the substrate. The docking experiments showed promising interactions between the substrate and the residues in the enzyme. Picture 2 illustrates methyl-3-amino-3-deoxy-α-D-altropyranoside **399a** docked into β-galactosidase. From the result one can see that the amino group is able to interact with both the glutamate residues, where Glu537

has been found to be a catalytic residue involved in binding. One can also see interaction between the hydroxyl groups on C-2 and C-6 with the sodium and magnesium ions.



Picture 2: Space filled model of Methyl-3-amino-3-deoxy-α-D-altropyranoside **399a** occupied in β-galactosidase *E.coli.*

3.6.4.1 Inhibition Results for Diamines 399b-g

The diamine derivatives **399b-g** were docked into the active site of β-galactosidase (*E.coli*) and similar interactions as described for **399a** were observed. The diamines **399b-g** were sent for testing, which were carried out by Professor R Nash and cowokers.[125] The results are summarised in Table 12.

	R group	IC_{50}
399a	H	-
399b	$H_2N(CH_2)_2$	-
399c	$H_2N(CH_2)_3$	-
399d	$H_2N(CH_2)_4$	0.15mM
399e	$H_2N(CH_2)_5$	0.63mM
399f	$H_2N(CH_2)_6$	-
399g	$H_2N(CH_2)_7$	0.98mM

Table 12; Comparison of β-galactosidase (bovine liver) IC_{50} values by **399b-g**

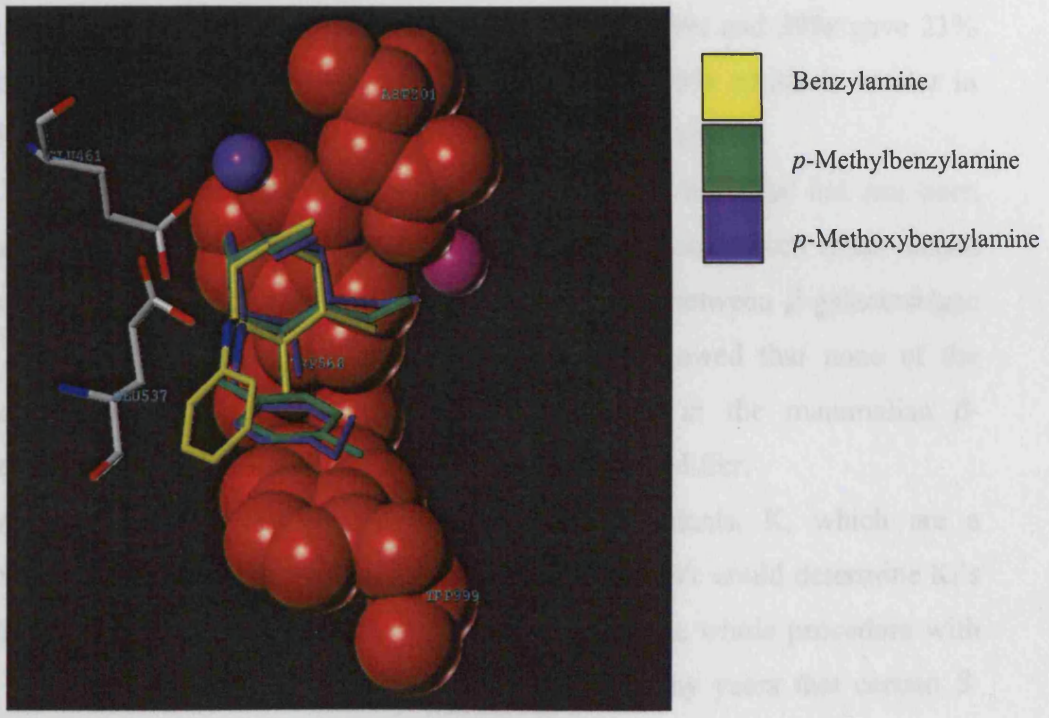
IC_{50} values portray the concentration of inhibitor required to inhibit 50% activity of the enzyme. Even though interactions were observed in the docking experiments, the

diamines displayed no inhibition of β-galactosidase from *E.coli*.. Instead inhibition was exhibited against β-galactosidase from bovine liver. IC_{50} values that were given to us from Aberystwyth for **399d, 399e** and **399g** were 0.15 mM, 0.63 mM and 0.98 mM respectively. From the IC_{50} values we can see that extending the carbon chain length between the amino functionalities sees a decrease in inhibition. Compounds **399b,c** showed minimal activity and **399f** gave 36% inhibition at a concentration of 0.14 mg/ml.

Since **399d** gave the lowest IC_{50} we decided to keep the carbon chain the same length but change the functional group at the end. Derivatives **399i** (butyl) and **399j** (hydroxyl) were synthesised and the former displayed 18% inhibition at a concentration of 0.14 mg/ml, while the latter showed minimal activity at the same concentration. The minimal activity and low percentage inhibition could be attributed to the lack of interaction between the residues in the active site and the functional group located at the end of the alkyl chain.

3.6.4.2 Inhibition Results for the Aromatic derivatives 399k-n

Three of the benzylamine derivatives **399k** (yellow) **399n** (purple) **399o** (green) were docked into the active site of β-gal. (*E.coli*). In the case of the *p*-methyl **399o** and *p*-methoxy **399n** derivatives π-π interactions could be seen between the phenyl group on the substrate and the phenyl group located on the tryptophan residue, suggesting a tighter binding ability of the substrate.



Picture 3: Stick model of **399k**, **399n** and **399o** occupied in β-galactosidase *E.coli*.

The derivatives in the title were sent for testing and the following results are displayed in Table 13. The samples that were sent for testing displayed no inhibition toward β-galactosidase from *E.coli* but in some cases inhibited β-galactosidase bovine liver. The *p*-methyl derivative **399o** gave an IC_{50} value of 0.67 mM, whereas the *p*-methoxy **399n** displayed no inhibition.

	Compound	**IC_{50}***
399k	$PhCH_2$	1.1mM
399l	$Ph(CH_2)_3$	70μM
399m	$Ph(CH_2)_4$	90μM
399o	*p*-$MePhCH_2$	0.67mM
399n	*p*-$OMePhCH_2$	-

Table 13. Comparison of β-gal (bovine liver) IC_{50} values by **399k-n** *Sample 313m was not completely dissolved

Considering the 1,4 diamino sugar **399d** gave the lowest IC_{50}, it was thought that a four carbon chain ending with a phenyl group would exhibit inhibition. Thus compounds **399k-m** were produced and tested. As we thought, these compounds displayed inhibition. Compound **399k** gave a value of 1.1 mM, compound **399l** gave an IC_{50} of 70 μM and compound **399m** (which was not completely dissolved) gave 90 μM a marked improvement on previous results. This improvement may have been due to the flexibility of the alkyl chain allowing the phenyl group to sit in a more parallel orientation in respect to the tryptophan residue. Other aromatic derivatives such as **399t** and **399r** gave 21% and 20% inhibition at 0.14 mg/ml respectively. Interestingly **399s** which is similar in structure to **399r** only showed minimal activity at the same concentration.

The X-ray crystal structure of β-galactosidase from bovine liver has not been reported, thus it makes it difficult to draw any specific conclusions from these results regarding the active site. Sequence alignment was performed between β-galactosidase (*E.coli*) and two mammalian β-galactosidases. The results showed that none of the residues found in the active site of *E.coli* was conserved in the mammalian β-galactosidase. Thus one can conclude that the active site residues differ.

For more information we can estimate inhibitor constants, K_i which are a quantitative measure of the inhibitory strength of the substrate. We could determine K_i's by first performing a standard K_m analysis and then repeating the whole procedure with the inhibitor in the assay buffer. It has been observed for many years that certain β-

glucosidases and galactosidases only hydrolyse correctly configured substrates, whereas others only show minor preference to the configuration at C(4).[126]

The amino alcohols were also tested against a number of other enzymes, the results collected in tabular form as shown. For the various assays against each enzyme the amino alcohols were used as supplied (described in table). From the raw data obtained the compounds displayed reasonable percentage inhibition against β-glucosidase. Thus each amino alcohol was tested again at a concentration of 1mg/ml. Compounds **399i**, **399k**, **399**, **399m**, **399u**, all displayed percentage inhibition values over 68%. These values are highlighted in bold. It is interesting to note that **399l** and **399m** also displayed activity against β-gal. bovine liver. All the compounds in the table either gave negative values, for α-glucosidase, *N*-acteyl-β-glucosaminidase, nargingnase and α-mannosidase, which could indicate a lack of substrate/active site residue interaction.

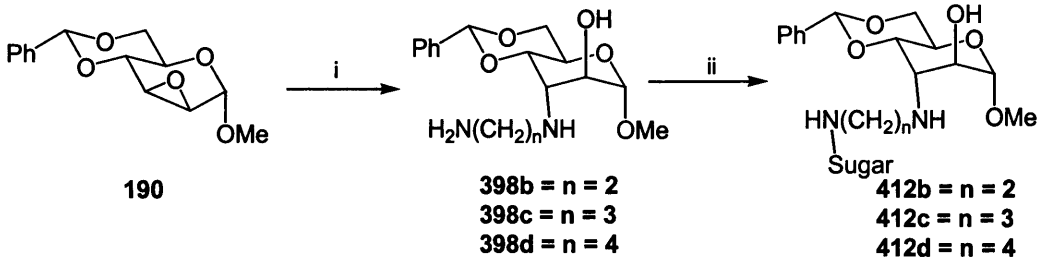
Amount as supplied (mg/ml):	*7.5*	*12.5*	*7.5*	*14.5*	*18*	*7.5*	*11.5*	*10*
Assay	**399o**	**399i**	**399h**	**399u**	**399w**	**399t**	**399j**	**399v**
α-glucosidase	-6.7	0.1	-1.5	-8.3	-3.2	-4.6	-9.6	2.3
β-glucosidase (samples as supplied)	59.3	87.2	67.6	79.2	52.6	67.6	85.1	41.1
β-glucosidase (samples at 1mg/ml)	9.6	**75.7**	25.7	**68.3**	-1.3	25.9	62.9	9.4
N-acetyl-β-glucosaminidase	-0.9	5.8	9.1	-2.6	4.8	4.6	5.5	4.6
Naringinase	9.2	11.3	18.0	17.8	12.2	8.0	5.6	6.7
α-mannosidase	-6.5	-29.0	-12.6	-27.1	-13.6	-27.1	-33.8	-15.8

Amount as supplied (mg/ml):	*9.5*	*6.5*	*15.5*	*2.5*	*3*	*5.5*	*5.5*	*3*
Assay	**399s**	**399q**	**399r**	**399f**	**399g**	**399k**	**399m**	**399l**
α-glucosidase	-12.8	-11.8	-4.1	-0.8	2.2	-3.8	-1.4	14.6
β-glucosidase (samples as supplied)	86.7	35.4	92.3	35.8	35.3	80.4	93.0	88.1
β-glucosidase (samples at 1mg/ml)	61.1	11.4	53.2	20.0	16.3	**78.3**	**85.5**	**76.3**
N-acetyl-β-glucosaminidase	-0.1	-15.3	1.6	0.5	-2.1	22.0	-6.5	-5.5
Naringinase	17.4	10.3	23.3	23.2	10.8	9.0	13.8	10.0
α-mannosidase	6.2	24.8	-14.5	-16.0	-0.4	-16.8	-19.8	-17.4

Table 14. Inhibition results of compounds **399f-m**, **399o** and **399q-w** on a range of glycosidases.

3.6.5 Bis sugars

From the literature,[108-110] there were numerous examples of bis-sugars displaying inhibitory properties. A number of bis-sugars were also synthesised.



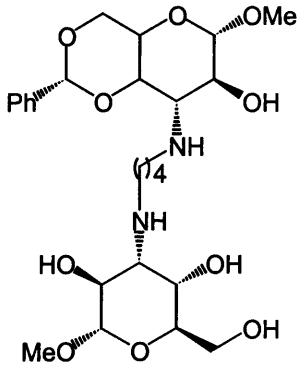
190

398b = n = 2
398c = n = 3
398d = n = 4

412b = n = 2
412c = n = 3
412d = n = 4

Scheme 34 Reagents and Conditions; i) various amines, 100^{0}C, 24h; ii) **394b-d**, 100^{0}C, 48h

Reaction of the epoxide **190** with various diamines afforded the amino alcohols **398b-d**. The protected amino alcohol was placed in a Youngs tube as before with the manno epoxide **400** and left to stir at 100^{0}C. The NMR(s) of the resulting products were uninterpretable, but the mass spectra gave one defined peak confirming the presence of the product. Purification was impossible via column chromatography. High performance liquid chromatography (HPLC) was then employed. An aqua C18, 5μM column was used with a flow rate of 0.2 ml/min using a 4:1 solvent mixture of acetonitrile and water. The HPLC gave two peaks for each bis-sugar made. The results were not reproducible on a quantitative scale. Bis-sugar **412d** was sent for testing and displayed no activity.



412d

This may either imply the substrate was impure or the active site of the enzyme is pocket shaped, thus not being able to accommodate such a large substrate.

3.7 Conclusion

The inhibition of glycosidase enzymes has been an important area in carbohydrate chemistry for many years. Inhibitors that are aza analogues of the intermediate oxonium ion involved in glycosidation are well known, such as nojirimycin **342**. As part of a continuing study on new azasugar glycosidase inhibitors we have studied the opening of two sugar epoxides with amino nucleophiles. We observed the expected *trans* di-axial opening of both the benzylidene protected and deprotected manno epoxide **190, 400.** This is in agreement with the wide literature precedence of *trans* di-axial opening. The stereochemistry of these compounds were confirmed by X-ray crystallography and careful ^{13}C comparison of the products achieved from the deprotected manno epoxide, showed that they were in agreement with *trans* di-axial opening.

To our surprise reaction of the deprotected allo epoxide **404** with a range of amines gave di-equatorial opening of the epoxide. Whilst trying to explain why this had happened we discovered two other groups who confirmed di-equatorial attack.[122,123] These results were confirmed further by an X-ray crystal structure and all the examples studied were correlated by ^{13}C chemical shifts.

Docking experiment were performed and these showed promising interactions between the inhibitor and the active site residues. The amino sugar derivatives were tested for glycosidase inhibition and IC_{50} activity in the mM and μM range were observed against β-galactosidase from bovine liver. Inhibition studies were also executed against a number of other glycosidase enzymes and resulted in inhibition percentages ranging from -1.3-85.5% at a concentration of 1mg/ml. A number of bis sugars were successfully synthesised, but purification was not possible.

The X-ray crystal structure of β-galactosidase from bovine liver has not been reported, thus the results obtained makes it difficult to draw any specific conclusions regarding the active site. Once the active site structure has been solved, this will enable a better understanding of the mechanism of galactosidase in more detail.

3.8 References

1. Hanessian, S., *"Total synthesis of Natural Products: The Chiron Approach,"* Pergamon Press, Oxford, **1983**
2. Varki, A., *Glycobiology*, **1993**, *3*(2), 97
3. Truscheit. E. Frommer,W.,Junge, B., Muller, L., Schmidt, D.D., Wingender, W., *Angew.Chem.Int.Ed.Engl.*, **1981**, *20*, 744
4. Boons, G.J (Ed)., *Carbohydyrate Chemistry*, Blackie Academic and Professional, **1998**
5. Richtmyer, N.K., *Methods Carbohydr.Chem.*, **1962**,*1*, 107
6. Schmidt, D., *Methods Carbohydr.Chem.*, **1963**, *2*, 318
7. Wolfman, M.L., Thompson, A., *Methods Carbohydr.Chem.*, **1963**, *2*, 427
8. Drauz, K., Kleeman, A., Martens, J., *Angew.Chem.Int.Ed.Engl.*, **1982**, *21*, 584
9. Szabo, W.A., Lee, H.T., *Aldrichimica Acta.*, **1980**, *13*, 13
10. Seebach, D in *Modern Synthetic Methods,* Scheffold, R (Ed)., Ato Salle Verlag, Frankfurt am Main, Germany, **1980**
11. Verheyden, J.P.H., Richardson, A.C., Bhatt, R.S., Grant, B.D., Fitch, W.L., Moffatt, J.G., *Pure Appl.Chem.*, **1978**, *51*, 1363
12. Wang, C.M., Buccini, J., TeRaa, J., *Can.J.Chem.*, **1968**, *46*, 3091
13. Paul, K.G., Johnson, F., FaVara, D., *J.Am.Chem.Soc.*, **1976**, *98*, 1285
14. Stork, G., Takahashi, T., Kawamoto, I., Suzuki, T., *J.Am.Chem.Soc.*, **1978**, *100*, 8272
15. Corey, E.J., *Pure Appl.Chem.*, **1969**, *14*, 30
16. Hodgson, J.*Bio/Technology*, **1991**, *9*, 609
17. Leonard, C.K., Spellman, M.W., Riddle, L., Harris, R.J., Thomas, J.N., Gregory, J.J., *J.Biol.Chem.*, **1990**, *265*, 10373
18. Hakomori, S., *Adv.Cancer.Res.*, **1989**, *52*, 257
19. Acker, R.F., Hartsell, S.E., *Sci.Am.*, June **1960**
20. Johansen, P.G., Marshall, R.D., Neuberger, A., *J.Biol.Chem.*, **1961**, *78*, 578
21. Hart, G.W., *Annu.Rev.Biochem.*, **1997**, *66*, 315
22. Drickamer, K., Taylor, M.E., *Annu.Cell.Biol.*, **1993**, 237
23. Rademacher, T.W., Parekh, R.B., Dwek, R.A., *Annu.Rev.Biochem.*,**1988**, *57*, 785
24. Yeh, Y., Feeney, R.E., *Chem.Rev.*, **1996**, *96*, 601
25. Muller, D.J., Macek, M.B., Shir, B.D., *Nature*, **1992**, *357*, 589
26. Landsteiner, K., *Zentralbl Bakeriol.Parastienkol.Infctionskrankh.Abt. 1.*, **1900**, *27*, 357
27. Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., (Eds) *Essentials of Glycobiology.*, **1999**, Laboratory Press, Cold Spring Harbor

28. Waekins, W.M., *Biochem.Soc.Trans.*, **1987**, *15*, 620
29. Fraser-Reid, B., Tatsuta, K.,Theim, J, (Eds), *Glycoscience – Chemistry and Chemical Biology.*, **2001**, *vol III*, Springer-Verlag Berlin Heidelberg, New York
30. Pohlentz, G., Klein, D., Schwarzmann, G., Schmtz, D.,Sandhoff, K., *Proc.Natl.Acad.Sci.USA.*, **1988**, *85*, 7044
31. Berten, S., Doren, K.R., Hirsh, D., *Genes.Rev.*, **1988**, *2*,1227
32. Neufeld, E.F., *Annu.Rev.Biochem.*, **1991**, *60*, 257
33. Legler, G., *Adv.Carbohydr.Chem.Biochem.*, **1990**, *48*, 319
34. Ganem, B., *Acc.Chem.Res.*, **1996**, *29*, 340
35. Hideya, Y., Chikako, S., Osamu, K., *Trends in Glycoscience and Glycotechnology.*, **2002**, *14*, 231
36. Leonetti, J.P., Rayner, B., Lemoutre, M., Gagnor, C., Milhaud, P.G., Imbach, J.L., Lebleir, B., *Gene*, **1988**, *72*, 323
37. Gewirtz, A.M., Antos, G., Ventwelli, D., Valpreda, S., Sims, R., Calabietta, B., *Science*, **1989**, *245*, 180
38. Alper, J., *Biotechnology*, **1993**, *11*, 1125
39. Nielsen, P.E., Christensen, L., *J.Am.Chem.Soc.*, **1996**, *118*, 2287
40. Wayner, R.E., *Nature*, **1994**, *373*, 333
41. Zhar, B.W., Puga, E., Sun, J.S., Garestier, T., Héléne, C., *J.Am.Chem.Soc.*, **1995**, *117*, 10425
42. Kemry, M.A., Brown, S.C., Berman, E., Shafer, R.H., *Biochemistry*, **1987**, *6*, 1058
43. Yu, H., Hurley, L.H., Kerwin, S.M., *J.Am.Chem.Soc.*, **1996**, *118*, 7040
44. Gruters, R.A., Neefjes, J.J., Tersmette, M., Degoede, R.E.Y., Tulp, A., Huisman, H.G., Miedema, F., Ploegh, H.L., *Nature,* **1987**, *330*, 74
45. Jacob, G.S., Bryant, M.L., *Persp.Drug Discov.Design,* **1993**, *1*, 211
46. Dwek, R.A., *Chem.Rev.,* **1996**, *96*, 683
47. Hakomori, S., *Cancer Res.,* **1985**, *45*, 2405
48. Goss, P.E., Baptisk, J., Fernandes, B., Baker, M., Dennis, J.W., *Cancer Rev.,* **1994**, *54*,1450
49. Davis, G., Henrissatt, B., *Structure,* **1995**, *3*, 853
50. Henrissatt, B., *Biochem.J.,* **1991**, *280*, 309
51. Koshland, D.E., *Biol.Rev.,* **1953**, *28*, 416
52. McCarther, J.D., Withers, S.G., *Curr.Opin.Struc.Biol.,* **1994**, *4*, 885
53. Henrissatt, B., Barroch, A.,*Biochem.J.,* **1993**, *293*, 781
54. Pauling, L., *Chem.Eng.News.*, **1946**, *24*, 1375; *Nature*, **1948**, *161*, 707

55. Jenks, W.P., *Adv.Enzymol.,* **1975**, *43*, 219

56. Matsui, M., Bianchard, J.S., Brewer, C.F., Hetire, E.J., *J.Biol.Chem.,* **1989**, *264*, 8714

57. Conchie, J., Levvy, G.A., *Biochem.J.,* **1957**, *65*, 389

58. Beer D., Vasella, A., *Helv.Chim.Acta.,* **1986**, *69*, 267

59. Ermert, P., Vasella, A., *Helv.Chim.Acta.,* **1991**, *74*, 2043

60. Tatsuta, K., Miura, S., Ohta, S, Gunji, H., *Tetrahedron Lett.,* **1995**, *36*, 1085

61. Legler, G., Pohl, S., *Carbohydr.Res.,* **1983**, *116*, 95

62. Ichikawa, Y., Igarashi, Y., *Tetrahedron Lett.,* **1995**, *36*, 4585

63. Legler, G., Herrchen, M., *Carbohydr.Res.,* **1983**, *116*, 95

64. Dong, W., Jespersen, T.M, Bols, M., Skrydstrup, T., Sierks, M.R, *Biochemistry,* **1996**, *35*, 2788

65. Tong, M.K., Papandreou, G., Ganem, B., *J.Am.Chem.Soc.,* **1990**, *112*, 6137

66. Legler, G., Finken, M.T., *Carbohydr.Res.,* **1996**, *292*, 103

67. Ganem, B., Papandreou, G., *J.Am.Chem.Soc.,* **1991**, *113*, 8984

68. Papandreou, G., Tong, M.K., Ganem, B., *J.Am.Chem.Soc.,* **1993**, *115*, 11682

69. Hoos, R., Naughton, A.B., Thiel, W., Vasella, A., Weber, W., Rupitz, K., Withers, S.G., *Helv.Chim.Acta.,* **1993**, *76*, 2666

70. Heightman, T.D., *O-Glycosidases Under Scrutiny:Synthesis of Inhibitors as Kinetic and Structural Probes,* **1998**, pg 29

71. Ishida, N., Kumagai, K., Niido, T., Tsuruaka, T., Yumato, H., *J.Antibiot.,* **1967**, *20*, 66

72. Inouge, S., Tsuruaka, T., Ito, T,Niida, T., *Tetrahedron.,* **1968**, *24*, 2125

73. Murro, S., Miyata, S., *Agric.Biol.Chem.,* **1980**, *44*, 219

74. Legler, G., in *Iminosugars as Glycosidase Inhibitors,* **1999**, Wiley-VCH, pg 39

75. Legler, G., in *Carbohydrate Mimics.,* **1997**, VCH-Weinheim, Chapter 24

76. Jespersen, T.M., Dong. W., Sierks, M.R., Skrydstup, T., Lundt, I., Bols, M., *Angew.Chem.Int.Ed.Engl.,* **1994**, *33*, 1778; Jespersen, T.M., Bols, M., Sierks, M.R., Skrydstup, T., *Tetrahderon,* **1994**, *50*, 13449

77. Trnka, T., Cerny, M., *Collect Czeh.Commun.,* **1971**, *36*, 2216

78. Kajimoto, T., Lui, K., Pederson, R.L., Zhong, Z., Ichikeiwa, Y., Porco.Jr., Wong, C.H., *J.Am.Chem.Soc.,* **1991**, *113*, 6191

79. Vasella, A.T., Heightmann, T.D., *Angew.Chem.Int.Ed.Engl.,* **1999**, *38*, 750

80. Schweden, J.,Borgmann, C., Legler, G., Bause, E., *Arch.Biochem.Biophys.,* **1986**, *248*, 335

81. Brookman, M., Lehmann, J., *Carbohydr.Res.,* **1977**, *53*, 21

82. Hősel, W.,Cann, E.E., *Trends Biochem.Sci.,* **1982**, *1*, 219

83. Ermert, P., Vasella, A., Weber, M., Rupitz, K., Withers, S.G., *Carbohydr.Res.,* **1993**, *250*, 113

84. Legler, G., *Biochim.Biophys.Acta.,***1978**, *524*, 94

85. Niwa, T., Tsurouko, T., Goi, H., Kodama, Y., Itoh, J., Inoues, S., Yamodo, Y., Niida, T., Nobe, M., Ogawa, Y., *J.Antibiot.,* **1984**, *37*, 1579

86. Miyake, Y., Ebata, M., *Agric.Biol.Chem.,* **1988**, *82*, 661

87. Evans, S.V., Fellows, L.E., Shing, T.K.M., Fleet, G.W.J., *Phytochemistry* **1985**, *24*, 1953

88. Paulsen, H., Sangster, A., Heyns, K., *Chem.Ber.,* **1967**, *100*, 802

89. Yagi,M., Kouno, T., Aoyagi, Y., Murai, H., *Nippon.Nogel.Kagaku.Kaishi.,* **1976**, *50*, 571

90. Kite, G.C., Fellows, L.E., Fleet, G.W.J., Lui, P.S., Scofield, A.M., Smith, W.G., *Tetrahedron Lett.,* **1988**, *29*, 6483

91. Fleet, G.W.J., Nicholas, S.J., Smith, P.W., Evans, S.V., Fellows, L.E., Nash, G.W.J., *Tetrahedron Lett.,* **1985**, *26*, 3127

92. Axamawaly, M.T.H., Fleet, G.W.J., Hannah, K.A., Namgoong, S.K., Sinnott, M.L., *Biochem.J.,* **1990**, *266*, 245

93. Saul, R., Chambers, J.P., Molyneux, R.J., Elbein, A.D., *Arch.Biochem.Biophys.,* **1983**, *221*, 593

94. Pan, Y.T., Her, H. saul, R., Sanford, B.A., Molyneux, R.J., Elbein, A.D., *Biochemistry,* **1983**, *22*, 3975

95. Molyneux, R.J., *J.Nat.Prod.,* **1990**, *53*, 609

96. Molyneux, R.J., Benson, M., Wong Cenci di Bello, J., Dorling, P., Winchester, B., *Biochem.J.,* **1983**, *215*, 693

97. , R.Y., Tropea, J.E., Elbein, A.D., *J.Nat.Prod.,* **1988**, *51*, 1198

98. Hohenschutz, L.D., Bell, Z.A., Jewess, P.J., LeWorthy, D.P., Pryce, R.J., Arnold, E., Clardy, J., *Phytochemistry,* **1981**, *20*, 811

99. Nash, R.J., Fellows, L.E., Bring, J.V., Fleet, G.W.J., Gurdhar, A., Ramsden, N.G., Peach, J.M., Hegarty, M.P., Scofield, A.M., *Phytochemistry,* **1990**, *29*, 111

100. Dickson, L.G., Leroy, E., Raymond, J.L., *Org.Biomol.Chem.,* **2004**, *2*, 1217

101. Murao, S., Sakai, T., Gubo, H., Nakayama, T., Shin, T., *Agri.Biochem.,* **1991**, *55*, 895

102. Sakuda, S., Isogai, A., Matsumoto, S., Suzuki, A., Koeski, K., *Tetrahedron Lett.,* **1986**, *27*, 2475

103. Cohen, E., *Arch.Insect.Biochem.Physiol.,* **1993**, *22*, 245

104. Yoshikawa, M., Murakomi, T., Yashiro, K., Matsuda, H., *Chem.Pharm.Bull.*, **1998**, *46*, 339

105. Yuasa, H., Kajimoto, T., Wong, C-H., *Tetrahedron Lett.*, **1994**, *35*, 8243

106. Merrer, Y.L., Fuzler, M., Dosbaa, I., Foglietti, M.J., Depezay, J.C., *Tetrahedron*, **1997**, 53, 16731

107. Robina, I., Gomez-Bujedo, S., Fernandez-Bolanos, J.G., Pozo, L.D., Demange, R., Picassas, T., Vogel, P., *Carbohydr.Lett.*, **2000**, *3*, 389

108. Lee, Y.C., Lee, R.T., *Acc.Chem.Res.*, **1995**, *28*, 321

109. Johns, B.A., Johnson,C.R., *Tetrahedron Lett.*, **1998**, *39*, 749

110. Duff, F.J., Vivien, V., Wightman, R.H., *Chem.Commun.*, **2000**, 2127

111. Wallenfels, K., Weil, R., *β-Galactosidase.*, **1972**, in Boyer, P., (Ed), *The Enyzmes*, 3rd ed, vol7,London, Academic Press

112. Peat, S., *Advanc.Carbohydr.Chem.*, **1946**, *2*, 37

113. Mills, quoted by Newth & Horner., *J.Chem.Soc.*, **1953**, 989

114. Fűrst & Plattner, *Abs.Papers.12th Internat.Congr.*,Pure.and Applied Chem. **1951**, New York, pg 409

115. Vega-Perez, M.; Candela, I.; Vega, M.; Iglesias-Guerra, F. *Carbohydr.Res.* **1995**, 279, C5

116. Myer, K.E., Walsh, P.J., Bergman., R.G., *J.Am.Chem.Soc.*, **1994**, *116*, 2669

117. Jones, K., McEvoy,J., Wood, W.W., Scott, I.L., *J.Chem.Research(M).*, **1987**, 1773

118. Laventine, D.M., Jenkins, P.R., Cullis, P.M., *Tetrahedron Lett.*, **2005**, *46*, 2295

119. Lemieux, R.U., Chu, N.J., *Abstracts of Papers Am.Chem.Soc.*, **1958**, *133*, 31N

120. Wolfe, S., Whangbo, M.H., Mitchell, D.J., *Carbohydr.Res.*, **1979**, *69*, 1

121. Smith, C., *M.Chem. Report*, **2001**

122. Wu, X., Kong, F., Lu, D., Li, G., *Carbohydr.Res.*, **1992**, *235*, 1992

123. Hollingsworth, R.I., Hrabak, E.M., Dazzo, F.B., *Carbohydr.Res.*, **1986**, *154*, 103

124. (a) Macleod, A.M., Linhorst, T., Withers, S.G., Warren, R.A., *Biochemistry*, **1994**, *33*, 6371; (b) Gebler, J.C., Aebersold, R., Withers, S.G., *J.Bio.Chem.*, **1992**, *267*, 11126

125. Nash, R., Molecular Nature Ltd, Plas Gogerddan, Aberystwyth,Ceredigion, SY23 3EB, UK

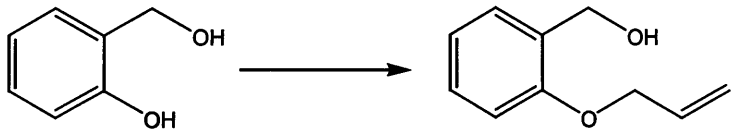
126. Love, D.R., Fisher, R., Bergquist, P.L., *Mol.Gen.GenetI.*, **1988**, *213*, 84

Chapter 4
Experimental

4.1 General Experimental

The synthesis of some compounds on a large scale proved to be laborious, and therefore experimental procedures for compounds prepared previously have been included where modifications to the published procedure have been employed, or little or no data for compounds was available. All reactions were performed under an atmosphere of nitrogen (unless otherwise stated) and solvent extractions were dried with anhydrous magnesium sulphate. Tetrahydrofuran and benzene were distilled from sodium-benzophenone. Diethyl ether was distilled from lithium aluminium hydride. Chloroform was distilled from phosphorus pentoxide and stored over molecular sieves. Dichloromethane was distilled from calcium hydride. Petroleum ether refers to the 40-60^{0}C boiling fraction. Flash column chromatography was performed on Sorbil C-60 silica gel (Crossfield Chemicals) 40-60 μM. Thin layer chromatography (TLC) analysis was conducted on pre coated silica sheets (60-254) with a 0.2 mm thickness, manufactured by Merck and Co. Melting points were obtained on a Kofler hotstage and are uncorrected. Elemental analyses were carried out by Butterworth Laboratories, Teddington, Middlesex. Infrared (IR) spectra were recorded using a Perkin Elmer FT-IR spectrometer, intensities are referred to as strong (s), medium (m), weak (w) or broad (br). Optical rotations were recorded on Perkin Elmer 341 Polarimeter. Mass Spectra were recorded on Kratos Concept Sector mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 250 (250 MHz ^{1}H, 62.9 MHz ^{13}C), Bruker AM 300 (300 MHz ^{1}H, 75.5 MHz ^{13}C), or Bruker DRX 400 (400 MHz ^{1}H, 100.6 MHz ^{13}C) spectrometer. NMR spectra recorded in $CDCl_3$ unless otherwise stated. All chemical shifts δ are given in ppm and were taken directly from the spectra; coupling constants *J* are given in hertz (Hz).

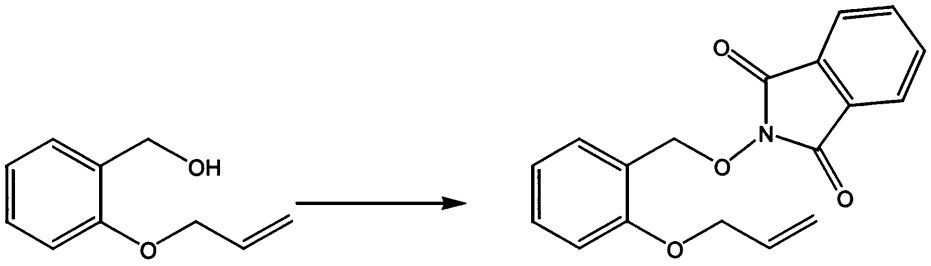
Ortho-allyloxybenzylalcohol (157)



156 157

O-hydroxybenzylalcohol **156** (24.8 g, 0.2 mol), allyl bromide (17.3 ml, 0.2 mol) and potassium carbonate (27.6 g, 0.2 mol) in acetone (100 ml) was heated to reflux temperature for 5 h. The resulting mixture was filtered and the filtrate extracted into diethyl ether (150 ml) washed with dilute sodium hydroxide (2 x 100 ml), dried and concentrated to give yellow oil. The oil was then purified using Kugelrhor (120^0C, 1.0 mbar) to give a clear, colourless oil **157** (12.24 g, 37%); R_f: 0.32 (petroleum ether:diethyl ether 1:2); ν_{max}(neat)/cm^{-1} 3361w, 1602m, 1490s, 996s, 750s; δ_H (250 MHz, $CDCl_3$) 2.56 (1H, br s, OH) 4.55 (2H, dt, *J* 1.6, 5.1, C*HH*CH=CH_2) 4.68 (2H, s, C*HH*OH) 5.27 (1H, dq, *J* 1.6, 10.6, CH=CH*H*) 5.39 (1H, dq, *J* 1.6, 17.2, CH=C*H*H) 6.04 (1H, ddt, *J* 5.1, 10.6, 17.2, CH_2CH=CH_2) 6.84 (1H, d, *J* 8.3, Ar-H) 6.94 (1H, dt, *J* 1.2, 7.6, Ar-H) 7.19-7.31 (2H, m, Ar-H); δ_C (62.9 MHz, $CDCl_3$) 62.09 (CH_2) 69.14 (CH_2) 111.89 (CH) 117.94 (CH_2) 121.28 (CH) 129.03,129.12 (2 x CH) 129.95 (C) 133.55 (CH) 156.70 (C)
This is a literature compound.[1]

Ortho-allyloxybenzyloxy-isoindale-1,3-dione (158)

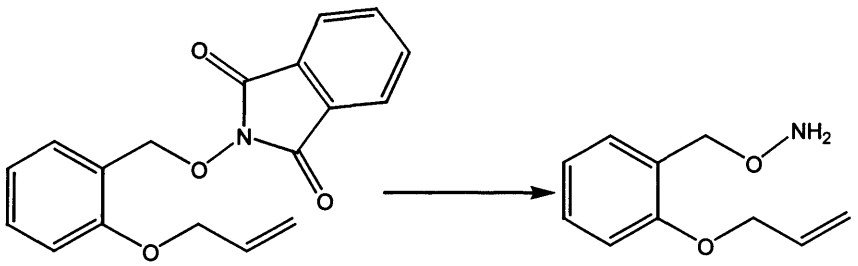


157 158

To a stirred solution of the ether **157** (9.00 g, 54.9 mmol), *N*-hydroxyphthalimide (8.95 g, 54.9 mmol), triphenylphosphine (14.4 g, 54.9 mmol) in THF (200 cm^3) diethylazodicarboxylate (9.51 ml, 60.4 mmol) was slowly added. The mixture was left to stir at room temperature for 24 h. The solution was diluted with diethyl ether (150 ml) washed with brine (3 x 100 ml) dried and evaporated. Chromatography on silica with petroleum ether:diethyl ether (1:1) as the eluent yielded **158** as a cream solid (10.05 g, 59%); mp 79-81^0C (petrol) R_f: 0.25 (petroleum ether:diethyl ether 1:1); ν_{max}(neat)/cm^{-1}

1721s, 1387m, 1132m, 761s; δ_H (250 MHz; $CDCl_3$) 4.47 (2H, dt, *J* 1.6, 5.1, C*HH*CH=CH_2) 5.19 (1H, dq, *J* 1.6, 10.5, CH=CH*H*) 5.29 (2H, s, C*HH*ON) 5.36 (1H, dq, *J* 1.6, 17.2, CH=C*H*H) 5.98 (1H, ddt, *J* 5.1, 10.5, 17.2, CH_2CH=CH_2) 6.84 (1H, d, *J* 8.3, Ar-H) 6.93 (1H, ddd, *J* 1.0, 7.5, Ar-H) 7.29 (1H, app ddt, *J* 1.0, 1.8, 8.3, Ar-H) 7.46 (1H, dd, *J* 1.8, 7.5, Ar-H) 7.67-7.77 (4H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 69.54 (CH_2) 74.88 (CH_2) 112.44 (CH) 117.66 (CH_2) 121.16 (CH) 123.24 (C) 123.69 (CH) 129.34 (C) 131.25 (CH) 132.52 (CH) 133.39 (CH) 134.67 (CH) 157.82 (C) 163.81 (C); *m/z* (FAB) 310 (MH^+, 100%) found MH^+, 310.1079; $C_{18}H_{15}O_4N$ requires 310.1079

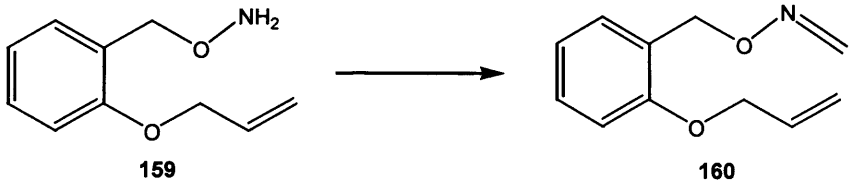
Ortho-allyloxybenzylhydroxylamine (159)



158 159

The phthalimide **158** (5.05 g, 16.34 mmol) and 98% hydrazine monohydrate (0.79 cm^3, 16.34 mmol) dissolved in ethanol (50 ml) was heated at reflux temperature for 2 h. The resulting mixture was poured into 3% sodium carbonate (150 ml) and extracted into diethyl ether (2 x 80 ml), dried and evaporated to give orange oil. Chromatography on silica with petroleum ether:diethyl ether (1:1) as the eluent yielded **159** as a yellow oil (6.67 g, 64%); R_f: 0.35 (petroleum ether:diethyl ether 1:1); ν_{max}(neat)/cm^{-1} 3315w, 2917w, 1490m, 1239m, 752s; δ_H (250 MHz; $CDCl_3$) 4.51 (2H, dt, *J* 1.6, 5.1, C*HH*CH=CH_2) 4.76 (2H, s, C*HH*ONH_2) 5.24 (1H, dq, *J* 1.6, 10.5, CH=CH*H*) 5.28-5.34 (2H, br s, NH_2) 5.39 (1H, dq, *J* 1.6, 17.2, CH=C*H*H) 6.02 (1H, ddt, *J* 5.1, 10.5, 17.2, CH_2CH=CH_2) 6.83 (1H, d, *J* 8.0, Ar-H) 6.93 (1H, dt, *J* 1.0, 7.4, Ar-H) 7.22 (1H, dt, *J* 1.6, 8.0, Ar-H) 7.33 (1H, dd, *J* 1.6, 7.4, Ar-H); δ_C (62.9 MHz; $CDCl_3$) 69.27 (CH_2) 73.38 (CH_2) 112.33 (CH) 117.56 (CH_2) 121.04 (CH) 126.49 (C) 130.33 (CH) 133.81 (CH) 157.05 (C); *m/z* (FAB) 180 (MH^+, 74%) found MH^+, 180.1024; $C_{10}H_{12}O_2N$ requires 180.1024

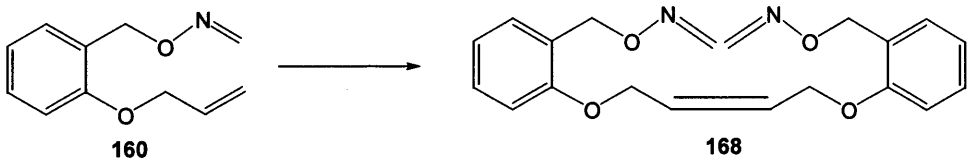
Formaldehyde *O*-(2-allyloxy-benzyl)-oxime (160)



159 160

The hydroxylamine **159** (1.0 g, 5.59 mmol) and formaldehyde (37 wt %) (0.48 cm^3, 6.15 mmol) in pyridine (10 ml) was stirred at room temperature for 24 h. Pyridine was removed under reduced pressure and the resulting solution taken up in diethyl ether (50 ml), washed with brine (4 x 50 ml), dried and evaporated to give yellow oil. Chromatography on silica with petroleum ether:diethyl ether (3:1) as the eluent yielded **160** as a yellow oil (0.65 g, 61%); R_f: 0.37 (petroleum ether:diethyl ether 1:1); ν_{max}(neat)/cm^{-1} 2871w, 1603m, 1491s, 1240s, 1014s; δ_H (250 MHz; $CDCl_3$) 4.53 (2H, dt, *J* 1.6, 5.0, C*HH*CH=CH_2) 5.21-5.27 (3H, overlapping dq, s, *J* 1.6, 10.6, CH=CH*H*, C*HH*ON=CH_2) 5.40 (1H, dq, *J* 1.6, 17.2, CH=C*H*H) 6.03 (1H, ddt, *J* 5.0, 10.6, 17.2, CH_2C*H*=CH_2) 6.42 (1H, d, *J* 8, ON=C*H*H) 6.84 (1H, d, *J* 8.0, Ar-H) 6.93 (1H, dt, *J* 1.0, 7.5, Ar-H) 7.06 (1H, d, *J* 8, ON=CH*H*) 7.23 (1H, dt, *J* 1.5, 8.0, Ar-H) 7.34 (1H, dd, *J* 1.5, 7.5, Ar-H); δ_C (62.9 MHz; $CDCl_3$) 69.26 (CH_2) 71.53 (CH_2) 112.25 (CH) 117.45 (CH_2) 121.03 (CH) 126.62 (C) 129.42 (CH) 130.09 (CH) 133.73 (CH) 137.69 (CH_2) 156.75 (C); *m/z* (FAB) 192 (MH^+, 12%) found MH^+ 192.1025; $C_{11}H_{13}O_2N$ requires 192.1024

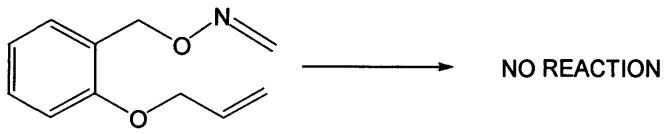
Formaldehyde O-{2-[4-(2-methyleneaminooxymethyl-phenoxy)-but-2-enyloxy]-benzyl}-(168)



160 168

Under an atmosphere of nitrogen, oxime **160** (50 mg, 0.00026 mmol) was placed in a round bottom flask. Benzene (5 ml) was added and the mixture stirred under nitrogen for 3 mins. Catalyst **13b** (1.1 mol%) was added and the resulting mixture stirred at 60^0C for 40h. The mixture was concentrated to yield a dark green oil. Chromatography on silica with petrol : diethyl ether (1:3) gave **168** (5 mg, 6%); *m/z* (ES) 377 (MH^+Na)

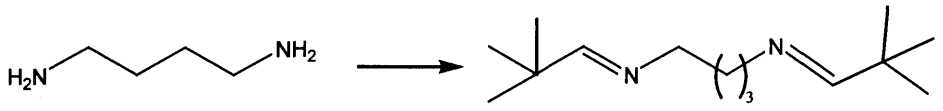
Attempted metathesis reaction



160

To the schrock catalyst **12b** (2 mol%) the oxime **160** (50 mg, 0.00026 mmol)was added. Nitrogen was bubbled through the flask as benzene (5 ml) was added. The reaction mixture was stirred at room temperature for 18h. The benzene was evaporated to yield a brown oil. TLC analysis showed only starting material to be present.

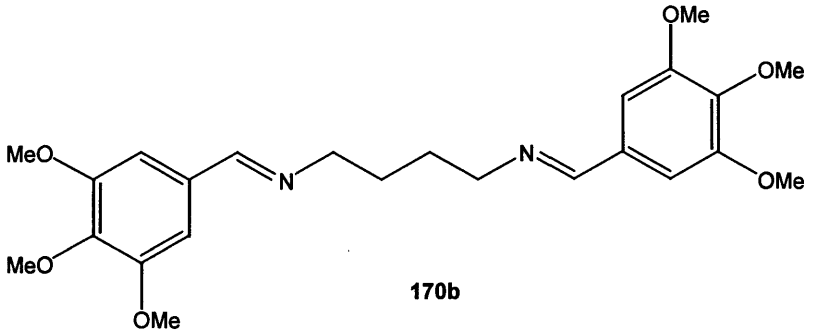
***N,N*-Bis-(2,2-dimethyl-propylidene)-butane-1,4-diamine (170a)**



169 **170a**

1,4 diaminobutane **169** (100 mg, 1.14 mmol) and trimethylacetaldehyde (0.25 mls, 2.28 mmol) in benzene (5 ml) was placed in a round bottom flask and refluxed for 4 h with the use of a Dean Stark trap. The reaction was concentrated to yield **170a** as a colourless oil (130 mg, 51%); ν_{max}(neat)/cm^{-1} δ_H (250 MHz; $CDCl_3$) 1.02 (18H, s, C(CH_3)$_3$ x 2) 1.52-1.60 (4H, m, NCH_2CH_2 x 2) 3.30-3.40 (4H, m, NCH_2CH_2 x 2) 7.54 (2H, s, N=C*H* x 2); δ_C (62.9MHz; $CDCl_3$) 27.16 (CH_3) 27.70 (CH_2) 36.07 (C) 61.17 (CH_2) 171.73 (CH); *m/z* (FAB) 255 (MH^+, 45%) found MH^+, 225.2331; $C_{14}H_{29}N_2$ requires 225.2331

***N,N*-Bis-(3,4,5-trimethoxybenzylidene)-butane-1,4-diamine (170b)**

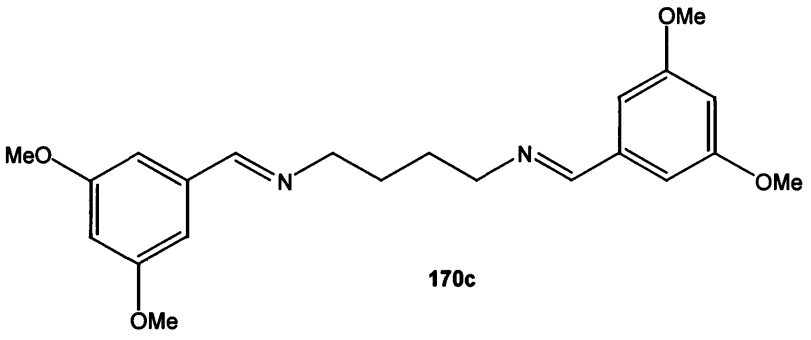


170b

1,4 diaminobutane **169**(310 mg, 3.52 mmol) and 3,4,5-trimethoxybenzaldehyde (1.38 g, 7.04 mmol) in benzene (5 ml) was placed in a round bottom flask and refluxed for 4 h

with the use of a Dean Stark trap. Benzene was removed under pressure to yield a cream solid which was recystallised from a mixture of methanol and petroleum ether to yield **170b** (660 mg, 42%), mp 114-115 ^{0}C (methanol); ν_{max}(neat)/cm^{-1} 2710m, 2660m, 1580s, 1330s, 1140s; δ_H (250 MHz; $CDCl_3$) 1.77-1.82 (4H, m, NCH_2CH_2 x 2) 3.62-3.69 (4H, m, NCH_2CH_2 x 2) 3.88 (6H, s, *p*-PhOMe) 3.90 (12H, s, *m*-PhOMe) 6.96 (4H, s, Ph) 8.18 (2H, s N=C*H* x 2); δ_C (62.9 MHz; $CDCl_3$) 29.14 (CH_2) 56.59 (CH_3) 61.29 (CH_3) 61.82 (CH_2) 105.43 (CH) 132.21 (C) 140.63 (C) 153.83 (C) 160.94 (CH); *m/z* (FAB) 445 (MH^+, 100%) found MH^+, 445.2338; $C_{24}H_{32}O_6N_2$ requires 445.2338; elemental analysis found C, 65.0; H, 7.2; N, 6.2% $C_{24}H_{32}O_6N_2$ requires C, 64.9; H, 7.3; N, 6.3%

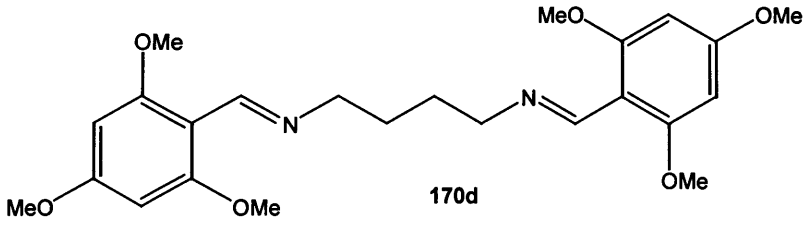
_N,N_-Bis-(3,5-trimethoxybenzylidene)-butane-1,4-diamine (170c)



170c

1,4 diaminobutane **169** (180 mg, 2.05 mmol) and 3,5-dimethoxybenzaldehyde (717 mg, 4.31 mmol) in benzene (5 ml) was placed in a round bottom flask and refluxed for 4 h with the use of a Dean Stark trap. Benzene was removed under pressure to yield a white solid which was recystallised from a mixture of methanol and petroleum ether to yield **170c** (420 mg, 53%), mp 102-103^{0}C (methanol); ν_{max}(neat)/cm^{-1} δ_H (250 MHz; $CDCl_3$) 1.75-1.80 (4H, m, NCH_2CH_2 x 2) 3.62-3.70 (4H, m, NCH_2CH_2 x 2) 3.81 (12H, s, OMe) 6.50-6.52 (2H, t, *J* 2.3 *p*Ar-H x 2) 6.88 (4H d, *J* 2.3 *o*Ar-H x 2) 8.18 (2H, s, N=C*H* x 2); δ_C (62.9 MHz; $CDCl_3$) 29.07 (CH_2) 55.88 (CH_3) 61.81 (CH_2) 103.71 (CH) 106.12 (CH) 138.78 (C) 161.30 (C) 161.34 (CH); *m/z* (FAB) 385 (MH^+, 30%) 220 (21); found MH^+, 385.2127; $C_{22}H_{28}O_4N_2$ requires 385.2127

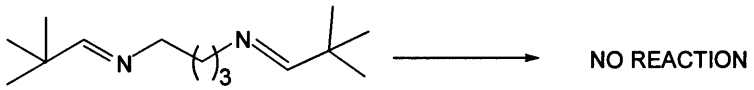
N,N-Bis-(2,4,6-trimethoxybenzylidene)-butane-1,4-diamine (170d)



170d

1,4 diaminobutane **169** (645 mg, 7.31 mmol) and 2,4,6-trimethoxybenzaldehyde (3.01 g, 15.35 mmol) in benzene (5 ml) was placed in a round bottom flask and refluxed for 4 h with the use of a Dean Stark trap. Benzene was removed under pressure to yield a yellow solid which was recystallised from a mixture of methanol and petroleum ether producing **170d** (2.85 g, 88%), mp 114-115^{0}C (methanol); ν_{max}(neat)/cm^{-1} 2945w, 1600m, 1125s, 815m; δ_H (250 MHz; $CDCl_3$) 1.72-1.81 (4H, m, NCH_2CH_2 x 2) 3.60-3.68 (4H, m, NCH_2CH_2 x 2) 3.81 (6H, s, *p*-PhOMe) 3.82 (12H, s, *o*-PhOMe) 6.11 (4H, s, Ar-H) 8.49 (2H, s, N=C*H* x 2); δ_C (75.5 MHz; $CDCl_3$) 28.85 (CH_2) 55.29 (CH_3) 55.9 (CH_3) 63.11 (CH_2) 90.62 (CH) 155.87 (CH) 160.74 (C) 162.29 (C) 187.78 (Ch); *m/z* (FAB) 445 (MH^+, 100%); found MH^+, 445.2339; $C_{22}H_{28}O_4N_2$ requires 445.2339; elemental analysis found C, 64.5; H, 7.1; N, 5.6% $C_{24}H_{32}O_6N_2$ requires C, 64.9; H, 7.3; N, 6.3%

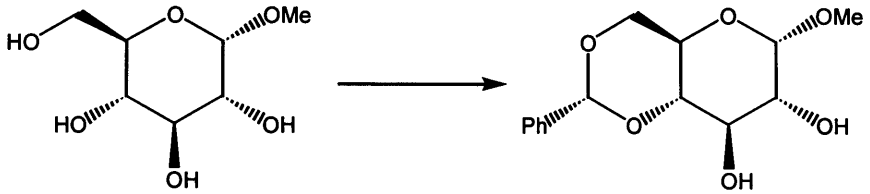
Attempted RCM



170a

The schrock catalyst **12b** (1 mol%) was placed in an NMR tube and the NMR taken to confirm the alkylidene proton. The imine **170a** (80 mg, 0.57mmol) was dissolved in the minimum amount of deuteriated benzene (1 ml) and then transferred to the NMR tube (this process was performed in the glove box). The reaction was left overnight in the fridge and heated the next day for 5h at 74^{0}C and then for 5h at 85^{0}C. The reaction was monitored *via* NMR. No reaction had taken place, starting material present.

Methyl-4,6-*O*-benzylidene- α-D-glucopyranoside(188)

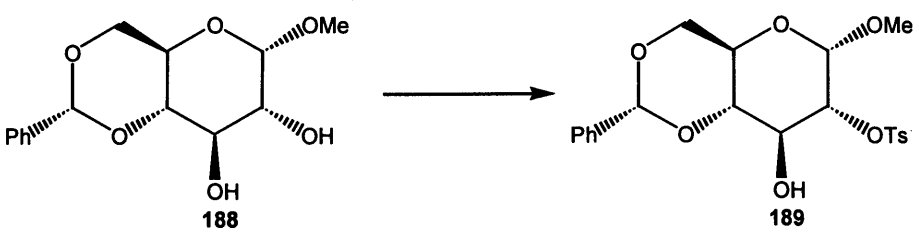


187 **188**

Methyl-α-D-glucopyranoside **187**(100 g, 0.52 mol), benzaldehyde dimethyl acetal (78 cm^3, 0.52 mol) in DMF (565 ml), and *para*-toluene sulphonic acid monohydrate (269 mg, 1.56 mmol) were placed in a round bottom flask fitted with a condenser to a water pump. The solution was heated to 65^0C for 3 h. DMF was removed under reduced pressure and the white solid dispersed in sodium hydrogen carbonate (560 cm^3 water in 11g carbonate) on a heating mantle. After cooling, the product was filtered, washed with water (400 ml) and dried in vacuo overnight over phosphorous pentoxide. The white solid was recrystallised from isopropanol (180 ml) and pyridine (3 ml) to give **188** (110 g, 75%); m.p. 165-166^{0}C (lit.,[2] 166-167^{0}C); δ_H (400 MHz; $CDCl_3$) 2.50 (2H, br s, OH) 3.41 (3H, s, OMe) 3.45 (1H, t, *J* 9.2, 4-H) 3.57 (1H, dd, *J* 4.0, 9.2, 2-H) 3.70 (1H, t, *J* 10.0, 6_{ax}-H) 3.77 (1H, dt, *J* 4.3, 10.0, 5-H) 3.88 (1H, t, *J* 9.2, 3-H) 4.26 (1H, dd, *J* 4.3, 10.0, 6_{eq}-H) 4.72 (1H, d, *J* 4.0, 1-H) 5.53 (1H, s, 7-H) 7.34-7.38 (3H, m, Ph) 7.46-7.49 (2H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 55.97 (CH_3, OMe) 62.77 (CH, 5-C) 69.33 (CH_2, 6-C) 72.16 (CH, 2-C) 73.28 (CH, 3-C) 81.33 (CH, 4-C) 100.18 (CH, 1-C) 102.35 (CH, 7-C) 126.72 (CH, Ph) 128.72 (CH, Ph) 129.65 (CH, Ph) 137.45 (C, Ph)

This is a literature compound[2]

Methyl-4,6-*O*-benzylidene-2-O-*p*-toluenesulphonyl-α-D-glucopyranoside (189)



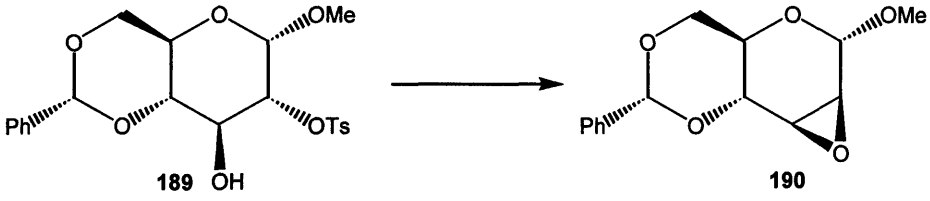
188 **189**

Methyl-4,6-*O*-benzylidene-α-D-glucopyranoside **188** (30.0 g, 0.11 mol), was dissolved in dry dichloromethane (300 ml). To this solution was added *N,N*-dimethyl-4-aminopyridine (2.6 g, 0.02 mol), and triethylamine (44 ml, 0.32 mol). This solution was cooled to 0^0C

and *para*-toluenesulphonyl chloride (22.29 g, 0.12 mol), added portion-wise. The reaction mixture was left to stir at 0^0C for 15 minutes and then at room temperature for 2 h. The reaction was quenched by the addition of water (250 ml), extracted into dichloromethane (2 x 200 ml), dried and evaporated to dryness. The resultant yellow syrup was dissolved in isopropanol (40 ml), and concentrated. This addition concentration was repeated until white foam was obtained. The product was finally precipitated by the addition of hot isopropanol, the white solid was then filtered, washed with isopropanol and dried in vacuo to give a white crystalline solid **189** (40.82 g, 85%); m.p.153-154^{0}C (lit.,[3] 153-155^{0}C) δ_H (250 MHz; $CDCl_3$) 2.41 (3H, s, Ts-Me) 3.35 (3H, s, OMe) 3.47 (1H, t, *J* 10, 4-H) 3.71 (1H, t, *J* 10, 6_{ax} -H) 3.80 (1H, dt, *J* 10, 4.8, 5-H) 4.14 (1H, t, *J* 10, 3-H) 4.28 (1H, dd, *J* 10, 4.8, 6_{eq}-H) 4.38 (1H, dd, *J* 10, 3.9, 2-H) 4.84 (1H, d, *J* 3.9, 1-H) 5.52 (1H, s, 7-H) 7.31 (5H, m, Ph) 7.52 (2H, m, *o*-Ph) 7.89 (2H, m, *o*-Ts); δ_C (62.9 MHz; $CDCl_3$) 22.1 (CH_3, Ts) 56.1 (CH_3, OMe) 62.3 (CH, 5-C) 68.8 (CH_2, 3-C) 69.2 (CH_2, 6-C) 79.9 (CH, 2-C) 81.4 (CH, 4-C) 98.6 (CH, 1-C) 102.4 (CH, 7-C) 126.6 (CH, Ph) 128.5 (CH, Ts) 128.7 (CH, Ph) 129.7 (CH, Ph) 130.2 (CH, Ts) 133.6 (C, Ts) 137.5 (C, Ph)

This is a literature compound[3]

Methyl-2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside (190)

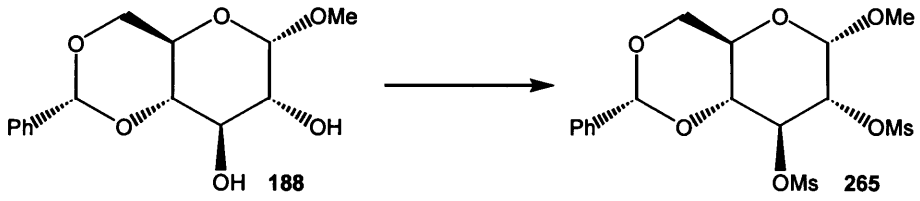


The tosylate **189** (35.0 g, 0.08 mol), was dissolved in dry DMF (200 ml), and cooled to 0^0C in an ice bath. Portions of sodium hydride (80% dispersion in mineral oil) (2.70 g, 0.09 mol) were added and the reaction allowed to stir at room temperature for 2 h. Ethanol (20.0 ml) was then added with cooling and the resulting solution poured into ice/water (100 ml). The resulting white precipitate was filtered and dried under suction for 1 h. The crude product was recrystallised from isopropanol (80 ml) to give a white crystalline solid **190** (19.96 g, 95%); m.p.143-144^{0}C (lit.[4], 145-147^{0}C); δ_H (250MHz; $CDCl_3$) 3.17 (1H, d, *J* 3.7, 2-H) 3.48 (4H, s, OMe, 4-H) 3.65-3.79 (3H, m, 3-H, 5-H, 6_{ax} -H) 4.29 (1H, dd, *J* 5.9,10, 6_{eq}-H) 4.91 (1H, s, 1-H) 5.55 (1H, s, 7-H) 7.22-7.61 (5H, m, Ph); δ_C (62.9MHz; $CDCl_3$) 50.9 (CH, 2-C) 54.2 (CH, 3-C) 56.2 (CH_3, OMe) 62.1 (CH, 5-

C) 69.8 (CH_2, 6-C) 75.3 (CH, 4-C) 97.3 (CH, 1-C) 102.8 (CH, 7-C) 126.6(CH, Ph) 128.8 (CH, Ph) 129.7 (CH, Ph) 137.5 (C, Ph)

This is a literature compound[4]

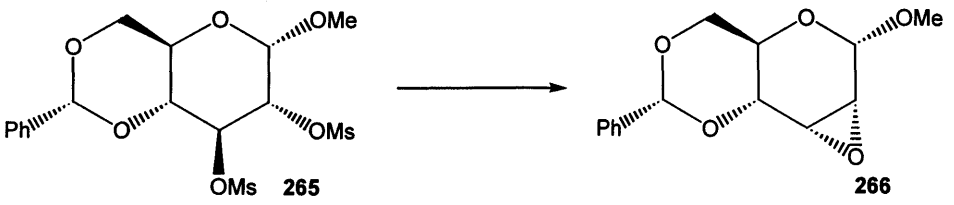
Methyl-4,6-*O*-benzylidene-2,3-di-*O*-methanesulfonyl-α-D-glucopyranoside (265)



Methyl-4,6-*O*-benzylidene-α-D-glucopyranoside **188** (40 g, 142 mmol) was dissolved in dichloromethane (200 ml) and triethylamine (50 ml, 264 mmol) and cooled in an ice bath. To this methanesulphonyl chloride (23 ml, 296 mmol) was added dropwise. The solution was allowed to warm to room temperature and left to stir for 19.5 h. The reaction was quenched by the addition of water (800 ml) and extracted using dichloromethane (2 x 250 ml), dried and evaporated to dryness. The resultant yellow solid was used without further purification. A small sample was purified by recrystallisation from $CHCl_3$ **265** (57.71 g, 93%); mp 164-166^{0}C (lit.[5], 163-164^{0}C); δ_H (250MHz; $CDCl_3$) 2.96 (3H, s, Ms-Me) 3.16 (3H, s, Ms-Me) 3.48 (3H, s, OMe) 3.70-3.82 (2H, overlapping, 4-H, 6_{ax}-H) 3.93 (1H, dt, *J* 4.6, 10.0 5-H) 4.33 (1H, dd, *J* 4.6, 10.0 6_{eq}-H) 4.63 (1 H, dd, *J* 3.7, 9.5, 2-H) 5.02 (1H, d, *J* 3.7, 1-H) 5.08 (1H, overlapping t, *J* 9.5, 3-H) 5.55 (1H, s, 7-H) 7.30-7.48 (5H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 39.19 (CH_3, Ms) 39.36 (CH_3, Ms) 56.49 (CH_3, OMe) 62.70 (CH, 5-C) 69.07 (CH_2, 6-C) 76.25 (CH, 3-C) 76.99 (CH, 2-C) 79.43 (CH, 4-C) 99.28 (CH, 1-C) 102.42 (CH, 7-C) 126.47 (CH, Ph) 128.88 (CH, Ph) 129.4 (CH, Ph) 136.6 (C, Ph)

This is a literature compound[5]

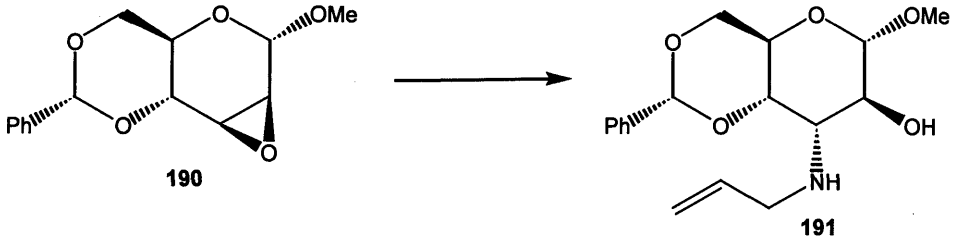
Methyl-2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside (266)



Sodium metal (10 g, 0.43 mol) was added cautiously to dry methanol (140 ml) with cooling. The dimesylate **265** (56.54 g, 0.13 mol) was dissolved in the minimum amount of dichloromethane and added to the methanol mixture via cannula. The resulting solid was allowed to stand for 7 days in the refrigerator with occasional stirring. The reaction was poured into water (100 ml) and saturated K_2CO_3 added (150 ml). The precipitated product was extracted into DCM (2 x 150 ml), dried and evaporated to dryness. The resulting solid was re-dissolved in dichloromethane and isopropanol (100 ml) to precipitate a white crystalline solid **266** (25.45 g, 74%) mp 200-201^{0}C (lit.[5] 199-200^{0}C); δ_H (250MHz; $CDCl_3$) 3.47 (3H, s, OMe), 3.45-3.51 (2H, overlapping, 2-H, 4-H) 3.69 (1H, t, *J* 10.2, 3-H) 3.96 (1H, dd, *J* 1.5, 9.1, 6_{ax}-H) 4.08 (1H, dt, *J* 5.6, 9.1, 5-H) 4.24 (1H, t, *J* 9.1, 6_{eq}-H) 4.87 (1H, d, *J* 2.6, 1-H) 5.56 (1H, s, 7-H) 7.30-7.53(5H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 51.14 (CH, 3-C) 53.5 (CH, 2-C) 56.3 (CH, 2-C) 56.3 (CH_3, OMe) 60.5 (CH, 5-C) 69.3 (CH_2, 6-C) 78.3 (CH, 4-C) 95.7 (CH, 1-C) 103.2 (CH, 7-C) 126.7 (CH, Ph) 128.7 (CH, Ph) 129.6 (CH, Ph) 137.6 (C, Ph)

This is a literature compound[5]

Methyl 3-allylamino-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (191)

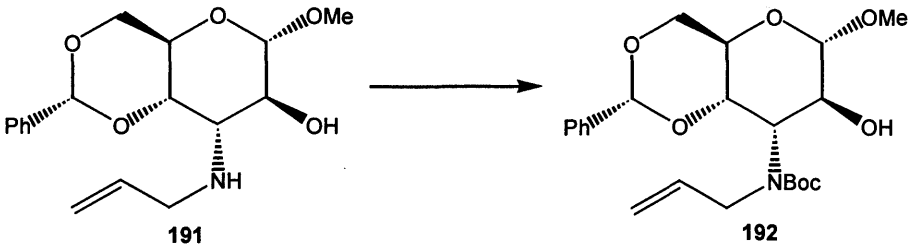


At 90^{0}C allyl amine (8.52 cm^3, 0.11 mol) was added to a solution of epoxide **190** (5.0 g, 18.94 mmol) and lithium perchlorate (8.03 g, 75.76 mmol) in acetonitrile (200 cm^3). After 1 h the solution turned light orange. The reaction was stirred at 90^{0}C for 22 h. The solution was cooled to room temperature and poured into ice cold water (150 cm^3). The resulting precipitate dissolved upon addition of diethyl ether (125 cm^3) and the organic phase washed with water (3 x 100 cm^3), dried and evaporated to give orange oil.

Purification by column chromatography using ethyl acetate and 0.5% ammonia solution as the eluent, gave **191** as an orange oil (5.59 g, 92%); R_f: 0.34 (petroleum ether:diethyl ether 1:1); $[\alpha]^{20}_D$ +96.9° (c. 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3329w, 2908w, 1454m, 1099s, 1041s, 916m, 697s; δ_H (250 MHz; $CDCl_3$) 2.49 (2H, br s, O*H*, N*H*) 3.21 (1H, t, *J* 2.6, 3-H) 3.33-3.43 (5H, overlapping ddt, s, *J* 1.4, 6.0, NHC*HH*CH, OMe) 3.80 (1H, t, *J* 10.0, 6_{ax}-H) 3.96 (1H, dd, *J* 1.0, 2.6, 2-H) 4.05-4.19 (2H, m, 4-H, 5-H) 4.30 (1H, app dd, *J* 4.1, 10.0, 6_{eq}-H) 4.58 (1H, s, 1-H) 5.07 (1H, dq, *J* 1.4, 10.2, CH=C*H*H) 5.17 (1H, dq, *J* 1.4, 17.2, CH=CH*H*) 5.57 (1H, s, 7-H) 5.89 (1H, ddt, *J* 6.0, 10.2, 17.2, C*H*=CH_2) 7.29-7.50 (5H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 51.67 (CH_2) 56.01 (CH_3, OMe) 57.96 (CH, 3-C) 59.23 (CH, 5-C) 69.83 (CH_2, 6-C) 70.65 (CH, 2-C) 76.95 (CH, 4-C) 102.52 (CH, 1-C) 102.66 (CH, 7-C) 116.42 (CH_2) 126.58 (CH, Ph) 128.66 (CH, Ph) 129.41 (CH, Ph) 137.69 (CH) 138.05 (C, Ph); *m/z* (FAB) 322 (MH^+, 100%) 290 (10) 154 (54) found MH^+, 322.1655; $C_{17}H_{23}O_5N$ requires 322.1654.

This is a literature procedure.[6]

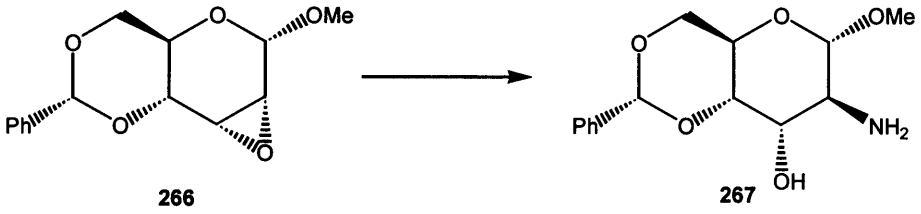
Methyl 3-allylamino-*N*-*tert*-butyloxycarbonyl-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (192)



Starting material **191** (1.85 mg, 5.76 mmol) was dissolved in dry dichloromethane (20 ml) and triethylamine (963 µl, 6.91 mmol) added. The mixture was left to stir for 30 mins. BOC anhydride (1.51 mg, 6.91 mmol) was added and the mixture left to stir at 50°C for 80 h. TLC showed no starting material present. The reaction mixture was washed with water (2 x 25 ml), dried and concentrated to leave a colourless oil which was purified by column chromatography using petroleum ether and diethyl ether (7:3 v/v) as the eluent, giving **192** (1.52 g, 63%); $[\alpha]^{20}_D$ +50.3° (c. 0.8 in MeOH); ν_{max}(neat)/cm^{-1} 3071w, 1740s, 1254m, 1096m; δ_H (400 MHz; $CDCl_3$) 1.52 (9H, s, C(CH_3)$_3$) 1.95 (1H, s, OH) 3.29 (1H, t, *J* 3.0, 3-H) 3.44 (3H, s, OMe) 3.47 (2H, dt, *J* 1.4, 6.0, NHC*HH*CH) 3.81 (1H, t, *J* 10.0, 6_{ax}-H) 4.04 (1H, dd, *J* 3.0, 10.0, 4-H) 4.22 (1H, dt, *J* 5.0, 10.0, 5-H) 4.32 (1H, dd, *J* 5.0, 10.0, 6_{eq}-H) 4.69 (1 H, d, *J* 1.0, 1-H) 4.83 (1 H, dd, *J* 1.0, 3.0, 2-H) 5.09 (1H, dq, *J* 1.4, 10.2,

CH=C*H*H) 5.23 (1H, dq, *J* 1.6, 17.2, CH=CH*H*) 5.61 (1 H, s, 7-H) 5.91 (1H, ddt, *J* 6.0, 10.2, 17.2, C*H*=CH_2) 7.34-7.41 (3H, m, Ph) 7.48-7.50 (2H, m, Ph); δ_C (100.6 MHz; $CDCl_3$) 28.16 (CH_3) 51.28 (CH_2) 55.86 (CH, 3-C) 56.12 (CH_3, OMe)58.97 (CH, 5-C) 69.75 (CH_2, 6-C) 73.84 (CH, 2-C) 77.09 (CH, 4-C) 83.33 (C) 99.97 (CH,1-C) 12.47 (CH, 7-C) 116.23 (CH_2) 126.58 (CH, Ph) 128.58 (CH, Ph) 129.34 (CH, Ph) 137.57 (C, Ph) 138.10 (CH) 152.87 (C); *m/z* (FAB) 422 (MH^+, 22%) 289 (12); found MH^+, 422.2179; $C_{22}H_{31}O_7N$ requires 422.2178.

Methyl 2-amino-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (267)

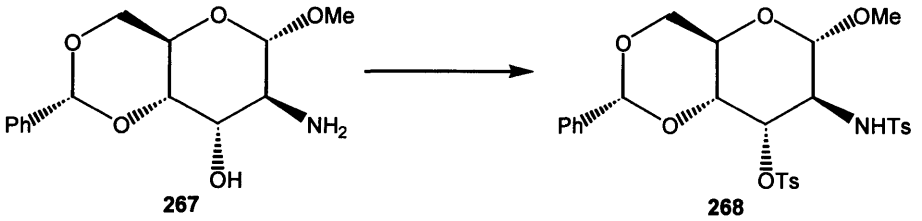


266 **267**

The epoxide **266** (0.5 g, 1.89 mmol) was heated with aqueous concentrated ammonia (12.5 ml) in a sealed tube (Youngs) for 65 h at 100^0C. On cooling long needle like crystals appeared which were dissolved in DCM (20 ml) and washed with water (2 x 20 ml), dried and concentrated to give a pink solid, which was recrystallised from ethanol to furnish an off white solid **267** (501 mg, 94%) mp 189-192^0C (lit.[7] 188-190^0C); δ_H (250 MHz; $CDCl_3$) 3.33 (1H, d, *J* 2.7, 2-H) 3.43 (3H, s, OMe) 3.82 (1H, t, *J* 10.0, 6_{ax}-H) 3.92 (1H, dd, *J* 2.7, 10.0, 4-H) 4.00 (1H, t, *J* 2.7, 3-H) 4.22 (1H, d app t, *J* 5.1, 10.0, 5-H) 4.34 (1H, dd, *J* 5.1, 10.0, 6_{eq}-H) 4.59 (1H, s, 1-H) 5.62 (1H, s, 7-H) 7.32-7.39 (3H, m, Ph) 7.46-7.53 (2H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 54.84 (CH, 2-C) 56.07 (CH_3, OMe) 58.91 (CH, 5-C) 69.69 (CH_2, 6-C) 71.21 (CH, 3-C) 76.86 (CH, 4-C) 102.75 (CH, 1-C) 103.82 (CH, 7-C) 126.67 (CH, Ph) 128.68 (CH, Ph) 129.653 (CH, Ph) 137.73 (C, Ph); *m/z* (FAB) 282 (MH^+, 100%) found MH^+, 282.1342 $C_{14}H_{19}O_5N$ requires 282.1341

This is a literature compound.[7]

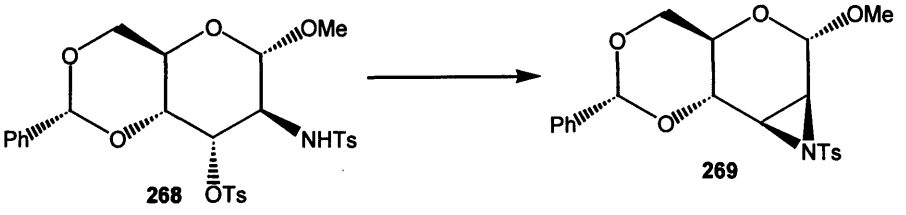
Methyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-(*p*-tolylsulfonamide)-3-O(*p*-tolylsulfonyl)-α-D-altropyranoside (268)



Protected from moisture, a solution of **267** (0.209 g, 0.74 mmol) and p-toluenesulphonyl chloride (0.564 g, 2.96 mmol) in pyridine was stirred at 50^0C for 55 h. The reaction mixture was poured onto ice (10 g) and the aqueous solution extracted with chloroform (3 x 20 ml). The combined extracts were washed with water (2 x 10 ml) dried and concentrated under reduced pressure to give a yellow solid. Chromatography on silica gel with DCM : 4% methanol as the eluent yielded **268** as a white solid (0.254 g, 58%) mp 196-198^{0}C (lit.[8] 198-199^{0}C); δ_H (250 MHz; $CDCl_3$) 2.34 (3H, s, *p*-MeTs) 2.47 (3H, s, *p*-MeTs) 3.29 (3H, s, OMe) 3.63 (1H, app t, *J* 9.1 6_{ax}-H) 3.75 (1H, dd, *J* 3.0, 9.6, 4-H) 3.90 (1H, dd, *J* 3.0, 7.8, 2-H) 4.16-4.25 (2H, m, 5-H, 6_{eq}-H) 4.28 (1H, s, 1-H) 4.66 (1H, t, *J* 3.0, 3-H) 4.76 (1H, d, *J* 7.8, N*H*) 5.38 (1H, s, 7-H) 7.05 (2H, d, *J* 8.1, Ph) 7.22-7.38 (7H, M, Ph) 7.69 (2H, d *J* 8.1, Ph) 7.83 (2H, d, *J* 8.4, Ph); δ_C (62.9 MHz; $CDCl_3$) 21.64 (CH3, *p*-MeTs) 53.85 (CH, 2C) 55.68 (CH_3, OMe) 58.37 (CH, 5-C) 68.83 (CH_2, 6-C) 73.02 (CH, 3-C) 74.74 (CH, 4-C) 100.47 (CH, 1-C) 101.88 (CH, 7-C) 126.23 (CH, Ph) 127.47 (CH, Ph) 128.00 (CH, Ph) 128.56 (CH, Ph) 129.38 (CH, Ph) 130.09 (CH, Ph) 135.93 (C, Ph) 136.90 (C, Ph) 144.53 (C, Ph); *m/z* (FAB) 590 (MH^+, 69%); found MH^+, 590.1518 $C_{28}H_{31}O_9NS_2$ requires 590.1518

This is a literature compound.[8]

Methyl-4,6-*O*-benzylidene-2,3-dideoxy-2,3-imino-*N*-(*p*-tolylsulfonyl)-α-D-mannopyranoside (269)

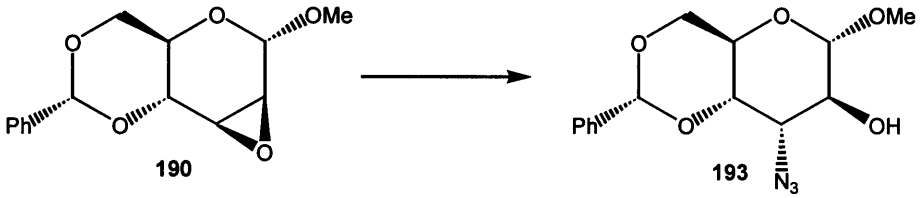


To a suspension of **268** (0.652 g,1.12 mmol) in methanol (6.5 ml) at room temperature, 1M methanolic sodium methoxide (4.4 ml) was added and a clear solution obtained. After standing for 1 h at room temperature the solution was neutralised by the addition of carbon

dioxide and diluted with water (20 ml). The resulting solution was extracted with chloroform (3 x 20 ml) and the combined extracts washed with water (2 x 20 ml), dried and concentrated to give orange oil. Chromatography on silica gel using petroleum ether : DCM (1:1) as the eluent yielded **269** as a colourless oil which solidified (238 mg, 57%) mp 128-130^{0}C (lit.[8] 129-131^{0}C); ν_{max}(neat)/cm^{-1}2912w, 1598w, 1328m, 1066s; δ_H (250 MHz; $CDCl_3$) 2.42 (3H, s, CH_3-Ts) 2.97 (1H, d, *J* 6.8, 2-H) 3.32 (1H, d, *J* 6.8, 3-H) 3.37 (3H, s, OMe) 3.59-3.71 (3H, m, 4-H, 5-H, 6_{ax}-H) 4.15-4.25 (1H, m, 6_{eq}-H) 4.74 (1H, s, 1-H) 5.46 (1H, s, 7-H) 7.31-7.37 (5H, m, Ph) 7.42-7.47 (2H, m, Ph-Ts) 7.82 (2H, br d, *J* 8.5, Ph-Ts); δ_C (62.9 MHz; $CDCl_3$) 22.09 (CH_3, Me-Ts) 38.47 (CH, 2-C) 41.79 (CH, 3-C) 55.91 (CH3, OMe) 62. (CH, 5-C) 69.61 (CH_2, 6-C) 75.04 (CH, 4-C) 96.45 (CH, 1-C) 102.71 (CH, 7-C) 126.64 (CH, Ph-Ts) 126.60 (CH, Ph-Ts) 128.76 (CH, Ph) 129.72 (CH, Ph) 130.72 (CH, Ph) 134.27 (C, Ph-Ts) 137.35 (C Ph) 145.55 (C, Ph-Ts); *m/z* (FAB) 418 (MH^+, 68%); found MH^+, 418.1324 $C_{21}H_{23}O_6NS$ requires 418.1325

This is a literature compound.[8]

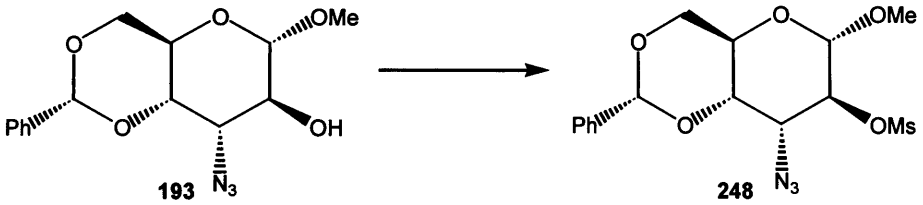
Methyl-3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (193)



Sodium azide (738 mg, 11.35 mmol), **190** (598 mg, 2.27 mmol) and ammonium chloride (243 mg, 4.54 mmol) in 2-methoxyethanol (12 ml) and water (1.5 ml) were heated at reflux for 22 h. The reaction was cooled and poured into ice water (40 ml). This mixture was extracted with chloroform (3 x 40 ml), dried and evaporated to give a yellow solid which was recrystallised from ethanol to give **193** (638 mg, 92%) mp.134-135^{0}C (lit[9]., 135-136^{0}C); $[\alpha]^{20}_D$ +48.1° (c. 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3460s, 2102s; δ_H (400 MHz; $CDCl_3$) 2.69 (1H, br s, OH) 3.39 (3 H, s, OMe) 3.77 (1H, overlapping t, *J* 10.0, 6_{ax}-H) 3.82 (1H, overlapping d, *J* 3.0, 2-H) 3.95 (1H, t, *J* 3.0, 3-H) 4.10 (1H, dd, *J* 3.0, 10.0, 4-H) 4.19-4.31 (2H, overlapping dt, dd, *J* 5.1, 10.0, 5-H, 6_{eq}-H) 4.50 (1H, s, 1-H) 5.58 (1H, s, 7-H) 7.36- 7.39 (3H, m,Ph) 7.48-7.50 (2H, m, Ph); δ_C (100.6 MHz; $CDCl_3$) 55.77 (CH_3, OMe) 58.95 (CH, 5-C) 60.06 (CH, 3-C) 69.11 (CH_2, 6-C) 69.58 (CH, 2-C) 75.84 (CH, 4-C) 101.39 (CH, 1-C) 102.36 (CH, 7-C) 126.20 (CH, Ph) 128.48 (CH, Ph) 129.39 (CH, Ph) 136.97 (C, Ph); *m/z* (FAB) 308 (MH^+, 78%) 282 (95); found MH^+, 308.1247; $C_{14}H_{17}N_3O_5$ requires 308.1246.

This is a literature compound.[9]

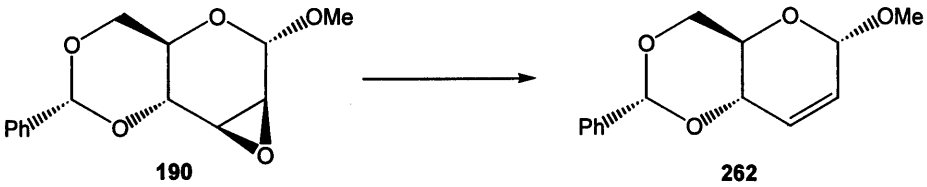
Methyl-3-azido-4,6-*O*-benzylidene-3-deoxy-2-methanesulphonate-α-D-altropyranoside (248)



The azidoaltroside **193** (1.47g, 4.77 mmol) was dissolved in pyridine (10 ml) and cooled to 0^0C. Methanesulfonyl chloride was added drop wise and the resulting solution kept overnight at room temperature. The solution was then poured into ice/water. The mixture was extracted with chloroform (2 x 20 ml) and the extract washed with cold dilute hydrochloric acid (10 ml) and water (20 ml) and dried to leave yellow oil. Column chromatography on silica gel using DCM as the eluent gave **248** as white solid (1.33 g, 72%) mp 132-134^{0}C (lit.[9] 135-137^{0}C) δ_H (250 MHz; $CDCl_3$) 3.12 (3H, s, OMe) 3.45 (3H, s, Me) 3.73-3.84 (1H, m, 6_{ax}-H) 4.11 (1H, dd, *J* 3.6, 9.4, 4-H) 4.24-4.37 (3H, m, 3-H, 5-H, 6_{eq}-H) 4.73 (1H, s, 1-H) 4.76 (1 H, d, *J* 2.1, 2-H) 5.62 (1H, s, 7-H) 7.35-7.40 (3H, m, Ph) 7.45-7.51 (2H, m, Ph); δ_C (72.5 MHz; $CDCl_3$) 38.65 (CH_3, Ms-CH_3) 56.01 (CH, 3-C) 58.21 (CH_3, OMe) 60.95 (CH, 4-C) 68.89 (CH, 6-C) 74.75 (CH, 2-C) 75.40 (CH, 5-C) 98.85 (CH, 1-C) 102.37 (CH, 7-C) 126.13 (CH, Ph) 128.41 (CH, Ph) 129.35 (CH, Ph) 136.79 (C, Ph)

This is a literature compound.[9]

Methyl-4,6-*O*-benzylidene-α-D-erythro-hex-2-enopyraonside (262)

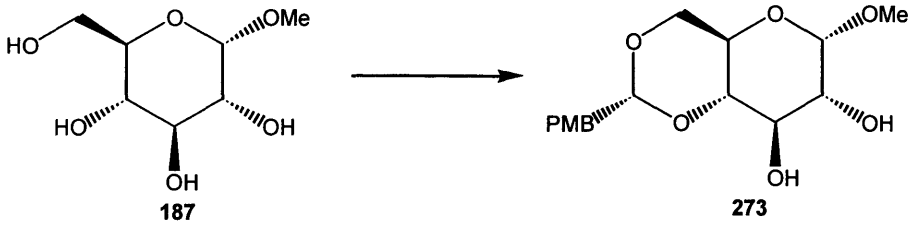


A stirred mixture of **190** (0.528 g 0.002 mmol), zinc-copper couple (0.65 g, 0.01 mmol), NaI (1.5 g, 0.01 mmol) DMF (5 ml) and dimethoxyethane (1 ml) was heated under reflux for 3 h. The mixture was poured into water (50 ml) and extracted with benzene (3 x 50 ml). The combined extracts were washed with water (3 x 50 ml) dried and evaporated producing white crystals of **262** (0.450g, 91%). A small sample was recrystallised from ethyl acetate and petrol; mp 120-122^{0}C (lit.[10], 119-120^{0}C) δ_H (250 MHz; $CDCl_3$) 3.45 (3H,

s, OMe) 3.80 (1H, t, *J* 10, 6ax-H) 3.89 (1H, dd, *J* 3.6, 10, 4-H) 4.13-4.17 (1H, m, 5-H) 4.30 (1H, dd, *J* 3.1, 10, 6eq-H) 4.88 (1H, s, 1-H) 5.55 (1H, s, 7-H) 5.72 (1H, dd, *J* 2.3, 10.3, 3-H) 6.12 (1H, br d, *J* 10.3, 2-H) 7.33-7.52 (5H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 56.34 (CH3, OMe) 64.34 (CH, 5-C) 69.85 (CH2, 6-C) 75.68 (CH, 4-C) 96.53 (CH, 1-C) 102.60 (CH, 7-C) 126.67 (CH, 2-C) 127.05 (CH, 3-C) 128.73 (CH, Ph) 129.56 (CH, Ph) 131.19 (CH, Ph) 137.78 (C, Ph)

This is a literature compound.[10]

Methyl-4,6-*O*(*p*-methoxybenzylidene)-α-D-glucopyranoside (273)

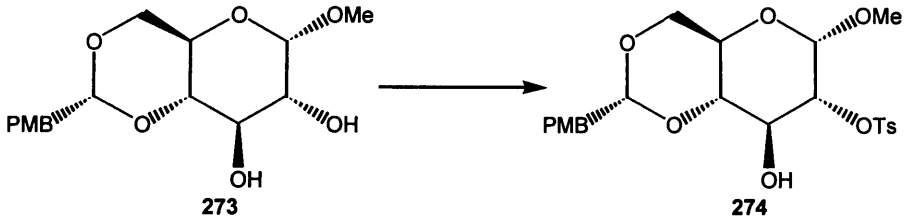


187 **273**

Methyl α-D-glucopyranoside **187** (30 g, 0.15 mol), *p*-anisaldehyde dimethyl acetal (26 ml, 0.15 mol) and *p*-toluenesulfonic acid monohydrate (0.12 g, 0.63 mmol) in DMF (80 ml) were placed in round bottom flask fitted with a condenser and heated at 80^0C for 24 h. Reaction was cooled and concentrated under reduced pressure to leave a yellow/orange solid. The resulting solid was dispersed in $NaHCO_3$ (0.68 g in 35 ml of H_2O). The solid is then filtered, dried and recrystallised from isopropanol to give **273** as a white solid (40.47 g, 84%); mp. 197-199^{0}C (lit[11]., 194^{0}C); $[\alpha]^{20}_D$ +82.1° (c. 1.0 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 2.23 (1H, d, *J* 9.5,OH) 2.66 (1H, s, OH) 3.46 (3H, s, OMe) 3.47 (1H, obscured t, *J* 9.2, 4-H) 3.63 (1H, ddd, *J* 3.9, 9.2, 2-H) 3.72 (1H, t, *J* 10.0, 6_{ax}-H) 3.79 (1H, apparent dd, *J* 4.1, 10.0, 5-H) 3.80 (3H, s, OMe-Ph) 3.92 (1H , t, *J* 9.2, 3-H) 4.27(1H, dd, *J* 4.1, 10.0, 6_{eq}-H) 4.80 (1H, d, *J* 3.9, 1-H) 5.49 (1H, s, 7-H) 6.88 (2H, m, Ph) 7.41 (2H, m, Ph); δ_C (100 MHz; $CDCl_3$) 55.31 (CH_3, OMe) 55.57 (CH_3, *p*-OMePh) 62.37 (CH, 5-C) 68.89 (CH_2, 6-C) 71.80 (CH, 3-C) 72.87 (CH, 2-C) 80.88 (CH, 4-C) 99.76 (CH, 1-C) 101.87 (CH, 7-C) 113.70 (CH, Ph) 127.62 (CH, Ph) 129.54 (C, Ph) 160.26 (C, Ph); *m/z* (FAB) 313 (MH^+, 41%) 273 (8); found MH^+, 313.1287; $C_{15}H_{20}O_7$ requires 313.1287.

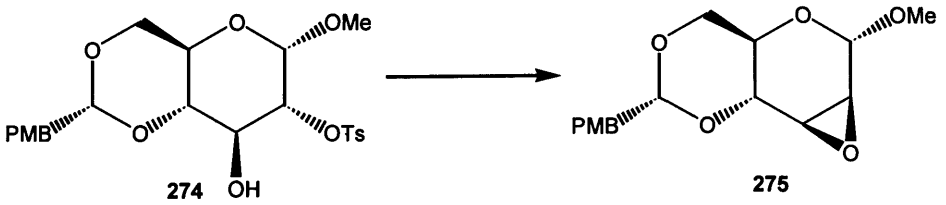
This is a literature compound.[11]

Methyl-4,6-*O*-(*p*-methoxybenzylidene)-2-*O*-*p*-toluenesulphonyl-α-D-glucopyranoside (274)



Methyl-4,6-*O* (*p*-methoxybenzylidene)-α-D-glucopyranoside **273** (5 g, 16.02 mmol) was dissolved in DCM (50 ml). Triethylamine (6.5 ml, 46.46 mmol) and DMAP (391mg, 3.2 mmol) was added. The mixture was cooled to 0^0C and *p*-toluenesulphonyl chloride (3.36 g, 17.6 mmol) was added portionwise. The reaction was stirred at 0^0C for 15 mins and then room temperature for 2 h. The reaction was quenched by the addition of water (2 x 50 ml) and the product extracted using DCM (2 x 50 ml). The solution was dried and concentrated under reduced pressure to leave yellow syrup, which was dissolved in IPA and concentrated. This process was repeated until a white foam was obtained. The product was precipitated by the addition of hot IPA, filtered and dried in vacuo to give **274** as white solid (5.46 g, 73%) mp 183-185^{0}C; δ_H (400 MHz; $CDCl_3$) 3.36 (3H, s, OMe) 3.44 (1H, t, *J* 9.4, 4-H) 3.69(1H, t, *J* 10, 6ax-H) 3.77-3.82(4H, overlapping dd, s, *J* 4.8,10, OMe, 5-H) 4.12 (1H, t, *J* 9.4, 3-H) 4.25 (1H, dd, *J* 4.8, 10, 6_{eq}-H) 4.35 (1H, dd, *J*, 3.8, 9.4, 2-H) 4.83 (1H, d, *J* 3.8, 1-H) 5.44 (1H, s, 7-H) 6.87 (2H, dd, *J* 2, 6.7, Ph) 7.33-7.38 (4H, m, Ph) 7.84 (2H, dd, *J* 2, 6.7, Ph) ;δ_C (75.5 MHz; $CDCl_3$) 21.7 (CH_3, CH_3-Ts) 55.31 (CH_3, OMe) 55.70 (CH_3, *p*-OMePh) 68.37 (CH, 3-C) 68.74 (CH_2, 6-C) 79.49 (CH, 2-C) 81.01 (CH, 4-C) 98.20 (CH, 1-C) 101.95 (CH, 7-C) 113.69 (CH, Ph) 127.58 (CH, Ph) 127.58 (CH, Ph) 128.11 (CH, Ph) 129.80 (CH, Ph) 133.29 (C, Ph); *m/z* (FAB) 467 (MH^+, 100%); found MH^+, 467.1376; $C_{15}H_{20}O_7$ requires 467.1376

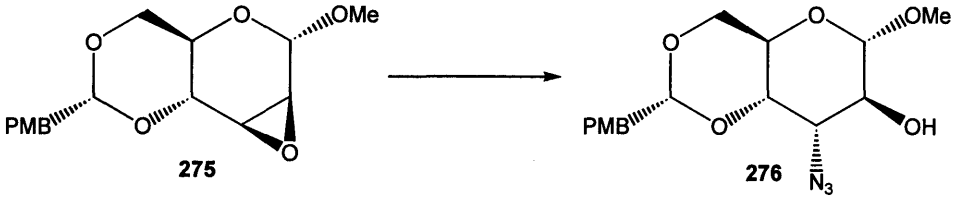
Methyl-2,3-anhydro-4,6-*O*-(*p*-methoxybenzylidene)-α-D-mannopyranoside (275)



The tosylate **274** (6 g, 12.88 mmol) was dissolved in dry DMF (60 ml) and the solution cooled to 0^0C. Portions of sodium hydride (60% dispersion in mineral oil) (1.55 g, 64.5

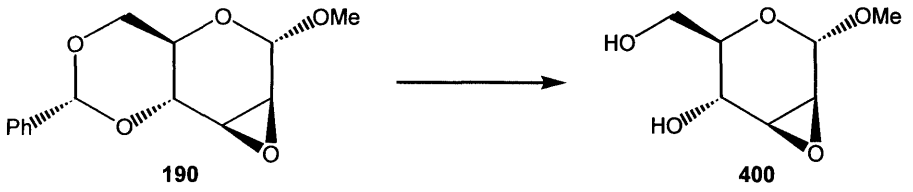
mmol) were added and the reaction was left to stir at room temperature for 2 h. Ethanol (6 ml) was added with cooling and the resulting solution poured into ice/water (50 ml). The precipitate was filtered, washed and dried under vacuo. The crude product was recrystallised from IPA (20 ml) to give **275** as a white solid (2.63 g, 70%) mp 145-147^{0}C δ_H (400 MHz; $CDCl_3$) 3.16 (1H, d, *J* 3.6, 3-H) 3.46 (1H, app, s, 4-H) 3.47 (3H, s, OMe) 3.64-3.72 (3H, m, 2-H, 5-H, 6_{ax}-H) 3.81 (3H, s, OMe-Ph) 4.24 (1H, app q, *J* 5.6, 6_{eq}-H) 4.89 (1H, s, 1-H) 5.53 (1H, s, 7-H) 6.89-6.91 (2H, m,Ph) 7.41-7.43 (2H, m, Ph); δ_C (75.5 MHz; $CDCl_3$) 50.56 (CH, 3-C) 53.85 (CH, 4-C) 55.32(CH_3, OMe) 55.76 (CH_3, OMe-Ph) 61.70 (CH, 5-C) 69.39 (CH_2, 6-C) 74.84 (CH, 2-C) 96.89 (CH, 1-C) 102.41(CH, 7-C) 113.75 (CH, Ph) 127.51 (CH, Ph) 129.57 (C, Ph) 160.28 (C, Ph); *m/z* (FAB) 295 (MH^+, 50%); found MH^+, 295.1182; $C_{15}H_{18}O_6$ requires 295.1182

Methyl-3-azido-3-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside (276)



275 (4.46 g, 15.18 mmol), sodium azide (4.93 g, 75.9 mmol) and ammonium chloride (1.62 g, 30.36 mmol) in a mixture of 2-methoxyethanol (50 ml) and water (5 ml) were heated under reflux for 24 h. The resulting orange solution was cooled and poured into ice/water (50 ml) and the mixture extracted using chloroform (2 x 50 ml) dried and co-evaporated with toluene. The oil was purified using column chromatography on silica gel using petrol:ethyl acetate (4:1) as the eluent. Fraction 20 eluent changed to hexane:ethyl acetate (1:1) producing **276** as a yellow solid (2.204 g, 43%); mp. 165-167^{0}C; δ_H (250 MHz; $CDCl_3$) 2.56 (1H, br s, OH) 3.41 (3H, s, OMe) 3.73-3.83 (4H, overlapping t, s, *J* 9.4, 6_{ax}-H, *p*-OMePh) 3.89 (1H, br s, 2-H) 4.01 (1H, t, *J* 3.0, 3-H) 4.08-4.14 (1H, app dd, *J* 3.0, 10.0, 4-H) 4.19-4.32 (2H, overlapping dt, dd, *J* 4.0, 10.0, 5-H, 6_{eq}-H) 4.55 (1H, s, 1-H) 5.55 (1H, s, 7-H) 6.87 (1H, t, *J* 2.4, Ph) 6.91 (1H, t, *J* 2.4, Ph) 7.40 (1H, t, *J* 2.4, Ph) 7.44 (1H, t, *J* 2.4, Ph); δ_C (62.9 MHz; $CDCl_3$) 55.70 (CH_3, OMe) 56.16 (CH_3, *p*-OMePh) 59.38 (CH, 5-C) 60.48 (CH, 3-C) 69.45 (CH_2, 6-C) 70.12 (CH, 2-C) 76.24 (CH, 4-C) 101.82 (CH, 1-C) 102.69 (CH, 7-C) 114.18 (CH, Ph) 127.94 (CH, Ph) 129.93 (C, Ph) 160.68 (C, Ph); *m/z* (FAB) 338 (MH^+, 94%); found MH^+, 338.1351; $C_{15}H_{19}O_6N_3$ requires 338.1352

Methyl-2,3-anhydro-α-D-mannopyranoside (400)

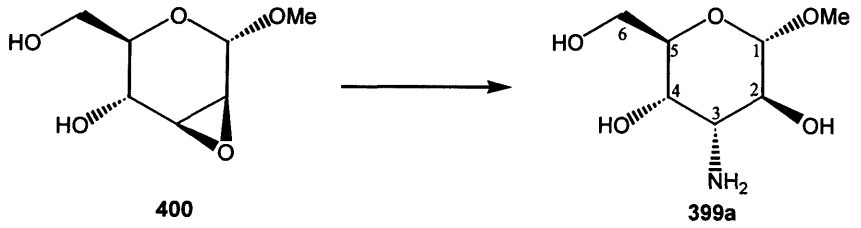


Methyl-2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside **190** (2.07 g, 7.8 mmol) was heated under reflux in a mixture of methanol (15 ml) and water (30 ml). Sulphuric acid (0.4 μl), 0.008 mmol) was added and the solution heated until all **190** had dissolved. Excess barium carbonate was added to the hot solution. After cooling to room temperature the solution was filtered through celite. Removal of the methanol under reduced pressure left an aqueous solution which was washed with toluene (2 x 15 ml). Concentration of the aqueous layer produced a clear oil which then solidified. Recrystallisation from DCM yielded **400** as a white solid (1.00 g, 73%);mp 80-81^{0}C (lit[12]., 81-82^{0}C) δ_H (250 MHz; $CDCl_3$) 2.34 (1H, s, OH) 3.03 (1H, d, *J* 5.3, OH) 3.13 (1H, d, *J* 3.7 3-H) 3.31 (1H, d, *J* 3.7, 2-H) 3.47 (3H, s, OMe) 3.50-3.57 (1H, dt, *J* 4.4, 9.0, 5-H) 3.73-3.81 (2H, m, 6-H) 3.89 (1H, dd, *J* 3.7, 9.0, 4-H) 4.89 (1H, s, 1-H); δ_C (62.9 MHz; $CDCl_3$) 50.28 (CH, 3-C) 55.84 (CH_3, OMe) 56.10 (CH, 2-C) 62.96 (CH, 4-C) 63.25 (CH_2, 6-C) 69.31 (CH, 5-C) 96.45 (CH, 1-C)
This is a literature compound.[12]

General procedure for epoxide opening

Methyl-2,3-anhydro-α-D-mannopyranoside **396** (100 mg, 0.57 mmol) together with the neat corresponding amine (0.62 mmol) was placed in a Youngs tube and stirred at 100 ^{0}C for 24 h under an atmosphere of nitrogen. The reaction was left to cool to room temperature and diluted with either dichloromethane or methanol. The mixture was then transferred to a round bottom flask and evaporated under reduced pressure to dryness. The product was purified using column chromatography.

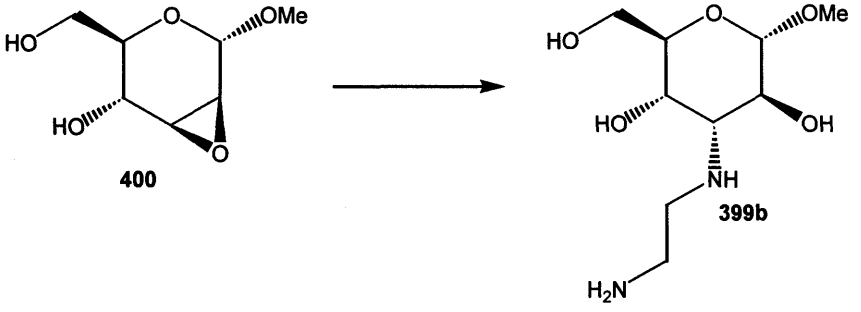
Methyl-3-amino-3-deoxy-α-D-altropyranoside (399a)



Methyl-2,3-anhydro-α-D-mannopyranoside **400** (190 mg, 1.08 mmol) and excess ammonia (S.G 0.88, 35%, 4 ml) was placed in a Youngs tube and stirred at 100^{0}C for 19 h. The solution was left to cool and then transferred to a round bottom flask and the excess ammonia removed under reduced pressure to leave orange oil. The oil was purified by column chromatography with dichloromethane : 2 M ammonia in methanol (1:1 v/v) as the eluent to yield the product **399a** (179 mg, 86%) as a yellow oil; ν_{max}(neat)/cm^{-1} 3351s, 2924m, 1601w, 1046m; δ_H (400 MHz; D_2O) 3.10 (1H, overlap dt, *J* 0.7, 4.2, 3-H) 3.44 (3H, s, OMe) 3.76-3.79 (1H, m, 6-H) 3.80-3.82 (1H,app q, *J* 2.9, 2-H) 3.84-3.90 (2H, m, 5-H, 6-H) 3.91 (1H, app dd, *J* 4.2, 8.4, 4-H) 4.66 (1H, br d, *J* 2.7, 1-H); δ_C (100.6 MHz; D_2O) 53.18 (CH, 3-C) 55.62 (CH_3, OMe) 61.37 (CH_2, 6-C) 64.74 (CH, 4-C) 70.22 (CH, 2-C) 70.46 (CH, 5-C) 101.27 (CH, 1-C); *m/z* (FAB) 194 (MH^+, 41%); found MH^+, 194.1029; $C_7H_{15}NO_5$ requires 194.1028.

This is a literature compound.[13]

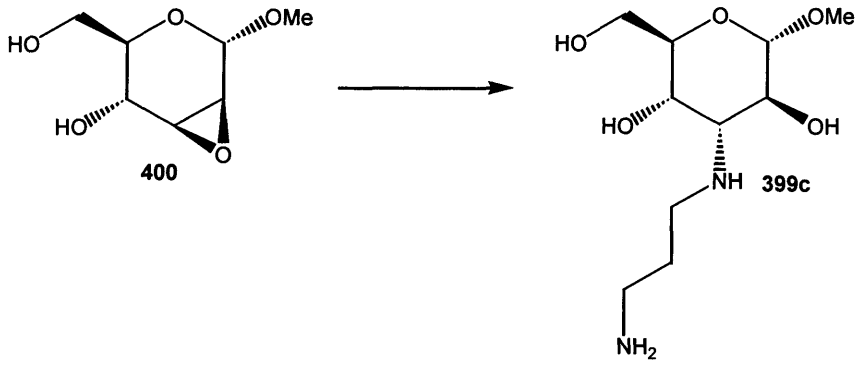
Methyl-3-(2-amino-ethylamino)-3-deoxy-α-D-altropyranoside (399b)



Methyl-2,3-anhydro-α-D-mannopyranoside **400** (250 mg, 1.42 mmol) and ethylene diamine (95 μl 1.42 mmol) was placed in a Youngs tube and stirred at 120^{0}C for 24 h. Upon cooling the solution solidified. The solid was diluted with methanol and transferred to a round bottom flask and the excess methanol removed under reduced pressure to leave orange oil. The oil was purified by column chromatography with dichloromethane : 2 M ammonia in methanol (1:1 v/v) as the eluent to yield the product **399b** (133 mg, 40%) as a

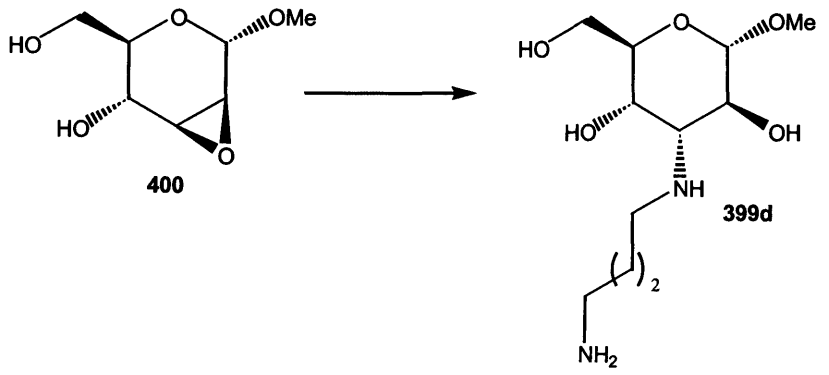
yellow oil; $[\alpha]^{20}_D$ +26.8° (c 0.4 in MeOH); ν_{max}(neat)/cm^{-1} 3360s, 2492m, 1644m, 1449m, 1047m; δ_H (250 MHz; D_2O) 2.75-2.87 (4H, m, $NH_2CH_2CH_2NH$, $NH_2CH_2CH_2NH$) 2.92 (1H, app t, *J* 5.5, 3-H) 3.51 (3H, s, OMe) 3.80-3.95 (4H, m, 2-H, 5-H, 2 x 6-H) 3.99 (1H, app t, *J* 5.5, 4-H) 4.71 (1H, d, *J* 3.6, 1-H); δ_C (75.7 MHz; D_2O) 40.48 (CH_2) 49.02 (CH_2) 55.85 (CH_3, OMe) 59.36 (CH, 3-C) 61.18 (CH_2, 6-C) 63.83 (CH, 2-C) 68.46 (CH, 5-C) 72.76 (CH, 4-C) 101.65 (CH, 1-C); *m/z* (ES) 237 (MH^+, 100%), 205 (25)187 (32); found MH^+, 237.1450 $C_9H_{20}N_2O_5$ requires 237.1451

Methyl-3-(3-amino-propylamino)-3-deoxy-α-D-altropyranoside (399c)



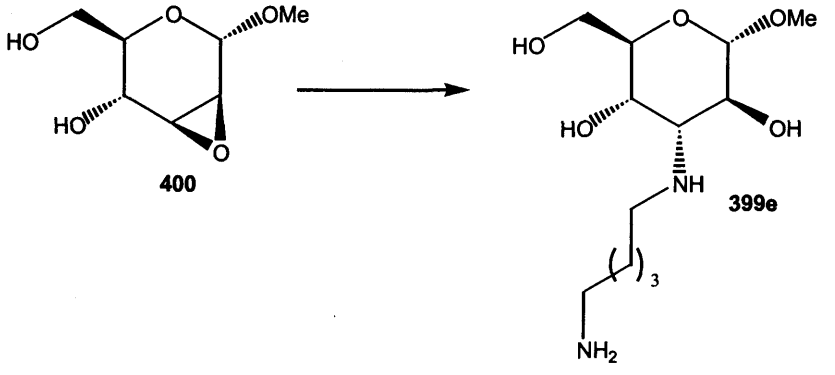
Methyl-2,3-anhydro-α-D-mannopyranoside **400** (209 mg, 1.19 mmol) and 1,3 diaminopropane (825 µl, 9.88 mmol) was placed in a Youngs tube and stirred at 100°C for 20 h. Upon cooling the solution solidified. The solid was diluted with methanol and transferred to a round bottom flask and the excess methanol removed under reduced pressure to leave orange oil. The oil was purified by column chromatography with dichloromethane : 2 M ammonia in methanol (1:1 v/v) as the eluent to yield the product **399c** (221 mg, 74%) as a yellow oil; $[\alpha]^{20}_D$ +92.5° (c 0.7 in MeOH); ν_{max}(neat)/cm^{-1} 3301m, 2920m, 1568m, 1036s, 974s; δ_H (400 MHz; D_2O) 1.62 (2H, quintet, *J* 7, $CH_2CH_2CH_2$) 2.67 (2H, app dd, *J* 2.1, 7.0, CH_2CH_2NH) 2.72 (2H, app tt, *J* 2.1, 7.0 , $NH_2CH_2CH_2$) 2.77 (1H, app dt, *J* 1.3, 5.0, 3-H) 3.37 (3H, s, OMe) 3.67-3.75 (3H, m, 2-H, 2 x 6-H) 3.78 (1H, app d app t, *J* 3.3, 7.0, 5-H) 3.87 (1H, app dd, *J* 5.0, 7.0, 4-H) 4.57 (1H, d, *J* 3.6, 1-H); δ_C (62.9 MHz; D_2O) 30.55 (CH_2) 39.06 (CH_2) 45.36 (CH_2) 55.89 (CH_3, OMe) 59.37 (CH, 3-C) 61.2 (CH_2, 6-C) 63.87 (CH, 4-C) 68.45 (CH, 2-C) 72.86 (CH, 5-C) 101.72 (CH, 1-C); *m/z* (FAB) 251 (MH^+, 100%), 220 (8), 201 (15); found MH^+, 251.1607 $C_{10}H_{22}N_2O_5$ requires 251.1607

Methyl-3-(4-amino-butylamino)-3-deoxy- α-D-altropyranoside (399d)



Methyl-2,3-anhydro-α-D-mannopyranoside **400** (121 mg, 0.68 mmol) and 1,4 diaminobutane (205 μl, 2.04 mmol) was placed in a Youngs tube and stirred at 120°C for 24 h. The mixture was diluted with methanol and transferred to a round bottom flask and the excess methanol removed under reduced pressure to leave a dark orange syrup. The syrup was purified by column chromatography with dichloromethane:10% 2 M ammonia in methanol as the eluent to give the product **399d** (155 mg, 87%) as a yellow oil; $[\alpha]^{20}_{D}$ +49.8° (c 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3329m, 2925m, 1469m, 1033s, 972s; δ_H (300 MHz; D_2O) 1.18- 1.26 (4H, m, $NHCH_2CH_2CH_2$, $CH_2CH_2NH_2$) 2.35-2.42 (4H, m, $NHCH_2CH_2$, $CH_2CH_2NH_2$) 2.56 (1H, app dd, *J* 4.5, 1.5, 3-H) 3.17 (3H, s, OMe) 3.45-3.54 (3H, m, 2-H, 2 x 6-H) 3.57 (1H, d app t, *J* 3.0, 7.0, 5-H) 3.65 (1H, dd, *J* 4.5, 7.0, 4-H) 4.35 (1H, d, *J* 3.6, 1-H); δ_C (75.5 MHz; D_2O) 26.32, 29.15 (2 x CH_2) 40.29 (CH_2) 47.00 (CH_2) 55.49 (CH_3, OMe) 58.90 (CH, 3-C) 60.74 (CH_2, 6-C) 63.52 (CH, 4-C) 68.17 (CH, 2-C) 72.73 (CH, 5-C) 101.31 (CH, 1-C); *m/z* (FAB) 265 found MH^+, 256.1764 $C_{11}H_{24}N_2O_5$ requires 265.1763

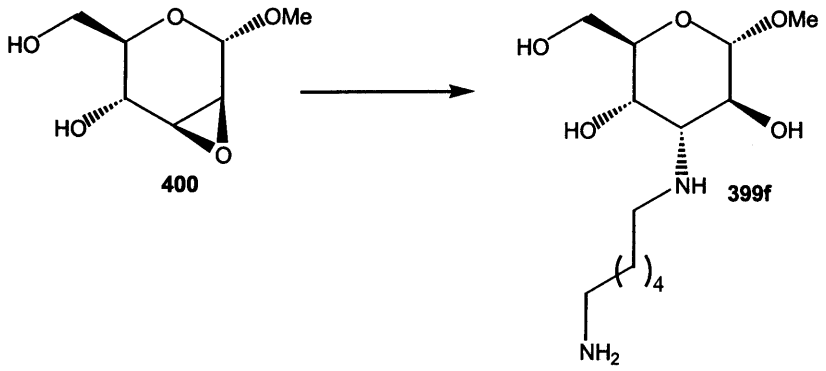
Methyl-3-(5-amino-pentylamino)-3-deoxy- α-D-altropyranoside (399e)



Methyl-2,3-anhydro-α-D-mannopyranoside **400** (230 mg, 1.31 mmol) and 1,5 diaminopentane (154 μl, 1.31 mmol) was placed in a Youngs tube and stirred at 120°C for

19.5 h. The mixture was diluted with methanol and transferred to a round bottom flask and the excess methanol removed under reduced pressure to leave a dark orange/brown syrup. The syrup was purified by column chromatography with dichloromethane:2 M ammonia in methanol (1:1 v/v) as the eluent to produce the product **399e** (121 mg, 44%) as a yellow/orange oil; $[\alpha]^{20}_D$ +135.1° (c 1.5 in MeOH); ν_{max}(neat)/cm^{-1} 3325m, 2927m, 2858w, 1568m, 1037s, 972s; δ_H (250 MHz; D_2O) 1.39-1.48 (2H, m, $HNCH_2CH_2CH_2$) 1.52-1.62 (4H, m, $HNCH_2CH_2$, $H_2NCH_2CH_2$) 2.68-2.79 (4H, m, $HNCH_2CH_2$, $H_2NCH_2CH_2$) 2.92 (1H, br d, *J* 4.0, 3-H) 3.52 (3H, s, OMe) 3.83-3.87 (2H, m, 6-H) 3.89 (1H, d, *J* 4.0, 2-H) 3.93 (1H, dt, *J* 3.0, 7.0, 5-H) 4.01 (1H, dd, *J* 4.0, 7.0, 4-H) 4.71 (1H, d, *J* 4.0, 1-H); δ_C (75.7 MHz; D_2O) 24.04 (CH_2) 28.94 (CH_2) 30.91 (CH_2) 40.61 (CH_2) 47.45 (CH_2) 55.87 (CH_3, OMe) 59.28 (CH, 3-C) 61.12 (CH_2, 6-C) 63.90 (CH, 2-C) 68.55 (CH, 4-C) 73.10 (CH, 5-C) 101.68 (CH, 1-C); *m/z* (FAB) 279 (MH^+, 100%); found MH^+, 279.1919 $C_{12}H_{26}N_2O_5$ requires 279.1920

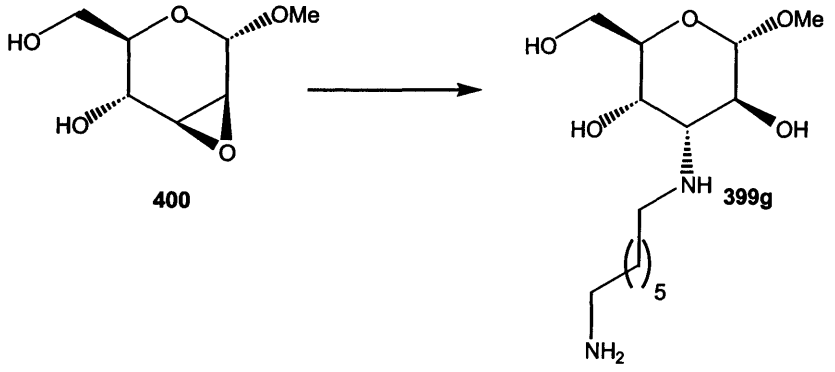
Methyl-3-(6-amino-hexylamino)-3-deoxy-α-D-altropyranoside (399f)



Methyl-2,3-anhydro-α-D-mannopyranoside **400** (100 mg, 0.57 mmol) and hexamethylenediamine (66 mg 0.57 mmol) was placed in a Youngs tube and stirred at 110°C for 24 h. The mixture was diluted with methanol and transferred to a round bottom flask and the excess methanol removed under reduced pressure to leave an orange syrup. The syrup was purified by column chromatography with dichloromethane:2 M ammonia in methanol (7:3 v/v) as the eluent to yield the product **399f** (49 mg, 30%) as a yellow oil; $[\alpha]^{20}_D$ +161.2° (c 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3327m, 2926s, 1564m, 1464s, 1038s, 972s; δ_H (250 MHz; D_2O) 1.27-1.35 (4H, app q, *J* 3.0, $HNCH_2CH_2CH_2$, $H_2NCH_2CH_2CH_2$) 1.40-1.54 (4H, app sextet, *J* 7.0 $HNCH_2CH_2CH_2$, $H_2NCH_2CH_2CH_2$) 2.55-2.66 (2H, app q, *J* 3.0, $HNCH_2CH_2$) 2.72-2.81 (3H, m, 3-H, $H_2NCH_2CH_2$) 3.39 (3H, s, OMe) 3.67-3.73 (2H, m, 2x 6-H) 3.75 (1H, d, *J* 3.0, 2-H) 3.80 (1H, dt, *J* 3.0, 6.7, 5-H) 3.88 (1H, dd, *J* 4.0, 6.7, 4-H) 4.58 (1H, d, *J* 3.0, 1-H); δ_C (62.9 MHz; D_2O) 26.04 (CH_2) 26.42 (CH_2) 29.04

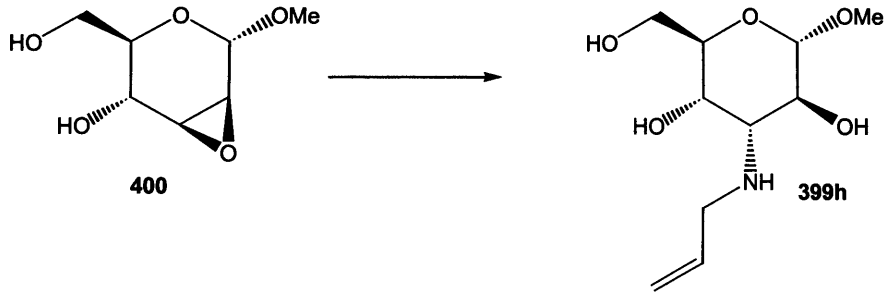
(CH_2) 29.56 (CH_2) 40.37 (CH_2) 47.49 (CH_2) 55.93 (CH_3, OMe) 59.32 (CH, 3-C) 61.17 (CH_2, 6-C) 63.95 (CH, 4-C) 68.60 (CH, 2-C) 73.18 (CH, 5-C) 101.73 (CH, 1-C); *m/z* (FAB) 293 (MH^+, 100%); found MH^+, 293.2077 $C_{13}H_{28}N_2O_5$ requires 293.2076

Methyl-3-(7-amino-heptylamino)-3-deoxy-α-D-altropyranoside (399g)



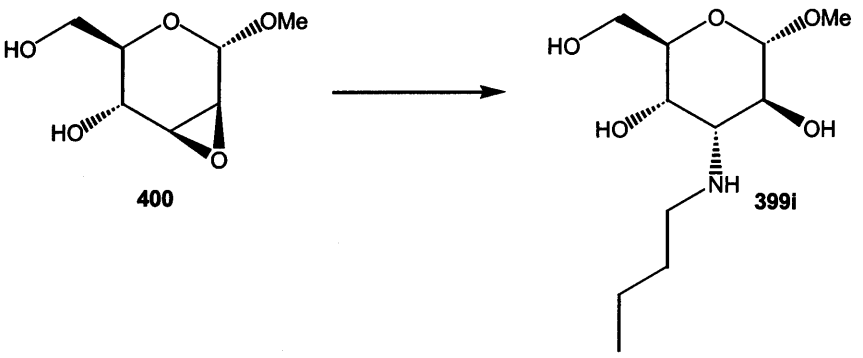
The previous procedure was followed using stated amounts and molar equivalents. The resulting orange oil was purified by column chromatography on silica dichloromethane:2 M ammonia in methanol (7:3 v/v) as the eluent to give **399g** (55 mg, 31%) as a yellow oil; $[\alpha]^{20}_D$ +60.6° (c 1.2 in MeOH); ν_{max}(neat)/cm^{-1} 3329m, 2919m, 2849m, 1646s, 1260w, 1032s, 972s, 831m; δ_H (250 MHz; D_2O) 1.29 (6H, app s, $HNCH_2CH_2CH_2$, $HNCH_2CH_2CH_2CH_2$, $H_2NCH_2CH_2CH_2$) 1.40-1.52 (4H, m, $HNCH_2CH_2$, $H_2NCH_2CH_2$) 2.55-2.66 (2H, m, $H_2NCH_2CH_2$) 2.73 (1H, t, *J* 7.0, $HNCHHCH_2$) 2.79 (1H, t, *J* 4.0, 3-H) 2.95 (1H, t, *J* 7.0, $HNCHHCH_2$) 3.39 (3H, s, OMe) 3.67-3.74 (2H, m, 2 x 6-H) 3.76 (1H, d, *J* 3.6, 2-H) 3.81 (1H, app ddd, *J* 2.3, 4.0, 9.6, 5-H) 3.89 (1H, dd, *J* 4.0, 7.0, 4-H) 4.59 (1H, d, *J* 3.6, 1-H); δ_C (62.9 MHz; D_2O) 26.15 (CH_2) 26.62 (CH_2) 28.56 (CH_2) 29.01 (CH_2) 29.45 (CH_2) 40.39 (CH_2) 47.56 (CH_2) 55.92 (CH_3, OMe) 59.33 (CH, 3-C) 61.18 (CH_2, 6-C) 63.93 (CH, 4-C) 68.58 (CH, 2-C) 73.15 (CH, 5-C) 101.72 CH, 1-C); *m/z* (FAB) 307 (MH^+, 68%) 185 (100) 174 (34); found MH^+, 307.2233 $C_{14}H_{30}N_2O_6$ requires 307.2233

Methyl-3-allylamino-3-deoxy-α-D-altropyranoside (399h)



The general procedure was followed using stated amounts and molar equivalents. The yellow oil was purified by column chromatography on silica using dichloromethane:15% 2 M ammonia in methanol as the eluent to give **399h** (122 mg, 92%) as a yellow oil; $[\alpha]^{20}_D$ +134.9° (c 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3334m, 2917m, 1644w, 1133m, 1038s, 971s; δ_H (250 MHz; C_6D_6) 3.37 (1H, apparent d, *J* 5.0, 3-H) 3.42 (3H, s, OMe) 3.58 (2H, app dd, *J* 6.2, 13.8, HNC*H*$_2$) 3.79 (1H, dt, *J* 3.0, 10.0, 5-H) 4.27-4.32 (2H, m, 2-H, 6-H overlap) ABx system centred at 4.42 (1H, pseudo *J* 3.0, 12.0, 6-H) 4.53 (1H, dd, *J* 5.0, 10.0, 4-H) 5.08 (1H, s, 1-H) 5.31 (1H, dd, *J* 1.6, 10.2, CH=C*H*H) 5.43 (1H, dd, *J* 1.6, 17.1, CH=CH*H*) 6.09 (1H, ddt, *J* 6.2, 10.2, 17.1, CH$_2$C*H*=CH$_2$); δ_C (62.9 MHz; C_6D_6) 51.82 (CH$_2$) 55.51 (CH$_3$, OMe) 59.92 (CH, 3-C) 61.78 (CH, 4-C) 62.74 (CH$_2$, 6-C) 68.12 (CH, 2-C) 70.93 (CH, 5-C) 102.6 (CH, 1-C) 116.63 (CH$_2$) 137.73 (CH); *m/z* (FAB) 234 (MH$^+$, 100%), 202 (40), 174 (7), 160 (20); found MH$^+$, 234.1342. $C_{10}H_{19}NO_5$ requires 234.1342

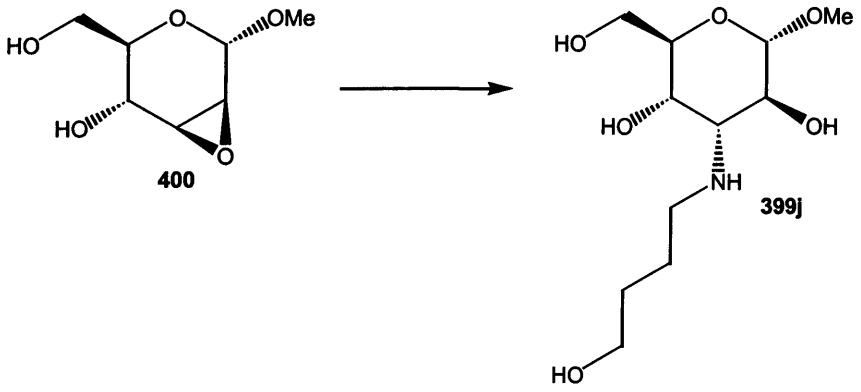
Methyl–3-(butylamino)-3-deoxy-α-D-altropyranoside (399i)



The general procedure was followed using stated amounts and molar equivalents. The orange oil was purified by column chromatography on silica using dichloromethane:5% 2 M ammonia in methanol as the eluent to give **399i** (130 mg, 92%) as an orange oil; $[\alpha]^{20}_D$ +127.1° (c 1.3 in MeOH); ν_{max}(neat)/cm^{-1} 3339m, 2928m, 1133s, 1037s, 971s; δ_H (250 MHz; C_6D_6) 1.08 (3H, t, *J* 7.1, C*H*$_3$CH$_2$) 1.45 (2H, app q, *J* 7.1, CH$_3$C*H*$_2$) 1.54-1.64 (2H, m, HNCH$_2$C*H*$_2$) 2.64 (1H, app quintet, *J* 6.2, HNC*H*H) 2.94 (1H, app quintet, *J* 6.2,

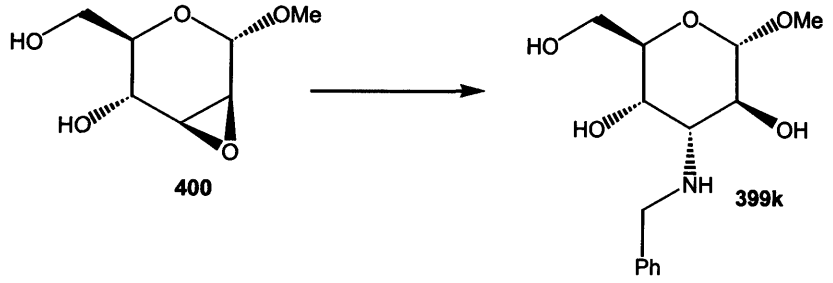
HNCH*H*) 3.28 (1H, app t, *J* 3.8, 3-H) 3.39 (3H, s, OMe) 3.72 (1H, app dt, *J* 3.9, 9.9, 5-H) 4.23-4.27 (2H, m, 2-H, 6-H) ABx centred at 4.37 (1H, dd, *J* 3.9, 12.0, 6-H) 4.47 (1H, dd, *J* 5.2, 9.9, 4-H) 5.03 (1H, s, 1-H); δ_C (62.9 MHz; C_6D_6) 14.47 (CH_3) 20.95 (CH_2) 33.35 (CH_2) 49.02 (CH_2) 55.51 (CH_3, OMe) 60.75 (CH, 3-C) 61.63 (CH, 4-C) 62.78 (CH_2, 6-C) 68.05 (CH, 2-C) 70.94 (CH, 5-C) 102.64 (CH, 1-C); *m/z* (FAB) 250 (MH^+, 100%) 218 (54), 190 (8), 176 (29); found MH^+, 250.1655. $C_{11}H_{23}NO_5$ requires 250.1655

Methyl–3(-4-hydroxy-butylamino)-3-deoxy -α-D-altropyranoside (399j)



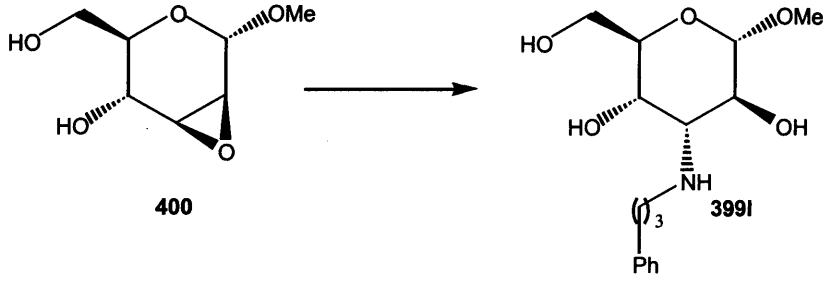
The general procedure was followed using stated amounts and molar equivalents. The orange oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (7:3 v/v) as the eluent to give **399j** (117 mg, 77%) as a yellow/orange oil; $[\alpha]^{20}_D$ +111.6° (c 1.7 in MeOH); ν_{max}(neat)/cm^{-1} 3331m, 2931m, 2482w, 1035s, 971s; δ_H (250 MHz; D_2O) 1.55-1.71 (4H, m, $HNCH_2CH_2$, $HOCH_2CH_2$) 2.71-2.85 (2H, m, $NHCH_2$) 2.93 (1H, dd, *J* 4.4, 6.2, 3-H) 3.52 (3H, s, OMe) 3.69 (2H, t, *J* 6.1, $HOCH_2CH_2$) 3.80-3.87 (2H, m, 6-H) 3.88 (1H, d, *J* 3.0, 2-H) 3.93 (1H, dd, *J* 3.0, 6.7, 5-H) 4.02 (1H, dd, *J* 4.4, 6.7, 4-H) 4.71 (1H, d, *J* 3.0, 1-H); δ_C (75.7 MHz; D_2O) 25.79 (CH_2) 29.5 (CH_2) 47.29 (CH_2) 55.81 (CH_3, OMe) 59.22 (CH, 3-C) 61.05 (CH_2, 6-C) 61.84 (CH_2) 63.8 (CH, 4-C) 68.42 (CH, 2-C) 72.98 (CH, 5-C) 101.59 (CH, 1-C); m/z (FAB) 265 (MH^+, 100%), 234 (12), 206 (8), 192 (9), 174 (7); found MH^+, 266.1603. $C_{11}H_{23}NO_6$ requires 266.1604

Methyl–3-(benzylamino)-3-deoxy-α-D-altropyranoside (399k)



Methyl-2,3-anhydro-α-D-mannopyranoside **400** (182 mg, 1.03 mmol) and benzylamine (113 μl, 1.03 mmol) was placed in a Youngs tube and stirred at 110^{0}C for 19 h. The mixture was diluted with methanol and transferred to a round bottom flask and the excess methanol removed under reduced pressure to leave an orange syrup. The syrup was purified by column chromatography with dichloromethane : 2 M ammonia in methanol (2:3 v/v) as the eluent to yield **399k** (218 mg, 75%) as a yellow oil; $[\alpha]^{20}_D$ +160.1° (c 0.7 in MeOH); ν_{max}(neat)/cm^{-1} 3323m, 2916m, 1454m, 1037s, 971s, 698s; δ_H (250 MHz; D_2O) 2.92 (1H, t, *J* 5.1, 3-H) 3.44 (2H, s, PhC*H*$_2$NH) 3.47 (3H, s, OMe) 3.77-3.94 (5H, m, 2-H, 4-H, 5-H, 2 x 6-H) 4.67 (1H, d, *J* 3.2, 1-H) 7.34-7.48 (5H, m, Ph); δ_C (62.9 MHz; D_2O) 51.46 (CH_2) 55.83 (CH_3, OMe) 58.31 (CH, 3-C) 61.24 (CH_2, 6-C) 63.43 (CH, 4-C) 68.08 (CH, 2-C) 72.45 (CH, 5-C) 101.64 (CH, 1-C) 127.81 (CH, Ph) 128.95 (CH, Ph) 129.12 (CH, Ph) 139.98 (C, Ph) *m/z* (FAB) 284 (MH^+, 63%) 252 (17) 91 (100); found MH^+, 284.1490 $C_{14}H_{21}NO_5$ requires 284.1499

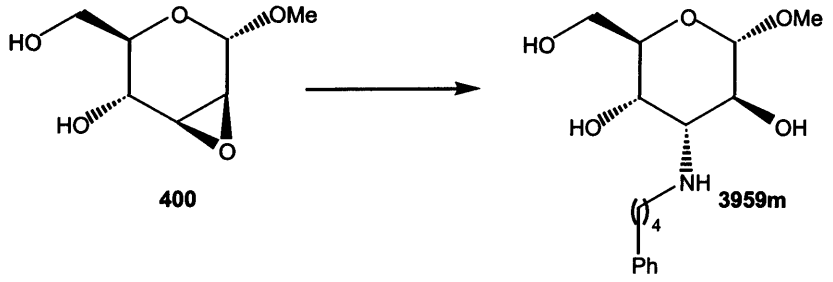
Methyl–3-(3-phenyl-1-propylamino)-3-deoxy-α-D-altropyranoside (399l)



Methyl-2,3-anhydro-α-D-mannopyranoside **400** (100 mg, 0.57 mmol) and 3-phenyl-1-propylamine (90 μl, 0.67 mmol) was placed in a Youngs tube and stirred at 222^{0}C for 24 h. The mixture was diluted with methanol and transferred to a round bottom flask and the excess methanol removed under reduced pressure to leave an orange syrup. The syrup was purified by column chromatography with ethyl acetate : 2 M ammonia in methanol (9.8:0.2 v/v) as the eluent to yield **399l** (120 mg, 68%) as a yellow oil; $[\alpha]^{20}_D$ +56.4° (c 0.8

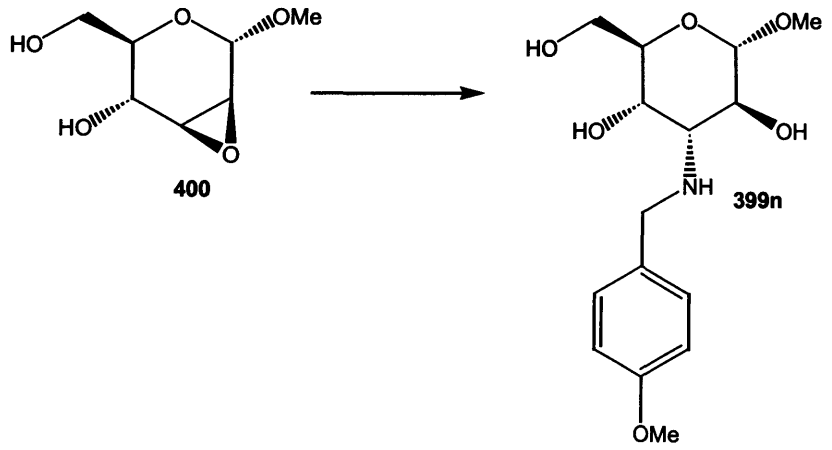
in MeOH); ν_{max}(neat)/cm^{-1} 3329m, 2926m, 1453m, 1103m 1037s, 745s; δ_H (300 MHz; $CDCl_3$) 1.80-1.88 (2H, m , $HNCH_2CH_2$) 2.55-2.68 (3H, m, $PhCH_2$, HNCHH) 2.84-2.95 (2H, m, 3-H, HNCHH) 3.50 (3H, br s, OH) 3.36 (3H, s, OMe) 3.43-3.49 (2H, m, 5-H, NH) 3.81-3.98 (4H, m, 2-H, 4-H, 2 x 6-H) 4.65 (1H, s, 1-H) 7.16-7.20 (3H, m, Ph) 7.25-7.30 (2H, m, Ph); δ_C (75.5 MHz; $CDCl_3$) 32.00 (CH_2) 33.30 (CH_2) 48.04 (CH_2) 55.44 (CH_3, OMe) 59.68 (CH, 3-C) 61.06 (CH, 4-C) 62.34 (CH_2, 6-C) 67.18 (CH, 2-C) 69.66 (CH, 5-C) 101.68 (CH, 1-C) 125.88 (CH, Ph) 128.38 (CH, Ph) 141.79 (C, Ph); *m/z* (FAB) 312 (MH^+, 26%) found MH^+, 312.1811 $C_{16}H_{25}NO_5$ requires 312.1811

Methyl–3-(4-phenylbutylamino)-3-deoxy-α-D-altropyranoside (399m)



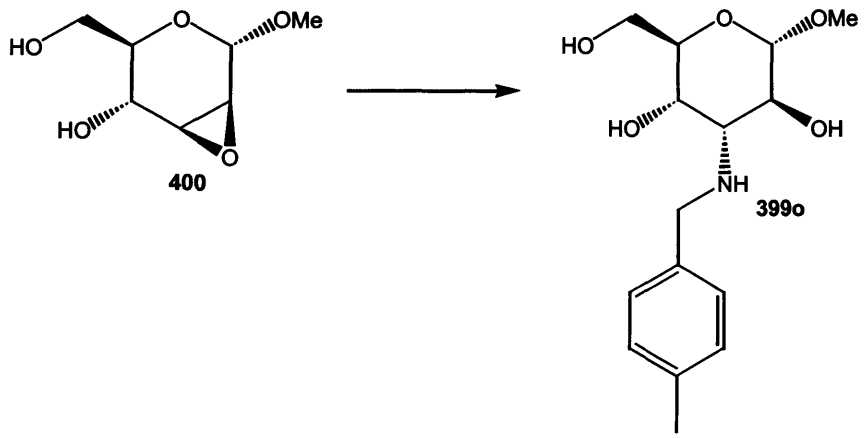
Methyl-2,3-anhydro-α-D-mannopyranoside **400** (100 mg, 0.57 mmol) and 4-phenylbutylamine (270 μl, 1.71 mmol) was placed in a Youngs tube and stirred at 125 ^{0}C for 72 h. The mixture was diluted with DCM and transferred to a round bottom flask and the excess DCM removed under reduced pressure to leave an orange syrup. The syrup was purified by column chromatography with ethyl acetate : 2 M ammonia in methanol (7:3 v/v) as the eluent to yield **399m** (173 mg, 94%) as a yellow oil; $[\alpha]^{20}_D$ +216.3° (c 1.6 in MeOH); ν_{max}(neat)/cm^{-1} 3334m, 2857m, 1453m, 1039s, 747s; δ_H (300 MHz; $CHCl_3$) 1.52 (2H, quin, *J* 6.8, $PhCH_2CH_2$) 1.65 (2H, quin, *J* 7.5, $HNCH_2CH_2$) 2.47-2.55 (2H, m, $PhCH_2$) 2.62 (1H, t, *J* 7.5, HNCHH) 2.81-2.90 (2H, m, 3-H, HNCHH) 3.17 (3H, br s, OH) 3.35 (3H, s, OMe) 3.39-3.45 (2H, m, 5-H, 6-H) 3.81-3.89 (4H, m, 2-H, 4-H, 6-H, NH) 4.64 (1H, s, 1-H) 7.15-7.19 (3H, m, Ph) 7.25-7.30 (2H, m, Ph); δ_C (75.5 MHz; $CHCl_3$) 28.85 (CH_2) 29.56 (CH_2) 35.64 (CH_2) 48.37 (CH_2) 55.39 (CH_3, OMe) 59.62 (CH, 3-C) 60.73 (CH, 4-C) 61.79 (CH_2, 6-C) 66.88 (CH, 2-C) 69.81 (CH, 5-C) 101.62 (CH, 1-C) 125.77 (CH, Ph) 128.32 (CH, Ph) 128.37 (CH, Ph) 142.27 (C, Ph); *m/z* (FAB) 326 (MH^+, 100%) 294 (28) found MH^+, 326.1968 $C_{17}H_{27}NO_5$ requires 326.1968

Methyl–3-(4-methoxy-benzylamino)-3-deoxy -α-D-altropyranoside (399n)



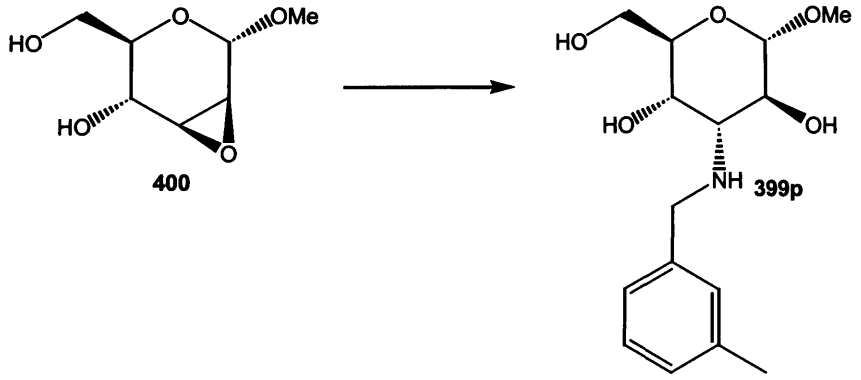
Methyl-2,3-anhydro-α-D-mannopyranoside **400** (94 mg, 0.53 mmol) together with *p*-methoxybenzylamine (207 μl, 1.59 mmol) was placed in a youngst tube and stirred at 100^{0}C for 31 h under an atmosphere of nitrogen. The reaction was left to cool to room temperature and diluted with methanol. The mixture was then transferred to a round bottom flask and evaporated to dryness. The product was purified using column chromatography on silica using ethyl acetate:petrol (4:1v/v). The ratio of the eluent was then changed to dichloromethane:2 M ammonia in methanol (7:3 v/v) to give **399n** as a yellow oil (152 mg, 92%); $[\alpha]^{20}_D$ +88.3° (c 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3332m, 2911m, 2836w, 1611m, 1512s, 1245s, 1030s, 972s, 820s; δ_H (250 MHz; MeOH) 3.04 (1H, t, *J* 4.2, 3-H) 3.56 (3H, s, OMe) 3.70-3.77 (1H app dtd, *J* 2.5, 2.9, 5.9, 5-H) 3.83-3.94 (3H, m, 4-H, 6-H, HNCH*H*) 3.96 (3H, s, PhOMe) 3.99-4.06 (3H, m, 2-H, 6-H, HNC*H*H) 4.77 (1H, s, 1-H) 7.07 (2H, d, *J* 8.0, Ph) 7.45 (2H, d, *J* 8.0, Ph); δ_C(62.9 MHz; MeOH) 53.08 (CH_2) 56.09 (CH_3, OMe) 56.13 (CH_3, *p*-OMePh) 60.46 (CH, 3-C) 63.74 (CH, 4-C) 63.86 (CH_2, 6-C) 68.86 (CH, 2-C) 72.61 (CH, 5-C) 103.64 (CH, 1-C) 115.35 (CH, Ph) 131.13 (CH, Ph) 133.82 (C, Ph) 160.81 (C, Ph); *m/z* (FAB) 274 (MH^+, 56%) 289 (10) 242 (6); found MH^+, 314.1604 $C_{15}H_{23}NO_6$ requires 314.1604

Methyl–3-(4-methyl-benzylamino)-3-deoxy-α-D-altropyranoside (399o)



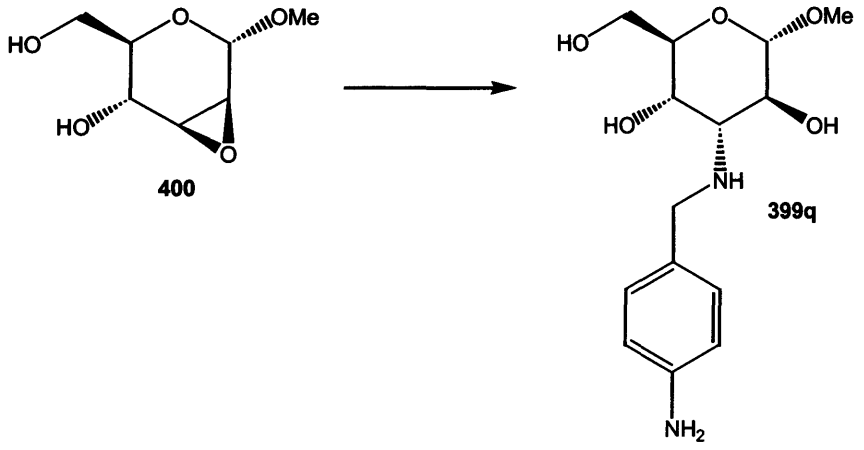
Methyl-2,3-anhydro-α-D-mannopyranoside **400** (215 mg, 1.22 mmol) and *p*-methylbenzylamine (155 μl 1.22 mmol) was placed in a Youngs tube and stirred at 100^{0}C for 18 h. The mixture was diluted with methanol and transferred to a round bottom flask and the excess methanol removed under reduced pressure to leave an orange oil. The orange oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (3:2 v/v) as the eluent to give **399o** (216 mg, 60%) as an orange oil; $[\alpha]^{20}_D$ +98.7° (c 0.9 in MeOH); ν_{max}(neat)/cm^{-1} 3335m, 2922m, 1647w, 1450w, 1038s, 972s; δ_H (250 MHz, D_2O) 2.21 (3H, s, C*H*$_3$) 2.98 (1H, t, *J* 4.6, 3-H) 3.42 (3H, s, OMe) 3.68-3.73 (2H, m, 5-H, HNC*H*H) 3.81-3.97 (5H, m, 2-H, 4-H, 2 x 6-H, HNCH*H*) 4.72 (1H, d, *J* 1.8, 1-H) 7.07 (2H, d, *J* 8, Ph) 7.23 (2H, d, *J* 8, Ph); δ_C (62.9 MHz, D_2O) 20 82 (CH_3, Me) 51.61 (CH_2) 55.60 (CH_3, OMe) 58.71 (CH, 3-C) 61.42 (CH_2, 6-C) 62.56 (CH, 4-C) 67.39 (CH, 2-C) 71.43 (CH, 5-C) 101.59 (CH, 1-C) 128.71 (CH, Ph) 129.41 (CH, Ph) 136.86 (C, Ph) 137 (C, Ph); *m/z* (FAB) 298 (MH^+, 74%), 289 (9), 266 (5), 242 (5), 224 (12); found MH^+, 298.1655. $C_{15}H_{23}NO_5$ requires 298.1655

Methyl–3-(3-methyl-benzylamino)-3-deoxy-α-D-altropyranoside (399p)



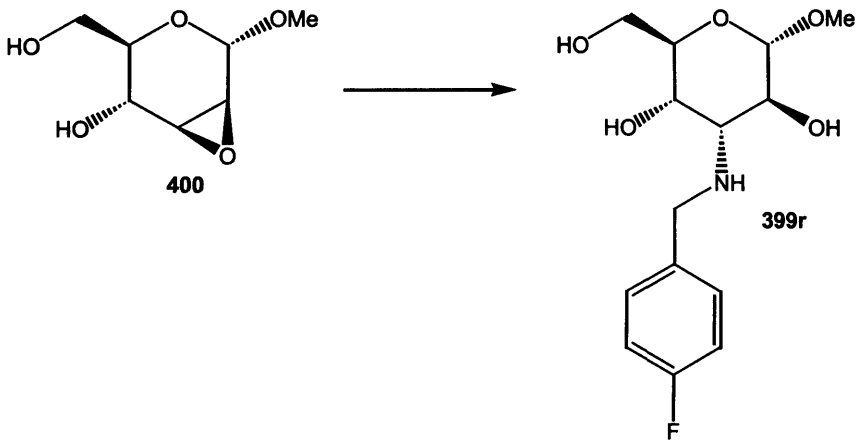
The general procedure was followed using stated amounts and molar equivalents. The orange oil was purified by column chromatography on silica using dichloromethane:20% 2 M ammonia in methanol as the eluent to give **399p** (153 mg, 92%) as an orange oil; $[\alpha]^{20}_{D}$ +77.8° (c 1.2 in MeOH); ν_{max}(neat)/cm^{-1} 3333m, 2917m, 2477m, 1645w, 1450m, 1193w, 1039s, 972s; δ_H (300MHz; C_6D_6) 2.31 (3H, s, C*H*$_3$) 3.29-3.36 (4H, br s, OMe, 3-H) 3.59-3.69 (1H, app d, *J* 10.0, 5-H) 3.77 (1H, d, *J* 2.6, HNC*H*H) 4.00 (1H, d, *J* 2.6, 7-HNCH*H*) 4.10-4.18 (1H, app d, *J* 10.0, 6-H) 4.19-4.29 (2H, m, 2-H, 6-H) 4.34-4.41 (1H, dd, *J* 5.0, 10.0, 4-H) 4.96 (1H, s, 1-H) 5.06 (1H, br s, NH) 7.05 (1H, app d, *J* 5.6, Ph) 7.23-7.33 (3H, m, Ph); δ_C (62.9 MHz; C_6D_6) 21.78 (CH_3, Me) 53.08 (CH_2) 55.77 (CH_3, OMe) 59.56 (CH, 3-C) 61.23 (CH, 4-C) 62.25 (CH_2, 6-C) 67.28 (CH, 2-C) 70.08 (CH, 5-C) 102.05 (CH, 1-C) 125.69 (CH, Ph) 128.44 (CH, Ph) 129.46 (CH, Ph) 138.55 (C, Ph) 140.19 (C, Ph); *m/z* (FAB) 298 (MH^+, 51%) 289 (11), 266 (5), 224 (6); found MH^+, 298.1655. $C_{15}H_{23}NO_5$ requires 298.1655

Methyl–3-(4-amino-benzylamino)-3-deoxy-α-D-altropyranoside (399q)



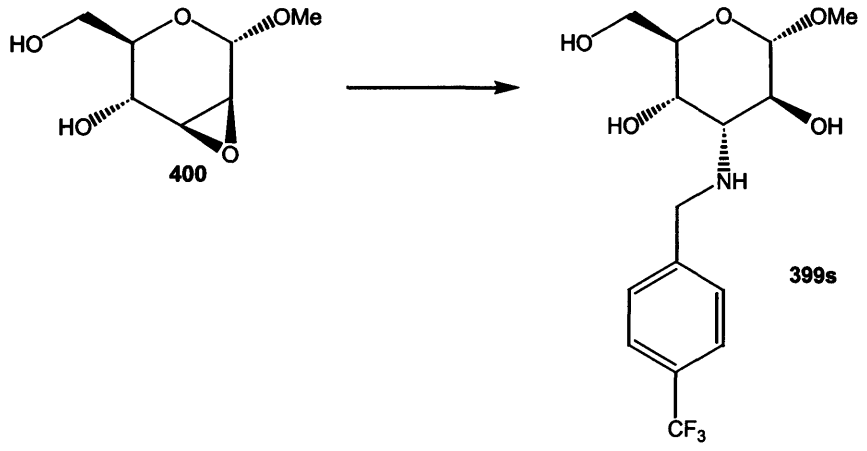
The general procedure was followed using stated amounts and molar equivalents. The reaction was left to cool to room temperature and diluted with methanol. The solution was then transferred to a round bottom flask and evaporated to dryness to give a brown oil. The oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (4:1 v/v) as the eluent to give **399q** (145 mg, 85%) as a brown oil; $[\alpha]^{20}_{D}$ +98.7° (c 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3334m, 2919w, 1614m, 1518m, 1036s, 971s; δ_H (250 MHz, $CDCl_3$) 3.00 (1H, dd, *J* 2.0, 4.0, 3-H) 3.58 (3H, s, OMe) 3.89 (2H, s, HNC*H*$_2$) 3.92 (1H, app t, *J* 3.0, 2-H) 3.96-4.04 (4H, m, 4-H, 5-H, 2 x 6-H overlap) 4.75 (1H, d, *J* 3.0, 1-H) 6.99 (2H, d, *J* 8.3, Ph) 7.36 (2H, d, *J* 8.3, Ph); δ_C (62.9 MHz, $CDCl_3$) 50.81 (CH_2) 55.86 (CH_3, OMe) 58.04 (CH, 3-C) 61.17 (CH_2, 6-C) 63.62 (CH, 4-C) 68.24 (CH, 2-C) 72.83 (CH, 5-C) 101.65 (CH, 1-C) 116.89 (CH, Ph) 130.12 (CH, Ph) 130.64 (C, Ph) 145.76 (C, Ph); *m/z* (FAB) 299 (MH^+, 80%) 265 (4), 242 (7), 209 (15); found MH^+, 299.1606. $C_{14}H_{22}N_2O_5$ requires 299.1607

Methyl–3-(4-fluoro-benzylamino)-3-deoxy-α-D-altropyranoside (399r)



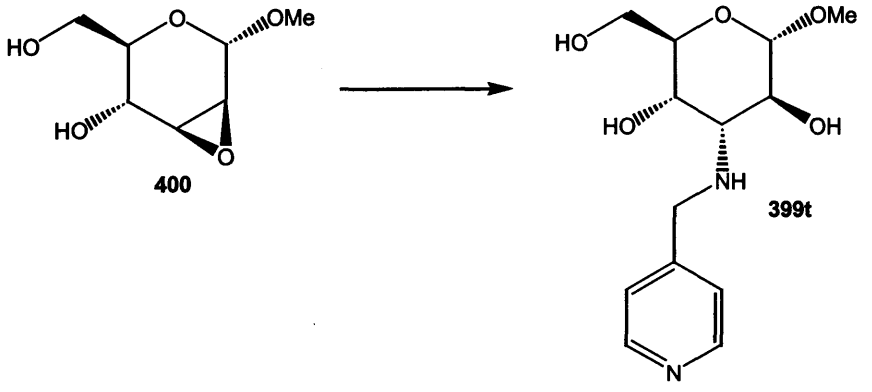
The general procedure was followed using stated amounts and molar equivalents. The oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (9:1 v/v) as the eluent to give **399r**(168mg, 98%) as an orange oil; $[\alpha]^{20}_{D}$ +95.5° (c 1.4 in MeOH); ν_{max}(neat)/cm^{-1} 3329m, 2911w, 2836w, 1509s, 1220s, 1039s, 764w; δ_H (250 MHz, $CDCl_3$) 2.93 (1H, t, *J* 3.8, 3-H) 3.32 (3H, s, OMe) 3.38-3.44 (1H, dt, *J* 3.5, 9.6, 5-H) 3.65 (1H, d, *J* 12.9, HNC*H*H) 3.75-3.94 (5H, m, 2-H, 4-H, 2 x 6-H, HNCH*H*) 4.63 (1H, s, 1-H) 6.96-7.04 (2H, m, Ph) 7.23-7.29 (2H, m, Ph); δ_C (62.9 MHz, $CDCl_3$) 52.25 (CH_2) 55.76 (CH_3, OMe) 59.39 (CH, 3-C) 61.25 (CH, 4-C) 62.29 (CH_2, 6-C) 67.27 (CH, 2-C) 70.04 (CH, 5-C) 102.07 (CH, 1-C) 115.74 (2CH, d, *J* 21.0, Ph) 129.24 (CH, d, *J* 8.0, Ph) 130.23 (CH, d, *J* 8.0, Ph) 136.05 (C, d, *J* 3.6, Ph) 162.46 (C, d, *J* 245.0, Ph) *m/z* (FAB) 302 (MH^+, 100%) 289 (10) 270 (12) 228 (13) 209 (4) 167 (8); found MH^+, 302.1403. $C_{14}H_{20}NO_5F$ requires 302.1404

Methyl–3-(4-trifluoromethyl-benzylamino)-3-deoxy-α-D-altropyranoside (399s)



The general procedure was followed using stated amounts and molar equivalents. The oil was purified by column chromatography on silica using dichloromethane:10% 2 M ammonia in methanol as the eluent to give **399s** (144 mg, 72%) as an orange/brown oil; $[\alpha]^{20}_{D}$ +84.5° (c 1.9 in MeOH); ν_{max}(neat)/cm^{-1} 3332w, 2915w, 1619w, 1323s, 1111s, 1064s, 822m; δ_H (250 MHz, $CDCl_3$) 2.96 (1H, t, *J* 3.6, 3-H) 3.32 (3H, s, OMe) 3.42 (1H, dt, *J* 2.7, 10, 5-H) 3.59-3.76 (5H, br m, 3 x OH, NH, HNC*H*H) 3.77-4.04 (5H, m, 2-H, 4-H, 2 x 6-H, HNCH*H*) 4.63 (1H, s, 1-H) 7.42 (2H, d, *J* 8.0, Ph) 7.57 (2H, d, *J* 8.0, Ph); δ_C (62.9 MHz, $CDCl_3$) 52.49 (CH_2) 55.81 (CH_3, OMe) 59.62 (CH, 3-C) 61.11 (CH, 4-C) 62.07 (CH_2, 6-C) 67.24 (CH, 2-C) 69.89 (CH, 5-C) 101.96 (CH, 1-C) 124.53 (C, Ph) 125.86 (CH, Ph) 128.88 (CH, Ph) 129.98 (C, Ph) 144.24 (C, Ph); *m/z* (FAB) 352 (MH^+, 88%) 332 (11) 320 (41) 291 (5) 278 (20) 244 (8) 217 (12); found MH^+, 352.1371. $C_{15}H_{20}NO_5F_3$ requires 352.1372

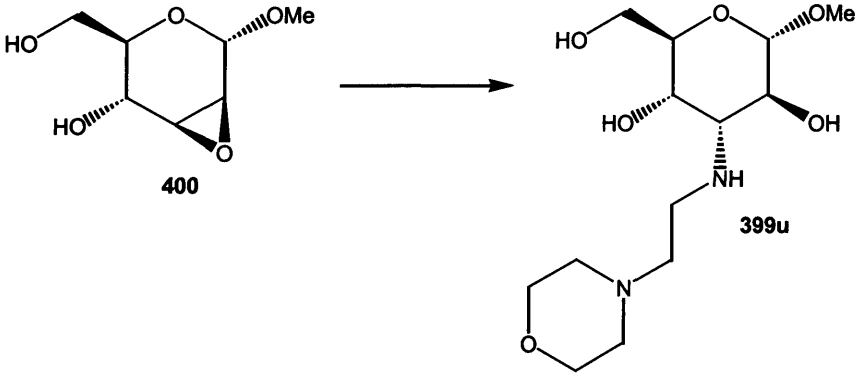
Methyl–3-(pyridine-4-ylmethylamino)-3-deoxy-α-D-altropyranoside (399t)



The general procedure was followed using stated amounts and molar equivalents. The oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in

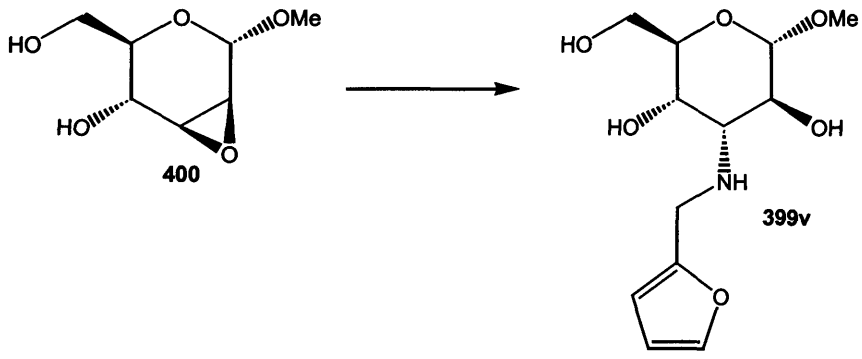
methanol (9:1 v/v)as the eluent to rid the impurity. The ratio of the eluent was then changed to (3:2 v/v) to give **399t** (140 mg, 86%) as an orange oil; $[\alpha]^{20}_{D}$ +77.8° (c 0.9 in MeOH); ν_{max}(neat)/cm^{-1} 3321m, 2917m, 2469w, 1603m, 1418m, 1042s; δ_H (250 MHz, D_2O) 2.98 (1H, dt, *J* 1.8, 3.9, 3-H) 3.57 (3 H, s, OMe) 3.83-4.11(7H, m, 2-H, 4-H, 5-H, 2 x 6-H, HNC*H*$_2$) 4.75 (1H, d, *J* 3.7, 1-H) 7.54-7.59 (2H, m, Ph) 8.57-8.64 (2H, m, Ph); δ_C (62.9 MHz; D_2O) 50.17 (CH_2) 55.87 (CH_3, OMe) 58.51 (CH, 3-C) 61.19 (CH_2, 6-C) 63.74 (CH, 4-C) 68.32 (CH, 2-C) 72.61 (CH, 5-C) 101.63 (CH, 1-C) 124.23 (CH, Ph) 149.10 (CH, Ph) 150.62 (C, Ph); *m/z* (FAB) 285 (MH^+, 38%) 253 (12), 211 (8); found MH^+, 285.1451. $C_{13}H_{20}N_2O_5$ requires 285.1451

Methyl–3-(2-morpholin-4-yl-ethylamino)-3-deoxy-α-D-altropyranoside (399u)



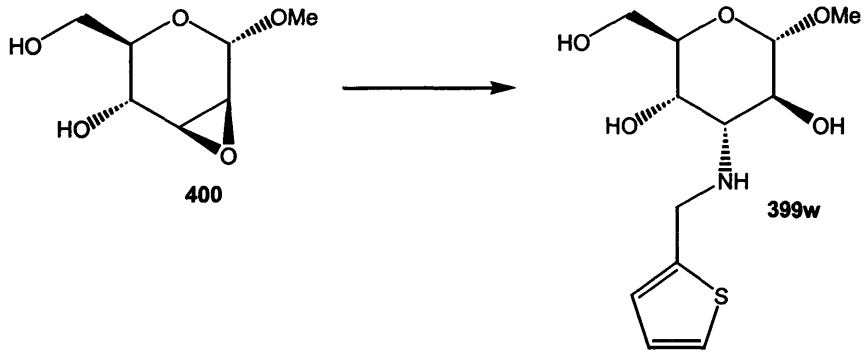
The general procedure was followed using stated amounts and molar equivalents. The oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (9:1 v/v) as the eluent to give **399u** (160mg, 92%) as an yellow solid; mp. 128-130°C; $[\alpha]^{20}_{D}$ +124.7° (c 1.3 in MeOH); ν_{max}(neat)/cm^{-1} 3363m, 2822m, 1149m, 1109s, 1045s, 919s; δ_H (250 MHz, $CDCl_3$) 2.28-2.40 (3H, m, OCH_2CHH, OCH_2CH_2) 2.48-2.64 (4 H, m, HNC*H*$_2$, $HNCH_2CH_2$) 2.83 (1H, t, *J* 4.0, 3-H) 2.96-3.08 (1H, m, OCH_2CHH) 3.37 (3H, s, OMe) 3.51 (1H, dt, *J* 3.4, 9.8, 5-H) 3.66-3.73 (4H, m, OCH_2CH_2 x 2) 3.79-3.92 (4H, m, 2 x 6-H, 2-H, 4-H) 4.64 (1H, s, 1-H); δ_C (62.9 MHz, $CDCl_3$) 44.56 (CH_2) 53.54 (CH_2) 55.62 (CH_3, OMe) 58.07 (CH_2) 59.74 (CH, 3-C) 62.28 (CH, 4-C) 62.62 (CH_2, 6-C) 67.19 (CH_2) 68.28 (CH, 2-C) 70.16 (CH, 5-C) 102.09 (CH, 1-C); *m/z* (FAB) 307 (MH^+, 100%) 185 (36); found MH^+, 307.1869. $C_{13}H_{26}N_2O_6$ requires 307.1869

Methyl–3-(furan-2-ylmethyl)amino-3-deoxy-α-D-altropyranoside (399v)



The general procedure was followed using stated amounts and molar equivalents. The resulting yellow oil was purified by column chromatography on silica using dichloromethane:10% 2 M ammonia in methanol as the eluent to give **399v** (150 mg, 96%) as a yellow oil; $[\alpha]^{20}_{D}$ +93° (c 1.3 in MeOH); ν_{max}(neat)/cm^{-1} 3356m, 331m8, 2922w, 1130m, 1060s, 1040s, 954m, 907s, 771s; δ_H (300 MHz; C_6D_6) 2.91 (3H, s, OMe) 2.99 (1H, s, 3-H) 3.12 (1H app d, *J* 9.9, 5-H) AB system centred at 3.53 (2H, pseudo *J* 14.0, HNCH_2) 3.68 (1H, s, 2-H) ABx system centred at 3.89 (2H, pseudo *J* 3.3, 12.0, 6-H) 4.09 (1H, dd, *J* 4.9, 9.9, 4-H) 4.56 (1H, s, 1-H) 5.94-5.99 (2H, m, Ar-H x 2) 7.02 (1H, app s, Ar-H); δ_C (75.7 MHz; C_6D_6) 44.09 (CH_2) 53.65 (CH_3, OMe) 58.22 (CH, 3-C) 59.87 (CH, 4-C) 60.77 (CH_2, 6-C) 66.37 (CH, 2-C) 68.80 (CH, 5-C) 100.56 (CH, 1-C) 106.15 (CH) 109.21 (CH) 140.79 (CH) 152.84 (C); *m/z* (FAB) 274 (MH^+, 100%), 242 (8), 221 (4), 200 (9), 147 (24); found MH^+, 274.1291. $C_{12}H_{19}NO_6$ requires 274.1290

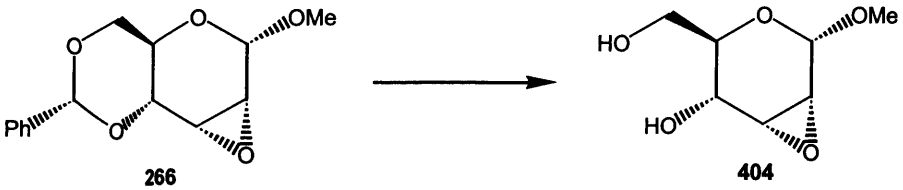
Methyl–3-thiophen-2-ylmethylamino-3-deoxy-α-D-altropyranoside (399w)



The general procedure was followed using stated amounts and molar equivalents. The orange oil was purified by column chromatography on silica using dichloromethane:10% 2 M ammonia in methanol as the eluent to produce **399w** (141 mg, 86%) as an orange oil; $[\alpha]^{20}_{D}$ +140.5° (c 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3392m, 2923w, 1134m, 1041s, 979s, 844s, 710s; δ_H (250 MHz; C_6D_6) 3.43 (3H, s, OMe) 3.49 (1H, app t, *J* 3.5, 3-H) 3.78 (1H,

app d, *J* 10.0, 5-H) 4.09-4.43 (4H, m, 2-H, 6-H, HNCH_2) partial ABx system centred at 4.44 (1H, pseudo *J*, 3.5, 11.8, 6-H) 4.57 (1H, dd, *J* 5.1, 10.0, 4-H) 5.09 (1H, s, 1-H) 7.10 (1H, dd, *J* 3, 5.1, Ar-H) 7.17 (1H, app d *J* 3.0, Ar-H) 7.25 (1H, dd, *J* 1.2, 5.1, Ar-H); δ_C (62.9 MHz; C_6D_6) 47.65 (CH_2) 55.52 (CH_3, OMe) 59.85 (CH, 3-C) 61.79 (CH, 4-C) 62.63 (CH_2, 6-C) 67.89 (CH, 2-C) 70.73 (CH, 5-C) 102.49 (CH, 1-C) 125.33 (CH) 125.98 (CH) 127.37 (CH) 144.78 (CH); *m/z* (FAB) 290 (MH^+, 38%) 242 (4), 216 (5); found MH^+, 290.1062. $C_{12}H_{19}NO_5S$ requires 290.1062

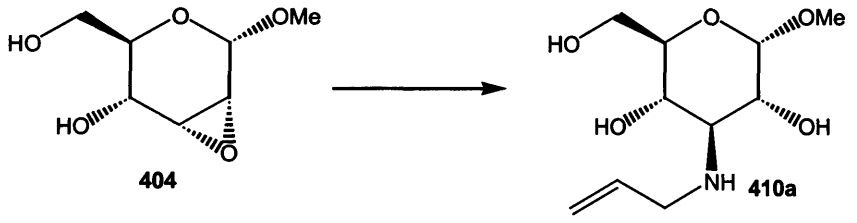
Methyl-2,3-anhydro-α-D-allopyranoside (404)



266 404

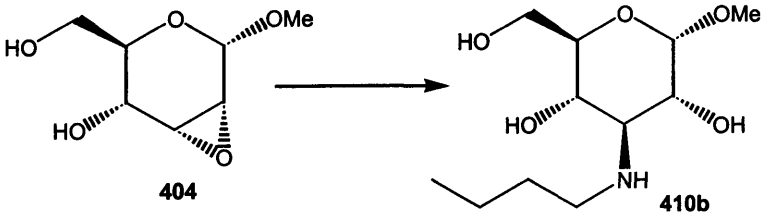
Methyl-2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside **266** (9.19 g, 0.4 mol) was heated under reflux in a mixture of methanol (56 ml) and water (185 ml). Sulphuric acid (0.37 ml, 5M) was added and the solution heated until all **266** had dissolved. Excess barium carbonate was added to the hot solution. After cooling to room temperature the solution was filtered through celite. Removal of the methanol under reduced pressure left an aqueous solution which was washed with toluene (2 x 15 ml). Concentration of the aqueous layer gave a clear oil which then solidified. Recrystallisation from acetone yielded **404** as a white solid (3.98 g, 65%) mp 110-112^{0}C(lit[12]., 112-113^{0}C) δ_H (250 MHz; $CDCl_3$) 3.28 (2H, s, OH) 3.46 (3H, s, OMe) 3.47 (1H, s, 3-H) 3.54 (1H, t, *J* 3.0, 2-H) 3.64 (1H, app ddd, *J* 3.6, 3.8, 9.4, 5-H) 3.71-3.85 (2H, 2 x 6-H) 3.93 (1H, app d, *J* 9.4, 4-H) 4.90 (1H, d, *J* 3.0, 1-H); δ_C (62.9 MHz; $CDCl_3$) 54.10 (CH_3, OMe) 54.96 (CH, 2-C) 55.47 (CH, 3-C) 61.55 (CH_2, 6-C) 65.23 (CH, 4-C) 6.02 (CH, 5-C) 94.58 (CH, 1-C)
This is a literature compound.[12]

Methyl-3-allylamino-3-deoxy-α-D-glucopyranoside (410a)



The general procedure was followed using stated amounts and molar equivalents. The yellow oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (9:1 v/v) as the eluent to give **410a** (30 mg, 22%) as a yellow oil; $[\alpha]^{20}_{D}$ +88.2° (c 0.7 in MeOH); ν_{max}(neat)/cm^{-1} 3313m, 2919w, 1644w, 1040s; δ_H (400 MHz; $CDCl_3$) 2.1-2.41 (4H, br s, 3 x OH, NH) 3.04 (1H, br t, *J* 10.0, 3-H) 3.30-3.33 (2H, m, HNC*H*$_2$) 3.43 (3H, s, OMe) 3.65-3.69 (1H, m, 5-H) 3.75 (1H, br t, *J* 10.0, 4-H) ABx system centred at 3.88 (2H, pseudo *J* 4.6, 11.8, 6-H) 3.94 (1H, app t, *J* 10.0, 2-H) 4.71 (1H, s, 1-H) 5.14 (1H, dq, *J* 1.4, 10.2, CH=C*H*H) 5.22 (1H, dq, *J* 1.4, 17.2, CH=CH*H*) 5.86 (1H, ddt, *J* 1.4, 10.2, 17.2, C*H*=CH$_2$); δ_C (100.62 MHz; $CDCl_3$) 50.95 (CH_2) 55.92 (CH_3, OMe) 58.62 (CH, 3-C) 62.99 (CH_2, 6-C) 64.81 (CH, 4-C) 69.21 (CH, 5-C) 70.46 (CH, 2-C) 101.14 (CH, 1-C) 117.28 (CH_2) 136.41 (CH); *m/z* (FAB) 234 (MH^+, 21%) 202 (17); found MH^+, 234.1341. $C_{10}H_{19}NO_5$ requires 234.1342

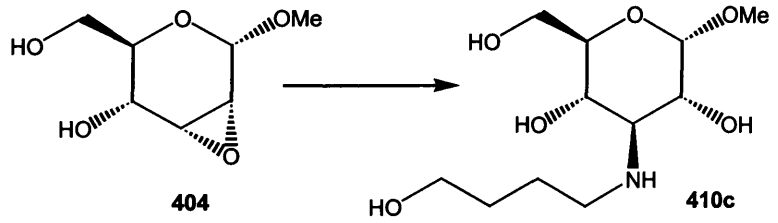
Methyl–3-(butylamino)-3-deoxy-α-D-glucopyranoside (410b)



The general procedure was followed using stated amounts and molar equivalents. The oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (9:1 v/v) as the eluent to give **410b** (29 mg, 21%) as an orange oil; $[\alpha]^{20}_{D}$ +67.2°(c 0.5 in MeOH); ν_{max}(neat)/cm^{-1} 3434w, 3281m, 2862m, 1136m 1047s, 962s, 796m; δ_H (400 MHz; $CDCl_3$) 0.92 (3H, t, *J* 7.3, C*H*$_3$) 1.30-1.39 (2H, app hextet, *J* 7.3, CH_3CH_2) 1.43-1.50 (2H, app quintet, *J* 7.3, $HNCH_2CH_2$) 2.25-2.45 (4H, br s, 3 x OH, NH) 2.62-2.72 (2H, m, HNC*H*$_2$) 3.01-3.02 (1H, br t, *J* 10.0, 3-H) 3.44 (3H, s, OMe) 3.65-3.69 (1H, m, 5-H) 3.72-3.75 (1H, dd, *J* 3.3, 10.0, 2-H) ABx system centred at 3.88 (2H,

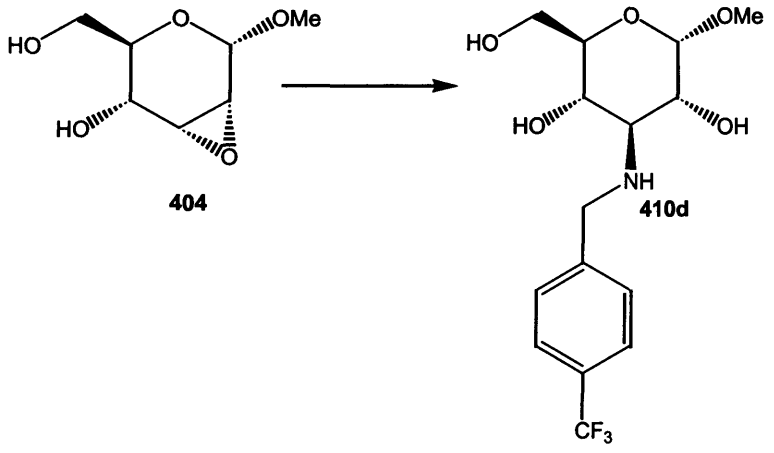
pseudo *J* 4.6, 11.8, 6-H) 3.94-3.96 (1H, m, 4-H) 4.71 (1H, s, 1-H); δ_C (100.62 MHz; $CDCl_3$) 14.31 (CH_3) 20.69 (CH_2) 32.65 (CH_2) 48.27 (CH_2) 55.88 (CH_3, OMe) 59.62 (CH, 3-C) 63.11 (CH_2, 6-C) 64.94 (CH, 4-C) 69.28 (CH, 5-C) 70.37 (CH, 2-C) 101.19 (CH, 1-C); *m/z* (FAB) 250 (MH^+, 13%) 218 (5); found MH^+, 250.1654. $C_{11}H_{23}NO_5$ requires 250.1655

Methyl–3(-4-hydroxy-butylamino)-3-deoxy-α-D-glucopyranoside (410c)



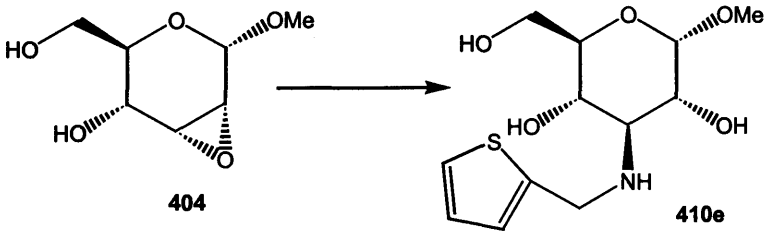
The general procedure was followed using stated amounts and molar equivalents. The orange oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (7:3 v/v) as the eluent to give **410c** (99 mg, 66%) as a yellow/orange oil; $[\alpha]^{20}_D$ +83.9° (c 1.0 in MeOH); ν_{max}(neat)/cm^{-1} 3318m, 2930m, 2480m, 1452w, 1194w, 1031s, 700w; δ_H (250 MHz; D_2O) 1.47-1.49 (4H, m, $HNCH_2CH_2CH_2$, $HNCH_2CH_2CH_2$) 2.69-2.80 (3H, overlapping t, *J* 6.7, 10.0, $HNCH_2$, 3-H) 3.29-3.37 (4H, overlapping s, t, *J* 10.0, 4-H, OMe) 3.47-3.52 (3H, m, $HOCH_2$, 2-H) 3.56 (1H, app ddd, *J* 2.0, 5.3, 10.0, 5-H) 3.56 (1H, dd, *J* 5.3, 12.0, 6-H) 3.77 (1H, dd, *J* 2.0 12.0, 6-H) 4.66 (1H, s, 1-H); δ_C (62.9 MHz; D_2O) 25.91 (CH_2) 29.49 (CH_2) 47.87 (CH_2) 55.46 (CH_3, OMe) 60.81 (CH, 3-C) 61.06 (CH_2, 6-C) 61.93 (CH_2) 68.64 (CH, 4-C) 70.47 (CH, 2-C) 72.33(CH, 5-C) 99.45 (CH, 1-C); *m/z* (FAB) 266 (MH^+, 100%) 234(8) 206 (7); found MH^+, 266.1603. $C_{11}H_{23}NO_6$ requires 266.1604

Methyl–3-(4-trifluoromethyl-benzylamino)-3-deoxy-α-D-glucopyranoside (410d)



The general procedure was followed using stated amounts and molar equivalents. The oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (4:1 v/v)as the eluent to give **410d** (76 mg, 38%) as a brown oil; $[\alpha]^{20}_{D}$ +99.7° (c 1.1 in MeOH); ν_{max}(neat)/cm^{-1} 3205w, 2921w, 1324m, 1108s, 1020s, 960m; δ_H (250 MHz; MeOH) 1.74 (1H, t, *J* 10.0, 3-H) 3.95 (1 H, app d, *J* 10.0, 4-H) 3.99 (3H, s, OMe) 4.08 (1H, dd, *J* 3.6, 10.0, 2-H) 4.11-4.17 (1H, app ddd, *J* 2.0, 5.0, 10.0, 5-H) 4.25(1H, dd, *J* 5.0, 11.7, 6-H) 4.39 (1H, dd, *J* 2.0, 11.7, 6-H) 4.69 (2H, s, HNC*H*$_2$) 5.22 (1H, d, *J* 3.6, 1-H) 8.12-8.21 (4H, m, Ph); δ_C (62.9 MHz; MeOH) 52.41 (CH_2) 54.47 (CH_3, OMe) 61.24 (CH, 3-C) 61.63 (CH_2, 6-C) 69.84 (CH, 4-C) 71.89 (CH, 2-C) 72.77 (CH, 5-C) 99.81 (CH, 1-C) 124.73 (C, Ph) 125.28 (CH, Ph) 128.96 (CH, Ph) 129.43 (C, Ph) 145.10 (C, Ph); *m/z* (FAB) 352 (MH^+, 92%) 320 (15) 289 (13) 242 (6) 217 (5) 174 (9); found MH^+, 352.1372. $C_{15}H_{20}NO_5F_3$ requires 352.1372

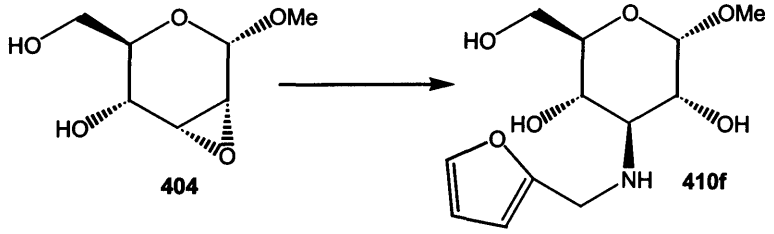
Methyl–3-(thiophen-2-ylmethyl-amino)-3-deoxy-α-D-glucopyranoside (410e)



The general procedure was followed using stated amounts and molar equivalents. The orange oil was purified by column chromatography on silica using dichloromethane:2 M ammonia (9:1 v/v) in methanol as the eluent to give **410e** (107 mg, 65%) as a yellow oil; $[\alpha]^{20}_{D}$ +98.7° (c 1.3 in MeOH); ν_{max}(neat)/cm^{-1} 3398w, 289w, 2582w, 1009s, 963m, 696s;

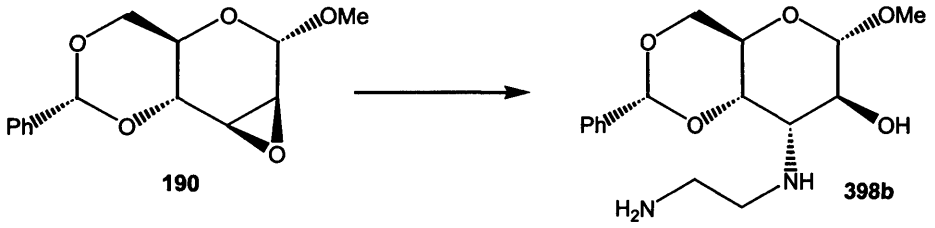
δ_H (250 MHz; D_2O) 2.91 (1H, t, *J* 9.9, 3-H) 3.34-3.44 (4H, m, OMe, 4-H) 3.54-3.63 (2H, m, 2-H, 5-H) 3.70 (1H, dd, *J* 5.3, 12.0, 6-H) 3.83 (1H, dd, *J* 2.2, 12.0, 6-H) 4.17 (2 H, s, HNC*H*$_2$) 4.71 (1H, s, 1-H) 7.01-7.04 (2H, m, SC=C*H*, SC=CHC*H*=CH) 7.36 (1H, dd, *J* 1.4, 5.0, SC=CHCH=C*H*); δ_C (62.9 MHz; D_2O) 46.69 (CH_2) 55.47 (CH_3, OMe) 59.71 (CH, 3-C) 61.06 (CH_2, 6-C) 69.15 (CH, 4-C) 71.02 (CH, 5-C) 72.24 (CH, 2-C) 99.41 (CH, 1-C) 125.79 (CH) 126.93 (CH) 127.60 (CH) 142.66 (C); *m/z* (FAB) 290 (MH^+, 22%) 242(4); found MH^+, 290.1062. $C_{12}H_{19}NO_5S$ requires 290.1062

Methyl–3-(furan-2-ylmethylamino)-3-deoxy-α-D-glucopyranoside (410f)



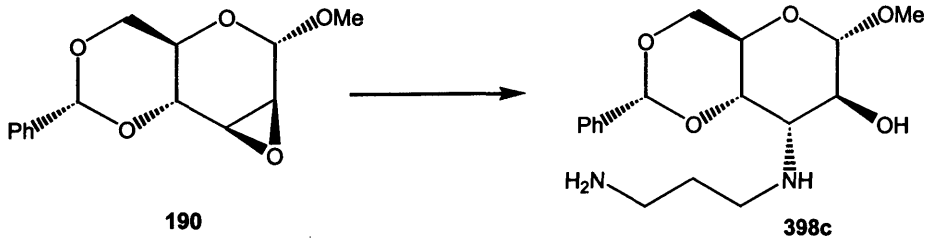
The general procedure was followed using stated amounts and molar equivalents. The orange oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (4:1 v/v) as the eluent to give **410f** (107 mg, 69%) as an orange solid; mp. 63-65^{0}C; $[\alpha]^{20}_D$ +80.4° (c 0.8 in MeOH); ν_{max}(neat)/cm^{-1} 3335m, 3207m, 2928m, 1115s, 1040s, 991s, 741s; δ_H (250 MHz; D_2O) 2.98 (1H, t, *J* 10.0, 3-H) 3.45-3.54 (4H, overlapping t, s, *J*,10.0, 4-H, OMe) 3.65 (1H, dt, *J* 3.6, 10.0, 2-H) 3.69-3.76 (1H, app ddd, *J* 2.3, 5.4, 10.0, 5-H) 3.83 (1H, dd, *J* 5.4, 12.0, 6-H) 3.95 (1H, dd, *J* 2.3, 12.0, 6-H) 4.09 (2H, s, 2 x 7-H) 4.84 (1H, s, 1-H) 6.44 (1H, d, *J* 3.2, Ar-H) 6.54 (1H, app dd, *J* 2.0, 3.2, Ar-H) 7.59 (1H, app d, *J* 2.0, Ar-H) ; δ_C (62.9 MHz; D_2O) 44.69 (CH_2) 55.46 (CH_3, OMe) 59.65 (CH, 3-C) 61.00 (CH_2, 6-C) 69.10 (CH, 4-C) 70.99 (CH, 2-C) 72.22 (CH, 5-C) 99.39 (CH, 1-C) 108.22 (CH) 110.82 (CH) 142.99 (CH) 153.26 (C); *m/z* (FAB) 274 (MH^+, 100%) 242(8) 200 (6); found MH^+, 274.1291. $C_{12}H_{19}NO_6$ requires 274.1291

Methyl-3-(2amino-ethylamino)-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (398b)



Methyl-2,3-anhydro-4,6-*O*- benzylidene-α–D-mannopyranoside **190** (824 mg, 3.12 mmol) and ethylenediamine (625 μl, 9.36 mmol) was placed in a Youngs tube and stirred for 19 h at 100°C. The solution was cooled down and transferred to a round bottom flask and concentrated under reduced pressure to leave a orange oil. The orange oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (7:3 v/v) as the eluent to give **398b** (278 mg, 28%); $[\alpha]^{20}_{D}$ +106.4° (c 1.5 in MeOH); ν_{max}(neat)/cm^{-1} 3375w, 2914m, 1660m, 1038s, 749s, 699s; δ_H (250 MHz; $CDCl_3$) 2.63-2.80 (3H, m, H_2NCH_2 HNC*H*H) 2.89-2.99 (1H, m, HNCH*H*) 3.14 (1H, s, 3-H) 3.36 (3H, s, OMe) 3.77 (1H, t, *J* 10.0, 6_{ax}-H) 3.86 (4H, br s, NH_2, NH, OH) 3.94 (1H, s, 2-H) 4.02-4.14 (2H, m, 4-H, 5-H) 4.25-4.30 (1H, app dd, *J* 4.5, 10.0, 6_{eq}-H) 4.60 (1H, s, 1-H) 5.54 (1H, s, 7-H) 7.32-7.37 (3H, m Ph) 7.42-7.46 (2H, m, PH); δ_C (62.9 MHz; $CDCl_3$) 41.41 (CH_2) 48.73 (CH_2) 55.81 (CH_3, OMe) 59.06 (CH, 3-C) 59.18 (CH, 5-C) 69.31 (CH, 2-C) 69.82 (CH_2, 6-C) 78.04 (CH, 4-C) 102.37 (CH, 7-C) 102.94 (CH, 1-C) 126.52 (CH, Ph) 128.69 (CH, Ph) 129.44 (CH, Ph) 138.03 (C, Ph); *m/z* (FAB) 325 (MH^+, 100%); found MH^+,325.1764. $C_{16}H_{24}N_2O_5$ requires 325.1764

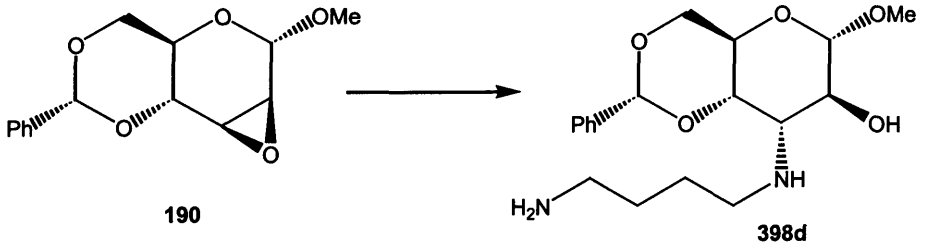
Methyl-3-(3-amino-propylamino)-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (398c)



Methyl-2,3-anhydro-4,6-*O*- benzylidene-α–D-mannopyranoside **190** (788 mg, 2.98 mmol) and 1,3 diaminopropane (995 μl, 11.92 mmol) was placed in a Youngs tube and stirred for

18.5 h at 100°C. The solution was cooled, diluted with dichloromethane and transferred to a round bottom flask and concentrated under reduced pressure to leave a orange oil. The orange oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (7:3 v/v) as the eluent to give **398c** (490 mg, 49%); $[\alpha]^{20}_{D}$ +101.8° (c 1.4 in MeOH); ν_{max}(neat)/cm^{-1} 3310w, 2911m, 1627w, 1380m, 1129, 1099s, 1038s, 753s; δ_H (250 MHz; $CDCl_3$) 1.55-1.65 (2H, app quintet, *J* 6.3, $HNCH_2CH_2$) 2.72-2.89 (4H, m, $HNCH_2$, H_2NCH_2) 3.13 (1H, br s, 3-H) 3.15-3.27 (4H, br s, NH_2, NH, OH) 3.35 (3H, s, OMe) 3.71-3.81 (1H, m, 6_{ax}-H) 3.93 (1H, app d, *J* 1.6, 2-H) 4.04-4.15 (2H, m, 4-H, 5-H) 4.26-4.30 (1H, m, 6_{eq}-H) 4.59 (1H, s, 1-H) 5.56 (1H, s, 7-H) 7.32-7.37 (3H, m, Ph) 7.43-7.47 (2 H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 32.62 (CH_2) 40.66 (CH_2) 46.85 (CH_2) 55.74 (CH_3, OMe) 59.23 (CH, 3-C) 59.34 (CH, 4-C) 69.23 (CH, 2-C) 69.86 (CH_2, 6-C) 78.18 (CH, 5-C) 102.46 (CH, 7-C) 102.98 (CH, 1-C) 126.58 (CH, Ph) 128.62 (CH, Ph) 129.38 (CH, Ph) 138.15 (C, Ph); *m/z* (FAB) 339 (MH^+, 100%) 308 (3) 289 (2); found MH^+, 339.1919. $C_{17}H_{26}N_2O_5$ requires 339.1920

Methyl-3-(4-amino-butylamino)-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (398d)



Methyl-2,3-anhydro-4,6-*O*-benzylidene-α–D-mannopyranoside **190** (1.229 g, 4.66 mol) and 1,4 diaminobutane (1.64 g, 18.64 mmol) was placed in a Youngs tube and stirred for 24 h at 100°C. The solution was cooled, diluted with dichloromethane and transferred to a round bottom flask and concentrated under reduced pressure to leave a yellow oil. The oil was purified by column chromatography on silica using dichloromethane:2 M ammonia in methanol (7:3 v/v) as the eluent to give **398d** (925 mg, 57%); $[\alpha]^{20}_{D}$ +98.4° (c 0.9 in MeOH); ν_{max}(neat)/cm^{-1} 3355w, 2927m, 2859m, 1379m, 1099s, 1040s, 967s, 699s; δ_H (250 MHz; $CDCl_3$) 1.59-1.76 (4H, app, s, $HNCH_2CH_2$, $H_2NCH_2CH_2$) 2.79-2.89 (2H, m, $HNCH_2$) 2.90-3.10 (2H, m, H_2NCH_2) 3.33 (1H, br s, 3-H) 3.44 (4H, br s, NH_2, NH, OH) 3.56 (3H, s, OMe) 3.97 (1H, t, *J* 9.6, 6_{ax}-H) 4.12 (1H, app d, *J* 1.6, 2-H) 4.22-4.35 (2H, m, 4-H, 5-H) 4.49 (1H, app dd, *J* 4.6, 9.6, 6_{eq}-H) 4.76 (1H, s, 1-H) 5.76 (1H, s, 7-H) 7.52-

7.58 (3H, m, Ph) 7.64-7.67 (2H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 27.82 (CH_2) 31.04 (CH_2) 41.92 (CH_2) 48.83 (CH_2) 55.79 (CH_3, OMe) 59.09 (CH, 3-C) 59.15 (CH, 4-C) 69.55 (CH, 2-C) 69.76 (CH_2, 6-C) 78.23 (CH, 5-C) 102.45 (CH, 7-C) 102.77 (CH, 1-C) 126.54 (CH, Ph) 128.57 (CH, Ph) 129.34 (CH, Ph) 138.11 (C, Ph); *m/z* (FAB) 353 (MH^+, 100%); found MH^+, 353.2076, $C_{18}H_{28}N_2O_5$ requires 353.2077

4.4 References

1. La Chapelle, M., St-Jacques, M., *Tetrahedron*, **1988**, *44*, 5033
2. Evans, M.E., *Carbohydr.Res.*, **1972**, *21*, 473
3. Hicks, D.R., Fraser-Reid, B., *Synthesis*, **1974**, 203
4. Pougny, J.R., Sinay, P., *J.Chem.Res.*, (M), **1982**, 186
5. Richtmeyer, N.K., Hudson, C.S., *J.Am.Chem.Soc.*, **1941**, *63*, 1727; Hicks, D.R.; Fraser-Reid, B., *Can.J.Chem.*, **1975**, *53*, 2017
6. Vega-Perez, M.; Candela, I.; Vega, M.; Iglesias-Guerra, F. *Carbohydr. Res.* **1995**, *279*, C5
7. Myer, W.H., Robertson, G.J., *J.Am.Chem.Soc.*, **1945**, 65
8. Baker, B.R., Hullar, T.L., *J.Org.Chem.*, **1965**, 4049
9. Guthrie, R.D., Murphy, D., *J.Chem.Soc.*, **1963**, 5288
10. Radatus, B.K., Clarke, I.S., *Synthesis.*, **1980**, 47
11. Johansson, R., Samuelsson, B., *J.Chem.Soc.Perkin Transaction I.*, **1984**, 2371
12. Jones, K., McEvoy,J., Wood, W.W., Scott, I.L., *J.Chem.Research(M).*, **1987**, 1773
13. Roberston, G.T., Myers, W.H., Tetlow, W.E., *Nature*, **1938**, *142*, 1076

Appendices

APPENDIX 1
X-Ray Crystallographic Data

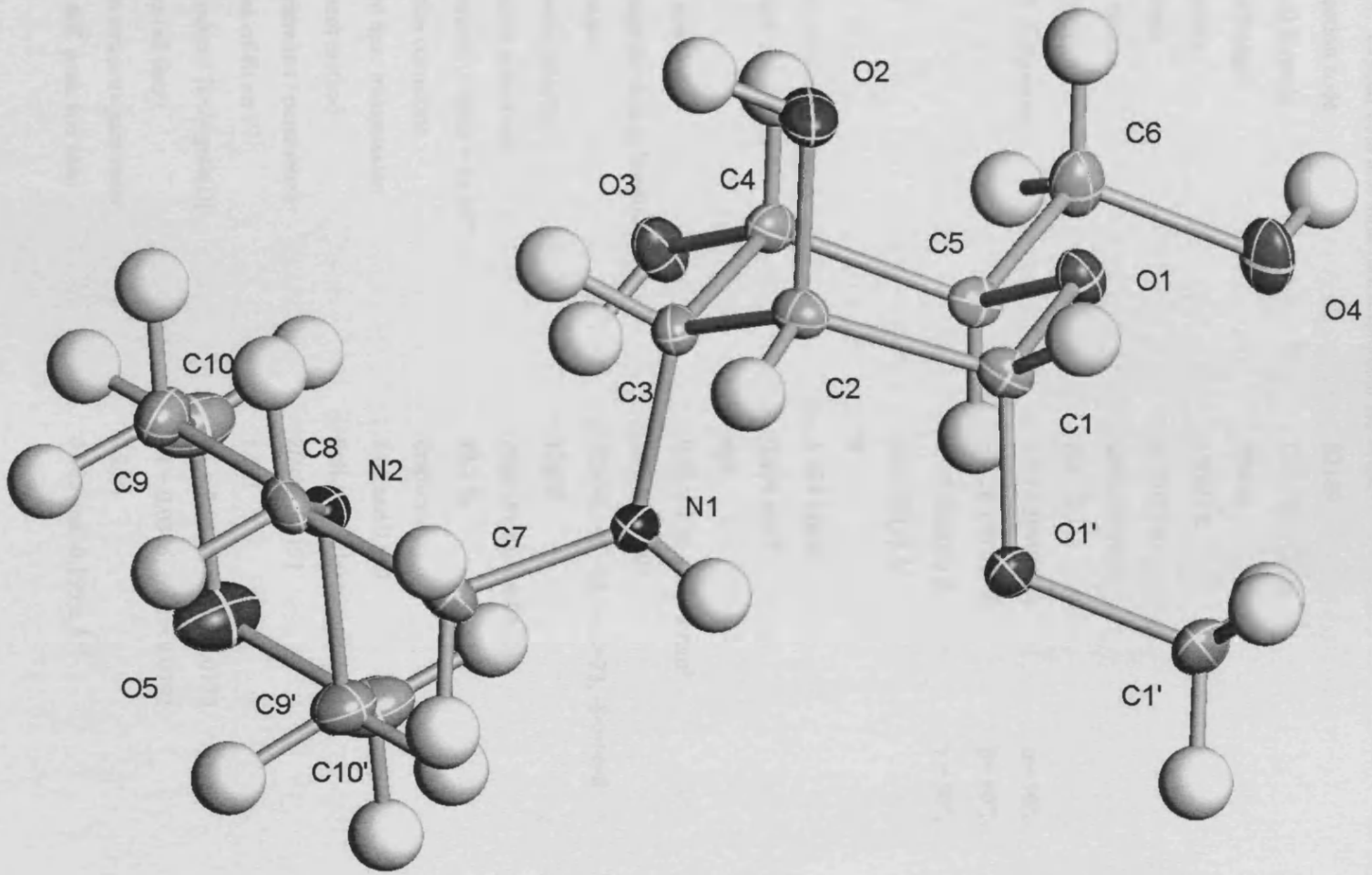

O2
C6
O3
C4
C5
O1
O4
C10
C3
C2
C1
C8
N2
C9
N1
C7
O1'
O5
C9'
C1'
C10'

Table 1. Crystal data and structure refinement for 2189.

Identification code	02189	
Empirical formula	C13 H26 N2 O6	
Formula weight	306.36	
Temperature	120(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2	
Unit cell dimensions	a = 10.2890(1) Å	α= 90°.
	b = 20.1092(2) Å	β= 90°.
	c = 7.4282(4) Å	γ = 90°.
Volume	1536.92(9) $Å^3$	
Z	4	
Density (calculated)	1.324 Mg/m^3	
Absorption coefficient	0.104 mm^{-1}	
F(000)	664	
Crystal size	0.30 x 0.15 x 0.075 mm^3	
Theta range for data collection	2.92 to 24.99°.	
Index ranges	-12<=h<=9, -23<=k<=23, -8<=l<=8	
Reflections collected	12656	
Independent reflections	2690 [R(int) = 0.0470]	
Completeness to theta = 24.99°	99.7 %	
Absorption correction	Empirical	
Max. and min. transmission	1.00 and 0.917	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2690 / 0 / 191	
Goodness-of-fit on F^2	1.041	
Final R indices [I>2sigma(I)]	R1 = 0.0324, wR2 = 0.0775	
R indices (all data)	R1 = 0.0346, wR2 = 0.0792	
Absolute structure parameter	0.2(9)	
Largest diff. peak and hole	0.185 and -0.199 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 2189. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	7215(1)	7202(1)	4179(2)	14(1)
O(2)	5276(1)	7669(1)	6671(2)	20(1)
O(3)	4281(1)	6813(1)	1520(2)	20(1)
O(4)	8297(1)	7226(1)	752(2)	22(1)
O(5)	1754(1)	5064(1)	-986(2)	30(1)
O(1')	7223(1)	6227(1)	5921(2)	17(1)
N(1)	4610(1)	6009(1)	4556(2)	15(1)
N(2)	2436(1)	5698(1)	2344(2)	16(1)
C(1)	6905(2)	6912(1)	5865(2)	15(1)
C(1')	8595(2)	6110(1)	5864(3)	26(1)
C(2)	5472(2)	6980(1)	6317(2)	15(1)
C(3)	4606(2)	6737(1)	4769(2)	15(1)
C(4)	5037(2)	7056(1)	2995(2)	15(1)
C(5)	6487(2)	6948(1)	2666(2)	14(1)
C(6)	6945(2)	7339(1)	1048(2)	20(1)
C(7)	3487(2)	5679(1)	5368(2)	18(1)
C(8)	2253(2)	5801(1)	4285(2)	18(1)
C(9)	1205(2)	5843(1)	1413(2)	21(1)
C(10)	1347(2)	5726(1)	-586(3)	28(1)
C(9')	2828(2)	5013(1)	1926(2)	21(1)
C(10')	2953(2)	4924(1)	-90(2)	26(1)

Table 3. Bond lengths [Å] and angles [°] for 2189.

O(1)-C(1)	1.419(2)
O(1)-C(5)	1.4446(19)
O(2)-C(2)	1.4234(19)
O(3)-C(4)	1.4302(19)
O(4)-C(6)	1.426(2)
O(5)-C(10)	1.426(2)
O(5)-C(10')	1.430(2)
O(1')-C(1)	1.4156(18)
O(1')-C(1')	1.433(2)
N(1)-C(7)	1.462(2)
N(1)-C(3)	1.4736(19)
N(2)-C(8)	1.469(2)
N(2)-C(9')	1.469(2)
N(2)-C(9)	1.472(2)
C(1)-C(2)	1.519(2)
C(2)-C(3)	1.535(2)
C(3)-C(4)	1.531(2)
C(4)-C(5)	1.527(2)
C(5)-C(6)	1.511(2)
C(7)-C(8)	1.523(2)
C(9)-C(10)	1.511(3)
C(9')-C(10')	1.513(2)
C(1)-O(1)-C(5)	115.12(11)
C(10)-O(5)-C(10')	109.91(14)
C(1)-O(1')-C(1')	112.71(12)
C(7)-N(1)-C(3)	113.81(13)
C(8)-N(2)-C(9')	112.06(13)
C(8)-N(2)-C(9)	108.80(13)
C(9')-N(2)-C(9)	108.86(13)
O(1')-C(1)-O(1)	112.03(13)
O(1')-C(1)-C(2)	107.81(12)
O(1)-C(1)-C(2)	112.09(13)
O(2)-C(2)-C(1)	105.49(13)
O(2)-C(2)-C(3)	111.45(13)
C(1)-C(2)-C(3)	111.66(13)

N(1)-C(3)-C(4)	108.85(13)
N(1)-C(3)-C(2)	113.30(13)
C(4)-C(3)-C(2)	110.08(13)
O(3)-C(4)-C(5)	111.12(13)
O(3)-C(4)-C(3)	111.02(13)
C(5)-C(4)-C(3)	111.17(13)
O(1)-C(5)-C(6)	105.84(12)
O(1)-C(5)-C(4)	109.35(12)
C(6)-C(5)-C(4)	110.96(13)
O(4)-C(6)-C(5)	110.15(13)
N(1)-C(7)-C(8)	111.59(13)
N(2)-C(8)-C(7)	112.87(14)
N(2)-C(9)-C(10)	110.35(15)
O(5)-C(10)-C(9)	112.30(15)
N(2)-C(9')-C(10')	110.07(14)
O(5)-C(10')-C(9')	111.36(15)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 2189. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	14(1)	16(1)	13(1)	2(1)	-2(1)	-3(1)
O(2)	19(1)	15(1)	27(1)	-6(1)	3(1)	-1(1)
O(3)	18(1)	22(1)	19(1)	5(1)	-8(1)	-5(1)
O(4)	18(1)	31(1)	17(1)	7(1)	4(1)	-3(1)
O(5)	37(1)	26(1)	27(1)	-9(1)	-11(1)	6(1)
O(1')	14(1)	16(1)	20(1)	3(1)	-3(1)	1(1)
N(1)	13(1)	14(1)	19(1)	1(1)	-2(1)	1(1)
N(2)	15(1)	14(1)	18(1)	-1(1)	-2(1)	0(1)
C(1)	19(1)	14(1)	11(1)	1(1)	-2(1)	-1(1)
C(1')	16(1)	21(1)	41(1)	8(1)	-5(1)	3(1)
C(2)	18(1)	13(1)	15(1)	-1(1)	1(1)	0(1)
C(3)	12(1)	14(1)	19(1)	-1(1)	0(1)	0(1)
C(4)	13(1)	14(1)	17(1)	1(1)	-5(1)	0(1)
C(5)	15(1)	14(1)	12(1)	0(1)	-5(1)	-1(1)
C(6)	16(1)	25(1)	18(1)	5(1)	-2(1)	-1(1)
C(7)	21(1)	14(1)	18(1)	2(1)	1(1)	-4(1)
C(8)	16(1)	16(1)	20(1)	0(1)	5(1)	-4(1)
C(9)	15(1)	20(1)	27(1)	-3(1)	-3(1)	2(1)
C(10)	32(1)	25(1)	27(1)	-4(1)	-11(1)	8(1)
C(9')	22(1)	18(1)	23(1)	-2(1)	0(1)	4(1)
C(10')	32(1)	24(1)	23(1)	-3(1)	-2(1)	9(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for 2189.

	x	y	z	U(eq)
H(2)	4503	7737	6870	31
H(3)	3916	6468	1815	30
H(4)	8537	7440	-128	33
H(1A)	5249	5859	4936	18
H(1)	7408	7140	6799	18
H(1'1)	9008	6345	6833	39
H(1'2)	8937	6265	4736	39
H(1'3)	8761	5642	5983	39
H(2A)	5278	6723	7405	18
H(3A)	3712	6879	5016	18
H(4A)	4888	7536	3089	18
H(5)	6669	6473	2497	16
H(6A)	6457	7203	-7	24
H(6B)	6792	7809	1246	24
H(7A)	3367	5844	6584	21
H(7B)	3651	5205	5438	21
H(8A)	1578	5503	4712	21
H(8B)	1961	6253	4489	21
H(9A)	961	6302	1627	25
H(9B)	523	5560	1888	25
H(10C)	520	5810	-1170	34
H(10D)	1976	6037	-1070	34
H(9'1)	2186	4705	2392	25
H(9'2)	3654	4916	2499	25
H(10A)	3624	5219	-543	32
H(10B)	3214	4471	-351	32

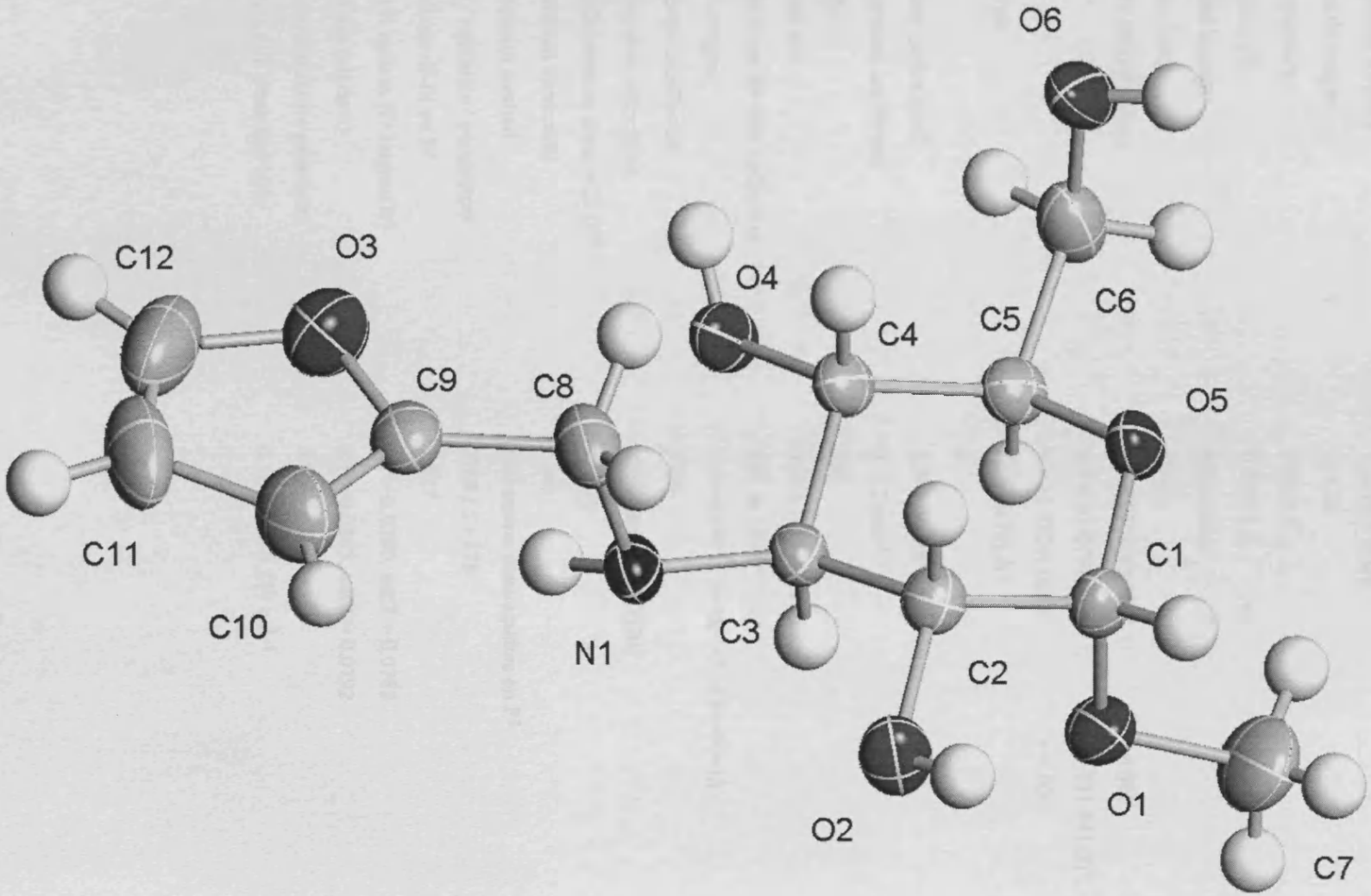

O6
C12
O3
O4
C4
C5
C6
C9
C8
O5
C11
C1
C10
N1
C3
C2
O2
O1
C7

Table 1. Crystal data and structure refinement for 03045.

Identification code	03045	
Empirical formula	C12 H19 N O6	
Formula weight	273.28	
Temperature	290(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 9.8736(13) Å	α= 90°.
	b = 6.0745(8) Å	β= 111.941(2)°.
	c = 11.7121(16) Å	γ = 90°.
Volume	651.58(15) $Å^3$	
Z	2	
Density (calculated)	1.393 Mg/m^3	
Absorption coefficient	0.112 mm^{-1}	
F(000)	292	
Crystal size	0.33 x 0.32 x 0.15 mm^3	
Theta range for data collection	1.87 to 25.00°.	
Index ranges	-11<=h<=11, -7<=k<=7, -13<=l<=13	
Reflections collected	4786	
Independent reflections	2268 [R(int) = 0.0320]	
Completeness to theta = 25.00°	99.8 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2268 / 1 / 176	
Goodness-of-fit on F^2	1.027	
Final R indices [I>2sigma(I)]	R1 = 0.0350, wR2 = 0.0783	
R indices (all data)	R1 = 0.0365, wR2 = 0.0792	
Absolute structure parameter	0.9(9)	
Largest diff. peak and hole	0.164 and -0.227 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 03045. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	3236(1)	10562(2)	3048(1)	30(1)
O(1)	1862(1)	6363(2)	5196(1)	37(1)
O(2)	4470(1)	7923(2)	5105(1)	41(1)
O(3)	3298(2)	12893(2)	698(1)	57(1)
O(4)	294(1)	9305(2)	1464(1)	41(1)
O(5)	1387(1)	4404(2)	3388(1)	31(1)
O(6)	8(2)	3576(2)	868(1)	52(1)
C(1)	2472(2)	5333(3)	4441(1)	31(1)
C(2)	3387(2)	6976(3)	4079(1)	30(1)
C(3)	2409(2)	8787(3)	3309(1)	28(1)
C(4)	1276(2)	7688(3)	2197(1)	29(1)
C(5)	420(2)	5990(3)	2601(1)	31(1)
C(6)	-665(2)	4732(3)	1558(2)	42(1)
C(7)	1225(3)	4886(4)	5774(2)	61(1)
C(8)	3992(2)	10055(3)	2218(2)	43(1)
C(9)	4360(2)	12083(3)	1713(2)	38(1)
C(10)	5513(2)	13396(4)	2039(2)	55(1)
C(11)	5176(3)	15117(4)	1173(2)	65(1)
C(12)	3854(4)	14767(4)	402(2)	70(1)

Table 3. Bond lengths [Å] and angles [°] for 03045.

N(1)-C(3)	1.4527(19)
N(1)-C(8)	1.462(2)
O(1)-C(1)	1.3906(18)
O(1)-C(7)	1.406(2)
O(2)-C(2)	1.4002(19)
O(3)-C(9)	1.351(2)
O(3)-C(12)	1.363(3)
O(4)-C(4)	1.4208(18)
O(5)-C(1)	1.4138(18)
O(5)-C(5)	1.4257(18)
O(6)-C(6)	1.411(2)
C(1)-C(2)	1.509(2)
C(2)-C(3)	1.518(2)
C(3)-C(4)	1.518(2)
C(4)-C(5)	1.517(2)
C(5)-C(6)	1.499(2)
C(8)-C(9)	1.469(2)
C(9)-C(10)	1.325(3)
C(10)-C(11)	1.407(3)
C(11)-C(12)	1.298(4)
C(3)-N(1)-C(8)	116.74(13)
C(1)-O(1)-C(7)	113.36(14)
C(9)-O(3)-C(12)	105.89(18)
C(1)-O(5)-C(5)	113.59(11)
O(1)-C(1)-O(5)	111.54(12)
O(1)-C(1)-C(2)	109.14(13)
O(5)-C(1)-C(2)	110.89(11)
O(2)-C(2)-C(1)	112.07(12)
O(2)-C(2)-C(3)	108.96(13)
C(1)-C(2)-C(3)	109.38(12)
N(1)-C(3)-C(4)	115.98(12)
N(1)-C(3)-C(2)	112.36(12)
C(4)-C(3)-C(2)	107.01(13)
O(4)-C(4)-C(5)	108.96(12)
O(4)-C(4)-C(3)	109.40(12)

C(5)-C(4)-C(3)	110.45(12)
O(5)-C(5)-C(6)	106.52(13)
O(5)-C(5)-C(4)	110.17(12)
C(6)-C(5)-C(4)	113.86(13)
O(6)-C(6)-C(5)	112.01(14)
N(1)-C(8)-C(9)	110.78(14)
C(10)-C(9)-O(3)	109.57(17)
C(10)-C(9)-C(8)	134.79(19)
O(3)-C(9)-C(8)	115.61(16)
C(9)-C(10)-C(11)	107.1(2)
C(12)-C(11)-C(10)	106.5(2)
C(11)-C(12)-O(3)	110.9(2)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 03045. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
N(1)	36(1)	23(1)	34(1)	1(1)	16(1)	1(1)
O(1)	43(1)	39(1)	34(1)	-2(1)	20(1)	-6(1)
O(2)	33(1)	38(1)	40(1)	5(1)	1(1)	-3(1)
O(3)	64(1)	55(1)	47(1)	6(1)	14(1)	2(1)
O(4)	52(1)	26(1)	31(1)	3(1)	1(1)	6(1)
O(5)	35(1)	22(1)	32(1)	2(1)	8(1)	1(1)
O(6)	89(1)	26(1)	32(1)	-3(1)	11(1)	2(1)
C(1)	32(1)	29(1)	29(1)	5(1)	8(1)	3(1)
C(2)	28(1)	30(1)	31(1)	2(1)	10(1)	2(1)
C(3)	34(1)	23(1)	30(1)	-1(1)	14(1)	1(1)
C(4)	36(1)	22(1)	29(1)	1(1)	9(1)	5(1)
C(5)	32(1)	26(1)	32(1)	0(1)	10(1)	5(1)
C(6)	41(1)	34(1)	41(1)	3(1)	5(1)	-5(1)
C(7)	72(1)	65(1)	59(1)	5(1)	41(1)	-15(1)
C(8)	55(1)	31(1)	54(1)	-2(1)	33(1)	-3(1)
C(9)	46(1)	37(1)	38(1)	-1(1)	23(1)	-2(1)
C(10)	52(1)	55(1)	63(1)	1(1)	26(1)	-10(1)
C(11)	92(2)	41(1)	89(2)	4(1)	64(2)	-10(1)
C(12)	114(2)	48(1)	60(1)	17(1)	46(2)	16(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for 03045.

	x	y	z	U(eq)
H(1A)	2608	11805	2708	36
H(2)	5051	6970	5488	61
H(4)	117	9055	735	61
H(6)	236	2340	1158	79
H(1)	3112	4148	4911	37
H(2A)	3855	6223	3583	36
H(3)	1887	9417	3797	34
H(4A)	1765	6965	1709	35
H(5)	-99	6738	3058	37
H(6A)	-1177	3696	1884	50
H(6B)	-1379	5746	1022	50
H(7A)	394	4194	5164	91
H(7C)	920	5672	6348	91
H(7B)	1927	3783	6204	91
H(8A)	3373	9136	1549	51
H(8B)	4879	9241	2662	51
H(10)	6382	13215	2712	66
H(11)	5782	16279	1156	78
H(12)	3353	15672	-262	84

APPENDIX 2
Inhibition Method/Protocol

Inhibition of glycosidase enzymes

A summary of the enzymes used is given below below:

Enzyme	Source	pH	Conc. (U ml^{-1})	Substrate (5 m*M*)
α-D-glucosidase EC 3.2.1.20	*Saccharomyces cerevisiae*	6.0	1.5	PNP-α-D-glucopyranoside
β-D-glucosidase EC 3.2.1.21	Almond (*Prunus* sp.)	5.0	0.2	PNP-β-D-glucopyranoside
α-D-mannosidase EC 3.2.1.24	Jack bean (*Canavalia ensiformis*)	4.5	0.2	PNP-α-D-mannopyranoside
Naringinase[1] EC 3.2.1.40	*Penicillium decumbens*	4.0	1.0	PNP-α-D-rhamnopyranoside
N-acetyl-β-D-glucosaminidase EC 3.2.1.52	Bovine kidney	4.25	0.2	PNP-N-acetyl-β-D-glucosaminide

[1] Naringinase is a preparation of α-rhamnosidase (EC 3.2.1.40) and approx. 10% β-glucosidase (EC 3.2.1.21).

All enzymes and substrates were bought from Sigma. Enzyme and substrate solutions were made using 0.2 *M* sodium phosphate buffers at the pH values recommended by the manufacturer (pH values given above).

Reagents

Enzyme inhibitor solutions

Compounds that were tested for enzyme inhibition were dissolved in distilled water at a concentration of 1 mg ml^{-1}. Where required, some compounds were dissolved in c. 20 μl of methanol before being diluted to 1 mg ml^{-1} using distlled water. Inhibitor solutions were frozen at –20°C when not in use.

0.2 *M* sodium phosphate buffers

Sodium phosphate buffers were made by mixing 0.2 *M* Na_2HPO_4 and 0.2 *M* NaH_2PO_4 solutions in various proportions to achieve the desired pH. Both component solutions were made using distilled water. Buffers were made as required, and stored at room temperature whilst in use. Stock solutions were replaced on a monthly basis, and were kept at 4°C to minimise bacterial growth.

5 m*M* substrate solutions

The substrates for the enzyme assays are listed in the table above. Substrate solutions were made in 20 ml batches, as required, using sodium phosphate buffer at the correct pH for the enzyme. Substrate solutions were kept at 4°C when not in use, and were discarded after a month.

0.4 *M* glycine solution, pH 10.4

Glycine solution was made by dissolving 15 g of glycine (Merck) in 400 ml of distilled water. The pH of the solution was increased to pH 10.4 using sodium hydroxide pellets, which were added to the stirred solution whilst the pH was monitored using a pH electrode. The volume of the solution was adjusted to 500 ml using distilled water.

Enzyme assay method

Enzyme assays were carried out as described in Griffiths (1998) (*Reference is: Griffiths, R. C. Ph. D. thesis, University of Wales (1998) Polyhydroxylated alkaloids and their ability to inhibit glycosidases*). All assays were carried out in triplicate, using water as a blank in place of the inhibitor. Reaction time was determined based on the length of time needed to give a final absorbance of 0.3 – 1.5 units. Linearity of the time course of the reaction was checked using a series of incubation times. Rate of colour development after the addition of Trinder glucose reagent was determined using a linear time course.

Assays using PNP-glycopyranoside substrates

The following were combined in the well of a flat-bottomed 96-well (300 µl) microtitre plate:

10 µl enzyme solution

10 µl inhibitor solution/distilled water

50 µl substrate solution

The reaction mix was incubated at 25 °C for 5 – 20 minutes, and was stopped using 70 µl of glycine solution. Absorbance at 405 nm was measured immediately in a microtitre plate reader (Molecular Devices VersaMax microplate reader).

Calculations

The extent of inhibition by compounds in the assays was calculated based on a control (uninhibited) reaction. Briefly, the percantage activity of the enzyme was calculated with reference to the control reaction for each assay, and the percentage inhibition was determined by subtraction from 100 percent.

APPENDIX 3
Inhibition Results

Test of compounds for inhibition of α and β galactosidase activity

Results

Most compounds inhibited β-galactosidase (bovine liver). Most compounds enhanced β-galactosidase (*E.coli*) activity. Compounds had no effect on α-galactosidase activity.

Results β-galactosidase (bovine liver).

Sample code	Inhibition	%Inhibition (.14mg/ml)*	IC50%**
CAD10.43	+		
CAD10.45	+		
CAD10.46	+	18	
CAD10.47	+		4.29mM
CAD10.48	+		
CAD10.49	+		48.6mM
CAD10.50	+	21	
CAD10.51A	+		18.8mM
CAD10.52	+		
CAD10.53	+		
CAD10.54	+		
CAD10.55	+		
CAD10.56	+		
CAD10.57	+	20	
CAD10.62	+	36	
CAD10.63	+		0.98mM
CAD10.65	+		1.1mM
CAD10.66	+		70uM
CAD10.67	+		90uM

* Samples without values showed minimal activity at this concentration.

**Sample 67 was not completely dissolved

Results β-galactosidase (*E.coli*)

Sample code	Enhanced activity
CAD10.43	+
CAD10.45	-
CAD10.46	++
CAD10.47	++
CAD10.48	+
CAD10.49	+
CAD10.50	+
CAD10.51A	++
CAD10.52	++
CAD10.53	++
CAD10.54	+
CAD10.55	+
CAD10.56	++
CAD10.57	+
CAD10.62	-
CAD10.63	-
CAD10.65	+
CAD10.66	+
CAD10.67	+

+ Increased rate
++ Approx. double rate

LEICESTER SYNTHETIC COMPOUNDS - enzyme inhibition data

Negative values may indicate enzyme stimulation

Amount as supplied (mg/ml):	*7.5*	*4*	*12.5*	*7.5*	*14.5*	*18*	*7.5*	*7*	*11.5*	*10*
Assay	CAD10.43	CAD10.45	CAD10.46	CAD10.47	CAD10.48	CAD10.49	CAD10.50	CAD10.51A	CAD10.52	CAD10.53
α-glucosidase	-6.7	-4.0	0.1	-1.5	-8.3	-3.2	-4.6	10.2	-9.6	2.3
β-glucosidase (samples as supplied)	59.3	23.2	87.2	67.6	79.2	52.6	67.6	63.1	85.1	41.1
β-glucosidase (samples at 1mg/ml)	9.6	22.7	75.7	25.7	**68.3**	-1.3	25.9	48.1	62.9	9.4
N-acetyl-β-glucosaminidase	-0.9	-3.1	5.8	9.1	-2.6	4.8	4.6	4.7	5.5	4.6
Naringinase	9.2	11.1	11.3	18.0	17.8	12.2	8.0	11.3	5.6	6.7
α-mannosidase	-6.5	-20.6	-29.0	-12.6	-27.1	-13.6	-27.1	-25.8	-33.8	-15.8

Amount as supplied (mg/ml):	*9.5*	*1.5*	*6.5*	*15.5*	*2.5*	*3*	*5.5*	*3*	*5.5*
Assay	CAD10.54	CAD10.55	CAD10.56	CAD10.57	CAD10.62	CAD10.63	CAD10.65	CAD10.66	CAD10.67
α-glucosidase	-12.8	-5.4	-11.8	-4.1	-0.8	2.2	-3.8	14.6	-1.4
β-glucosidase (samples as supplied)	86.7	49.0	35.4	92.3	35.8	35.3	80.4	88.1	93.0
β-glucosidase (samples at 1mg/ml)	61.1	33.7	11.4	53.2	20.0	16.3	**78.3**	**76.3**	**85.5**
N-acetyl-β-glucosaminidase	-0.1	-0.9	-15.3	1.6	0.5	-2.1	22.0	-5.5	-6.5
Naringinase	17.4	12.3	10.3	23.3	23.2	10.8	9.0	10.0	13.8
α-mannosidase	6.2	-15.6	24.8	-14.5	-16.0	-0.4	-16.8	-17.4	-19.8

LEICESTER SAMPLES - AS SUPPLIED IN 2ML

Sample code	Mr	Amount mg/ml	Concentration (uM)	Conc.(uM) @ 1mg/ml
CAD10.43	297	7.5	25.3	3.4
CAD10.45	308	4	13.0	3.2
CAD10.46	249	12.5	50.2	4.0
CAD10.47	233	7.5	32.2	4.3
CAD10.48	306	14.5	47.4	3.3
CAD10.49	289	18	62.3	3.5
CAD10.50	284	7.5	26.4	3.5
CAD10.51A	466	7	15.0	2.1
CAD10.52	265	11.5	43.4	3.8
CAD10.53	273	10	36.6	3.7
CAD10.54	351	9.5	27.1	2.8
CAD10.55	304	1.5	4.9	3.3
CAD10.56	298	6.5	21.8	3.4
CAD10.57	301	15.5	51.5	3.3
CAD10.62	292	2.5	8.6	3.4
CAD10.63	306	3	9.8	3.3
CAD10.65	273	5.5	20.1	3.7
CAD10.66	311	3	9.6	3.2
CAD10.67	325	5.5	16.9	3.1