Rare Monosaccharides and Biologically Active Iminosugars from Carbohydrate Chirons

Daniel Best

St. John's College

University of Oxford

2010

A thesis submitted in partial fulfilment of the requirement for the degree of Doctor of Philosophy (D. Phil.)

Abstract

Rare Monosaccharides and Biologically Active Iminosugars from Carbohydrate Chirons

Daniel Best, St. John's College, University of Oxford
DPhil Thesis, Michaelmas Term 2010

Iminosugars are polyhydroxylated alkaloids, and can be viewed as sugar analogues in which the endocyclic oxygen atom has been replaced with nitrogen. These compounds are highly medically relevant and their biological activity is largely due to their inhibition of glycosidases. Several examples of the iminosugar class are currently marketed as drugs, and many more are in earlier stages of development for a variety of diseases and disorders. The most fruitful approaches to the chemical synthesis of iminosugars have utilised carbohydrate starting materials as optically pure chiral building blocks, or chirons. Most of the monosaccharides are not readily available, but the relatively few naturally abundant cheap sugars have been exploited as chirons for over a century. The availability of the rare sugars is growing with the development of a new biotechnological approach to their synthesis, known as Izumoring.

This thesis is primarily concerned with the chemical synthesis of iminosugars from carbohydrate starting materials. The synthesis of unnaturally functionalised sugar polyols and their suitability as substrates for the Izumoring process is also discussed.

Chapter 1 provides a brief general overview of the history, natural occurrence and therapeutic application of iminosugars. General strategies for their synthesis from carbohydrate chirons are discussed.

Chapter 2 concerns divergent syntheses of several iminosugar targets from both enantiomers of glucuronolactone and their biological evaluation. A new scaleable synthesis of the natural product 1-deoxynojirimycin is presented that has since been adopted for commercial purposes, as well as an efficient strategy for the synthesis of both enantiomers of 2,5-dideoxy-2,5-imino-mannitol and their novel amino acid analogues. Access to hexosaminidase inhibiting acetamido-substituted piperidines is presented, including 2-acetamido-1,5-imino-1,2,5-trideoxy-D-galactitol, which has been found to be one of the few known potent and specific inhibitors of α-*N*-acetyl-galactosaminidase. This inhibitory profile may allow the compound's use for further investigation of a strategy for cancer treatment.

Chapter 3 concerns the synthesis of carbon branched pyrrolidines and their biological evaluation. A novel and highly potent α-glycosidase inhibitor has been discovered, synthesised by a strategy that utilises the benzhydryl ether as key protecting group. A mild method for the introduction of this protecting group has been shown to be general to a range of sterically congested and/or acid/base sensitive carbohydrate lactones.

Chapter 4 concerns the synthesis of deoxygenated and fluorinated sugar alcohols and their successful biotechnological transformation into ketoses by the Izumoring process.

Publications arising from this work are included in the **Appendix**.

Acknowledgements

First and foremost, I would like to extend my enormous gratitude to Prof. George W. J. Fleet. I have thoroughly enjoyed my time in the Fleet group and working for such an enthusiastic, immensely supportive and knowledgeable supervisor – a phenomenally *great* guy.

Many thanks to all the members of the Fleet group, past and present. Special thanks to Dr. Sarah F. Jenkinson for advice, discussions, X-ray work, collaboration on the isoDMDP project, and truly heroic proof reading. Thanks to: Gabe for discussions, proof reading, and picking up the fluoro-Izumoring project; Elizabeth for biological testing and taking on the enantiomeric synthesis of DNJNAc; Andreas for biological testing, proof reading and discussion; Chen for work on the enantiomeric DNJ/DMDP syntheses; and Phoom for work on the enantiomeric DGJNAc synthesis.

Many thanks to my sponsors, Summit plc, and Dr. Francis X. Wilson for advice and discussions. Thanks to Dr. Graeme Horne for proof reading and advice.

A huge thank you to all of the Dextra Labs team, particularly Dr. Alex C. Weymouth Wilson for supervision during my placement, and the supply of starting materials. Thanks also to Dr. Robert A. Clarkson and Dr. Nigel A. Jones for further advice, discussions and the L-glucose work.

Massive appreciation must be expressed to the inhumanly efficient Prof. Atsushi Kato and his group for mixture separation and biological testing.

Many thanks go to Prof. Ken Izumori and his group for collaboration on the Izumoring projects.

For an excellent and unique academic and cultural experience in Iceland, I am very grateful to Prof. Sigthor Petursson – and of course Colleen for incredibly generous hospitality.

Many thanks to: Dr. Mark R. Wormald for NMR work in the Izumoring project; Dr. Terry D. Butters for discussion and biological testing; Dr. Robert J. Nash for mixture separation and purification; Dr. Scott C. Garman and group for protein crystallography work; Prof. Frederic Becq and Dr. Caroline Norez for CF assays.

I am very grateful to the all the analytical service staff at the University of Oxford.

The support of my family and friends has been a great help, I am particularly grateful for the support from my parents. I must of course thank Maria for more than I can list here, including all the discussions, telling me to “split it” – sound advice, and putting up with all the chemistry.

<u>**Contents**</u>

Nomenclature **i**
Abbreviations **ii**

1. General Overview **1**

1.1. Natural Occurrence and Significance of Iminosugars 1
1.2. Therapeutic applications 4
1.3. Synthetic Methods 19
1.4. References 34

2. Iminosugars from glucuronolactone **38**

2.1. Introduction 38
2.2. 1-Deoxynojirimycin (1,5-Cyclisation) 47
2.3. DMDP and Sugar Amino Acids (2,5-Cyclisation) 50
2.4. Hexosamine Analogues (2,6-Cyclisation) 55
2.5. Conclusions and Future Work 66
2.6. Experimental 67
2.7. References 110

3. Branched Pyrrolidines: The Synthesis of isoDMDP **114**

3.1. Introduction 114
3.2. The Benzhydryl Protecting Group 117
3.3. isoDMDP by Inramolecular S_N2 119
3.4. isoDMDP by Intramolecular Reductive Amination 125
3.5. Conclusions and Future Work 130
3.6. Experimental 130
3.7. References 163

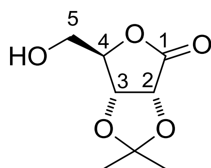
4. Modified Hexitols as Izumoring Substrates **166**

4.1. Introduction 166
4.2. 5-Deoxy Izumoring 169
4.3. 1/6-Fluoro Izumoring 172
4.4. Conclusions and future work 176
4.5. Experimental 177
4.6. References 187

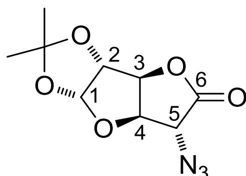
Appendix: Publications Arising from this Thesis **190**

Nomenclature

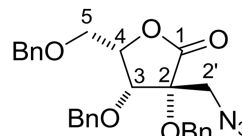
Conventions for the systematic naming and numbering of carbohydrate derivatives are consistent with those recommended by IUPAC.* Examples are given below.



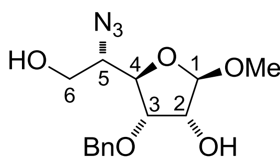
2,3-*O*-Isopropylidene-D-ribono-1,4-lactone



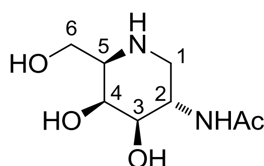
5-Azido-5-deoxy-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone



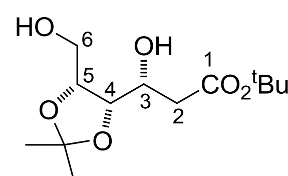
2-*C*-Azidomethyl-2,3,5-tri-*O*-benzyl-L-lyxono-1,4-lactone



Methyl 5-azido-3-*O*-benzyl-5-deoxy-α-L-talofuranoside



2-Acetamido-1,5-imino-1,2,5-trideoxy-D-galactitol



tert-Butyl 2-deoxy-4,5-*O*-isopropylidene-D-*arabino*-hexanoate

* McNaught, A. D. *Carbohydr. Res.* **1997**, *297*, 1-92

Abbreviations

+ve	Positive (in MS data)
-ve	Negative (in MS data)
a	Apparent (in NMR data)
Ac	Acetyl
allo-DNJ	1-Deoxyallonojirimycin, 1,5-dideoxy-1,5-imino-D-allitol
altro-DNJ	1-Deoxyaltronojirimycin, 1,5-dideoxy-1,5-imino-D-altritol
aq	Aqueous
Ar	Aromatic (in NMR data)
$B_{AC}2$	Bimolecular base catalysed hydrolysis with acyl C-O bond cleavage
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
br	Broad (in IR and NMR data)
Bu	n-Butyl
Bzh	Benzhydryl, diphenylmethyl
c	Concentration (in optical rotation data)
Cat	Catalytic
Cbz	Benzyloxycarbonyl
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane regulator
CMT	Chaperone mediated therapy
Cy	Cyclohexyl
d	Days; doublet/double (in NMR data)
d.r.	Diastereomeric ratio
DAB	1,4-Dideoxy-1,4-imino-D-arabinitol
DALDP	2,5-Dideoxy-2,5-imino-D-altritol
DAST	Diethylaminosulfur trifluoride
DCM	Dichloromethane
DGDP	2,5-Dideoxy-2,5-imino-D-glucitol
DGJ	1-Deoxygalactonojirimycin, 1,5-dideoxy-1,5-imino-D-galactitol
DGJNAc	2-Acetamido-1,5-imino-1,2,5-dideoxy-D-galactitol
DGLDP	2,5-Dideoxy-2,5-imino-D-galactitol
DIAD	Diisopropyl azodicarboxylate
DIBAL	Diisobutylaluminium hydride
DIJ	1-Deoxyidonojirimycin, 1,5-dideoxy-1,5-imino-D-iditol
DMDP	2,5-Dideoxy-2,5-imino-D-mannitol
DMF	Dimethylformamide
DMJ	1-Deoxymannonojirimycin, 1,5-dideoxy-1,5-imino-D-mannitol
DMJNAc	2-Acetamido-1,5-imino-1,2,5-dideoxy-D-mannitol
DMSO	Dimethylsulfoxide
DNJ	1-Deoxnojirimycin, 1,5-dideoxy-1,5-imino-D-glucitol
DNJNAc	2-Acetamido-1,5-imino-1,2,5-dideoxy-D-glucitol
DRB	1,4-Dideoxy-1,4-imino-D-ribitol
DTE	D-Tagatose-3-epimerase
DTJ	1-Deoxytalonojirimycin, 1,5-dideoxy-1,5-imino-D-talitol
ECM	Extracellular matrix
ER	Endoplasmic reticulum
ESI	Electrospray ionisation
Et	Ethyl
FG	Fagomine, 1,2-dideoxynojirimycin, 1,5-imino-1,2,5-trideoxy-D-*arabino*-hexitol
GalNAc	*N*-Acetyl-D-galactosamine,
GSL	Glycosphingolipid
gulo-DNJ	1-Deoxygulonojirimycin, 1,5-dideoxy-1,5-imino-D-gulitol

h	Hours
HMBC	Heteronuclear multiple bond coherence
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IC_{50}	Half maximal inhibitory concentration
IFG	Isofagomine, 2-*C*-hydroxymethyl-1,5-imino-1,2,5-trideoxy-D-xylitol
Industrial methylated spirits	19:1 EtOH/MeOH
IPA	Isopropanol
IR	Infrared
isoDAB	1,4-Dideoxy-2-*C*-hydroxymethyl-1,4-imino-D-threitol
isoDGDP	1,4-Dideoxy-2-*C*-hydroxymethyl-1,4-imino-L-xylitol
isoDMDP	1,4-Dideoxy-2-*C*-hydroxymethyl-1,4-imino-D-arabinitol
isoDRB	1,4-Dideoxy-2-*C*-hydroxymethyl-1,4-imino-D-erythritol
isoLAB	1,4-Dideoxy-2-*C*-hydroxymethyl-1,4-imino-L-threitol
K_i	Inhibition constant
LAB	1,4-Dideoxy-1,4-imino-L-arabinitol
LDA	Lithium diisopropylamide
Lit	Literature value
LRMS	Low resolution mass spectrometry
m	Multiplet (in NMR data); medium intensity (in IR data)
MS	Mass spectrometry
m.p.	Melting point
MAF	Macrophage activating factor
Me	Methyl
Mesylate	Methanesulfonate
min	Minutes
Ms	Methanesulfonyl
*N*B-DNJ	*N*-Butyl-DNJ
n-Bu	n-Butyl
NJ	Nojirimycin, 5-amino-5-deoxy-D-glucopyranose
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
PCC	Pyridinium chlorochromate
PDH	Polyol dehydrogenase
PET	Positron emmission topography
Ph	Phenyl
PNP	Purine nucleoside phosphorylase
PPTS	Pyridinium *p*-toluenesulfonate
*p*TSA	*p*-Toluenesulfonic acid
Py	Pyridine
q	Quartet/quadruple (in NMR data)
R_f	Retention factor
RT	Room temperature
s	Singlet (in NMR data); strong intensity (in IR data)
Sat	Saturated
S_N1	Unimolecular nucleophilic substitution
S_N2	Bimolecular nucleophilic substitution
SRT	Substrate reduction therapy
t	Triplet/triple (in NMR data)
TBAF	Tetra-*N*-butylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
TBS	*tert*-Butyldimethylsilyl
^{t}Bu	*tert*-Butyl
TEA	Triethylamine

Tf	Trifluoromethanesulfonyl
TASF	Tris(dimethylamino)sulfonium difluorotrimethylsilicate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Tosylate	*p*-Toluenesulfonate
Triflate	Trifluoromethanesulfonate
Ts	*p*-Toluenesulfonyl
UDP	Uridine diphosphate

1. General Overview

Iminosugars are broadly defined as sugar analogues in which the endocyclic hemiacetal oxygen atom has been replaced with nitrogen, i.e. polyhydroxylated alkaloids. They are widespread in plants and have been isolated from fermentation broths of various bacterial strains. Compounds of this class are often biologically active, generally inhibiting glycosidases by virtue of their structural and electronic resemblance to the transition states of glycoside hydrolysis.[1,2] While most iminosugars act as transition state mimics, and therefore in a competitive manner, there are examples of iminosugars that interfere with carbohydrate processing enzymes in a non-competitive fashion.[3] Glycosidases are involved in a huge number of biological processes, including those associated with a number of diseases and disorders, and inhibitors of these enzymes constitute viable therapeutic targets for the treatment of various medical conditions.[4] The dense stereochemical complexity coupled with the potential medical applications of iminosugar inhibitors makes them challenging and rewarding synthetic targets.[5,6] This thesis is primarily concerned with iminosugar synthesis from carbohydrate starting materials.

1.1. Natural Occurrence and Significance of Iminosugars

There are five main categories into which iminosugar natural products fall: the monocyclic five-membered pyrrolidines and six-membered piperidines; and the bicyclic [3.3.0] pyrrolizidines, [4.3.0] indolizidines and [3.2.1] nortropanes (**Figure 1.1**).[7] Interest in the field of iminosugars was sparked with the isolation of the piperidine 5-deoxy-5-amino-D-glucopyranose, or nojirimycin [NJ] **1.1**, from a *Streptomyces* culture in the mid sixties.[8] It was not until 1973 that iminosugars were first reported from plant sources with the isolation of fagomine [FG] **1.2**,[9] which is the 1,2-dideoxy analogue of NJ **1.1**. Shortly afterwards came the isolation of 1-deoxy-NJ [DNJ] **1.3** from mulberry trees,[10] which had already been synthesised a decade earlier with the discovery of NJ **1.1**,[11] and it

was later isolated from bacterial culture.[12] The pyrrolidine 2,5-dideoxy-2,5-imino-D-mannitol [DMDP] **1.4** was also first isolated around this time,[13] and has since been found in a very wide range of plant sources.[7] FG **1.2**, DNJ **1.3** and DMDP **1.4**, so called iminocyclitols, all lack the hemiaminal function of NJs, which greatly increases their stability. As a result, much of the focus in the synthetic field is associated with these iminocyclitols, this thesis being no exception. Following these early discoveries, the array of known naturally occurring iminosugars, especially from plant sources, has exploded.

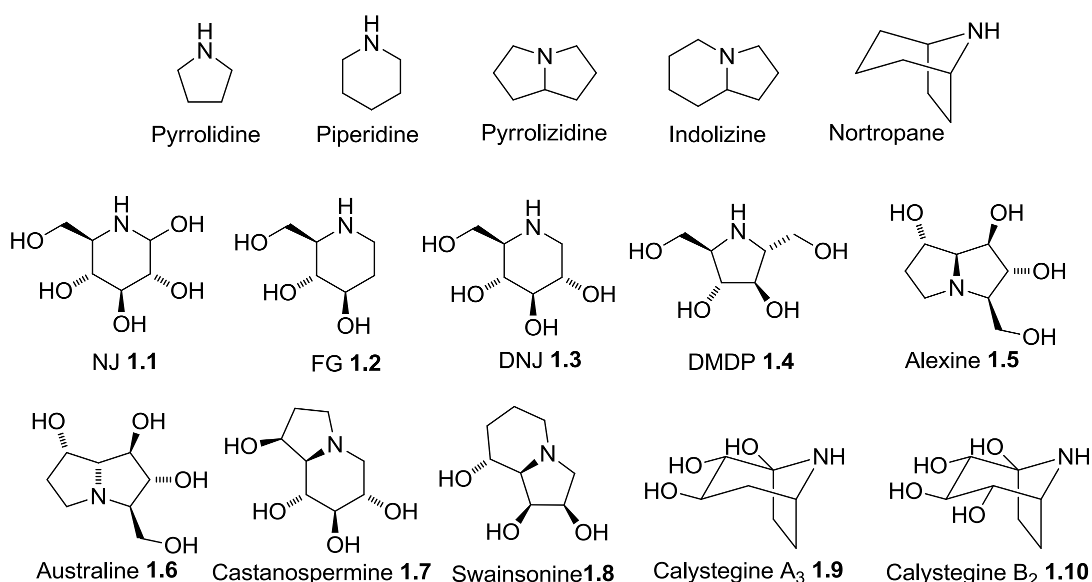


Figure 1.1: Generic iminosugar scaffolds and examples of naturally occurring iminosugars

The first isolated indolizidines were swainsonine **1.8**[14] and castanospermine **1.7**[15] from Australian legumes *Swainsona canescens* and *Castanospermum australe* around 1980, which had been known to be toxic to livestock for some time. The first pyrrolizidines appeared towards the end of the decade in the form of alexine **1.5**[16] and australine **1.6**[17] from *Alexa leiopetala* and *C. australe*, along with the first polyhydroxylated nortropanes, the calystegines,[18] from *Calystegia sepium.* When specifically screened for, iminosugars can often be found in common plants in which their existence was hitherto not considered. Many potatoes are particularly rich sources of nortropane

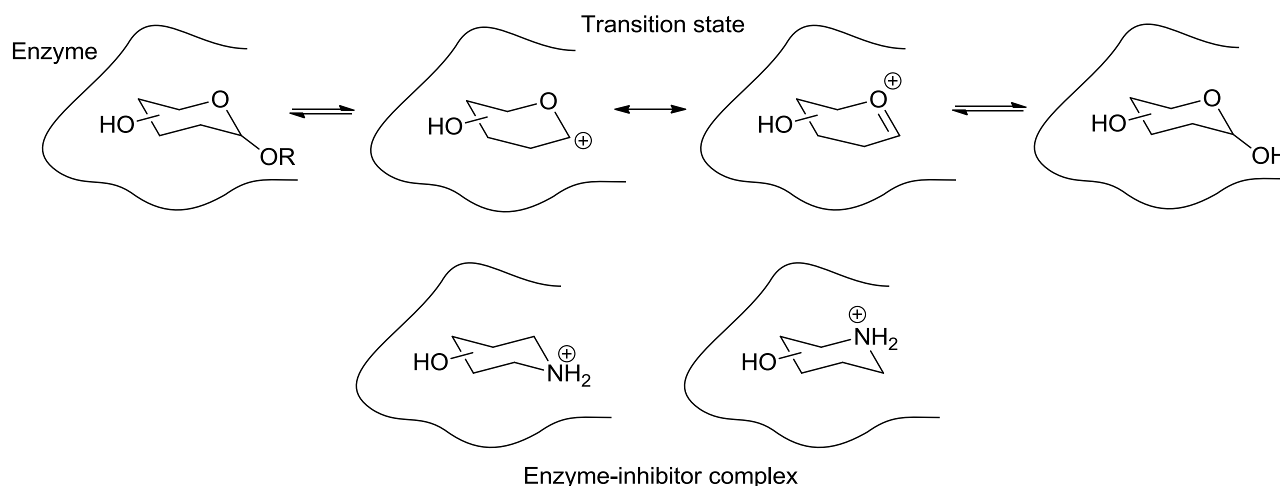
iminosugars,[19] which survive cooking, including the various processes in the manufacture of crisps, and are consumed regularly by an enormous proportion of the world's population. Many traditional herbal medicines contain relatively high levels of iminosugars and it has been suggested that it may be these nitrogen heterocycles that confer the health benefits associated with the plant extracts.[20,21] Mulberry leaves, which are a rich source of the glucosidase inhibitor DNJ **1.3**, are used in traditional Chinese medicine to treat type II diabetes.[7] The *N*-hydroxyethyl DNJ derivative miglitol is a liscenced drug that serves the same purpose. Some animals have been found to store iminosugars; the pupae of the Swallowtail moth *Urania fulgens* feed on iminosugar rich plants and store the alkaloids into maturity, even the eggs laid by the adult moths contain iminosugars.[22] The precise reason for the accumulation of iminosugars in animals is not certain, but a plausible explanation is a defence mechanism associated with the toxicity of some iminosugars, or even simply reducing the nutritional value of an insect through inhibition of the predator's digestive enzymes. The Death's-head hawk-moth (*Acherontia atropus*) stores calystegines, including calysegines A_3 **1.9** and B_2 **1.10**, gained from the potatoes that nourished its pupa,[19] and bees accumulate iminosugars from iminosugar-containing nectars; some African varieties of honey show relatively high levels of iminosugars and may have health benefits associated with them.[23] The UK's common bluebell *Hyacinthoides non-scripta* is a rich source of iminosugars and grows in woodland traditionally fenced off for the protection of livestock, to which it is toxic.[24] On the other hand, it has been observed that badgers are quite partial to the common bluebell, and given the antibacterial properties of some iminosugars this could be interpreted as self-medicating behaviour in an animal renowned as a carrier of tuberculosis, particularly in the south-west of England – though this would be quite a leap of deduction.

1.2. Therapeutic Applications

The therapeutic applications of iminosugars are wide ranging[25,26] and include their use to treat cancer, infectious diseases, neurodegenerative diseases, lysosomal strorage disorders, cystic fibrosis and non-insulin-dependent (type II) diabetes. It is beyond the scope of this thesis to discuss them all in detail; selected examples of the therapeutic applications of iminosugars will be introduced in this section.

1.2.1. Mechanisms of Glycosidase Inhibition

The biological activity of iminosugars is largely due to glycosidase inhibition. The mechanisms of enzymatic glycoside hydrolysis generally involve the build-up of positive charge in the transition state (**Scheme 1.1**).[1]



Scheme 1.1: A basic representation of transition state mimicry

Iminosugars are transition state mimics; being protonated at physiological pH, the resultant ammonium cation mimics the carbocation/oxocarbenium character of the transition state for glycoside hydrolysis. Selectivity of an iminosugar inhibitor for different enzymes arises through the configuration of the hydroxyl groups and any other exocyclic substituents compared to the natural substrate, as well as charge distribution. An iminosugar bearing an identical or closely related

arrangement of hydroxyls to a natural sugar is often a specific inhibitor for the processing enzymes associated with that sugar. One would naturally reason that these hydroxyl groups are involved in binding to the active site and require a particular configuration to do so effectively. For example, the configuration of DNJ **1.3** is *gluco*, its structural resemblance to D-glucose is obvious and similarly the *galacto* configured DNJ analogue 1,5-dideoxy-1,5-imino-D-galactitol [DGJ] **1.11** has a clear structural resemblance to D-galactose (**Figure 1.2**). Both DNJ **1.3** and DGJ **1.11** are potent inhibitors of α-glucosidases and -galactosidases, respectively.

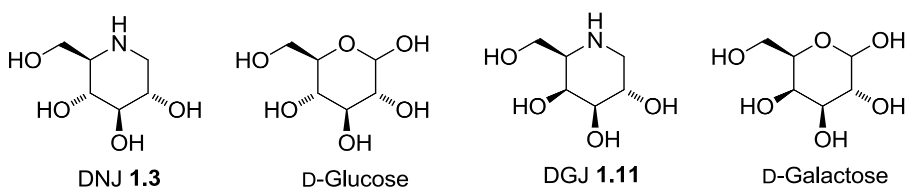
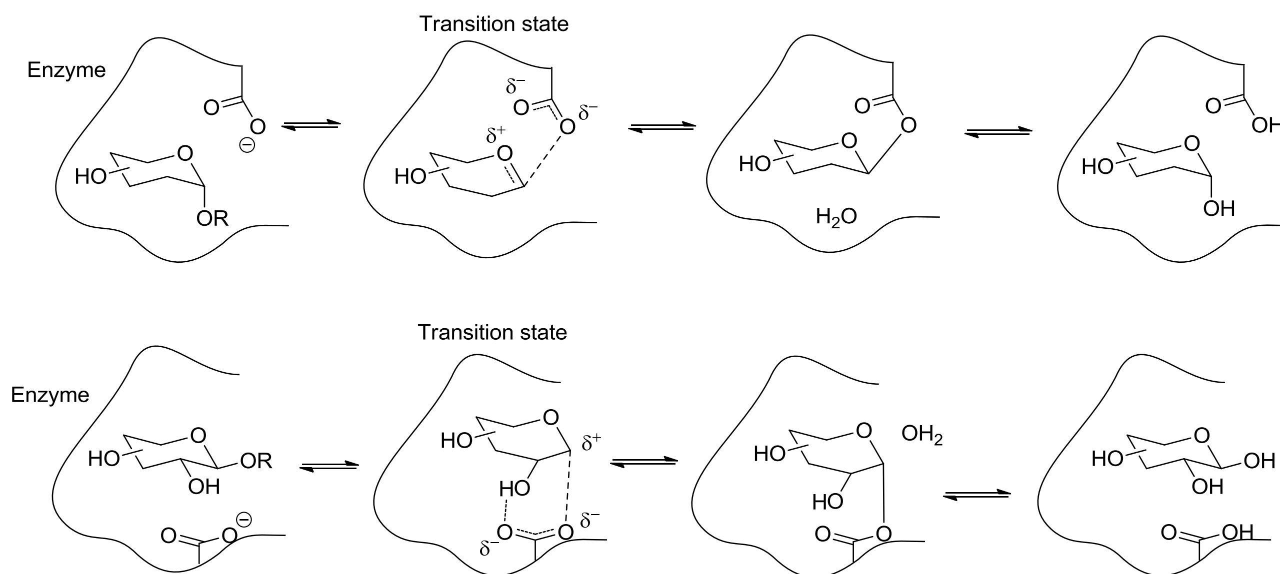


Figure 1.2: DNJ, DGJ and their structural resemblance to natural sugars

It is interesting to note that DNJ **1.3** and DGJ **1.11** are both selective for α-glycosidases, showing much weaker inhibition for β-glucosidases and -galactosidases despite their structural resemblance to the natural substrates.[27] This is a charge distribution effect, the origin of which lies in the different mechanisms of enzymatic α- and β-glycoside hydrolysis. Taking the more common retaining glycosidases as an example,[2] the mechanism of hydrolysis involves glycosylation of a nucleophilically catalytic residue of the active site, commonly a carboxylate, as the rate determining step followed by displacement of the catalytic residue with water (**Scheme 1.2**). The configuration of the anomeric position is inverted twice in this mechanism, leading to net retention of the starting anomeric configuration. In the case of the α-glycosidases, the nucleophilic residue is positioned in such a way that a build-up of positive charge at the endocyclic oxygen is favoured as the anomeric group is ejected, resulting in a transition state with oxocarbenium character. This oxocarbenium

character is mimicked by DNJ **1.3** and DGJ **1.11** where the endocyclic oxygen has been replaced by an ammonium cation.



Scheme 1.2: General mechanism for retaining α-glycosidases (top) and β-glycosidases (bottom) showing electronic differences in the transition states

Conversely, in the case of β-glycosidases the endocyclic oxygen lone pair is not antiperiplanar to the anomeric leaving group, and the nucleophilic residue is positioned in such a way that it interacts predominantly with the anomeric centre and the adjacent exocyclic hydroxyl, favouring carbocation character at the anomeric position in the transition state (**Figure 1.3**).

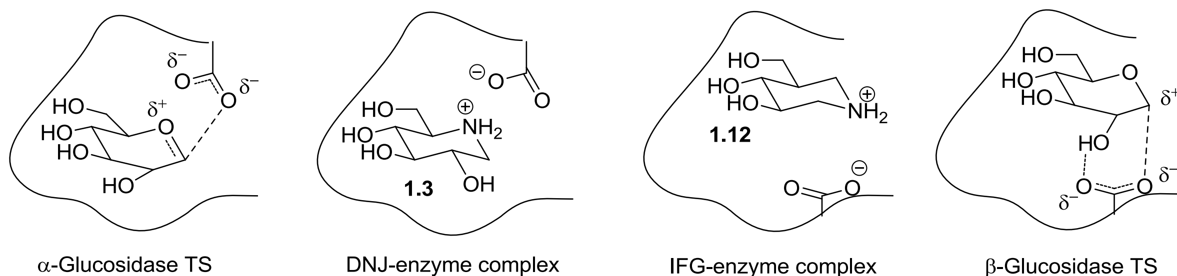


Figure 1.3: Rationalisation of the different selectivities of DNJ **1.3** and IFG **1.12**

Replacing the anomeric position with nitrogen generally results in β-selective inhibitors often referred to as the 1-*N*-iminosugars. Bearing a configuration of hydroxyl groups analogous to glucose, isofagomine [IFG] **1.12** is a 1-*N*-iminosugar designed specifically for β-glucosidase

activity, and is indeed a very potent inhibitor with the intended selectivity.[28,29] Similarly, the *galacto* analogue of IFG is a potent and selective β-galactosidase inhibitor.[30,31]

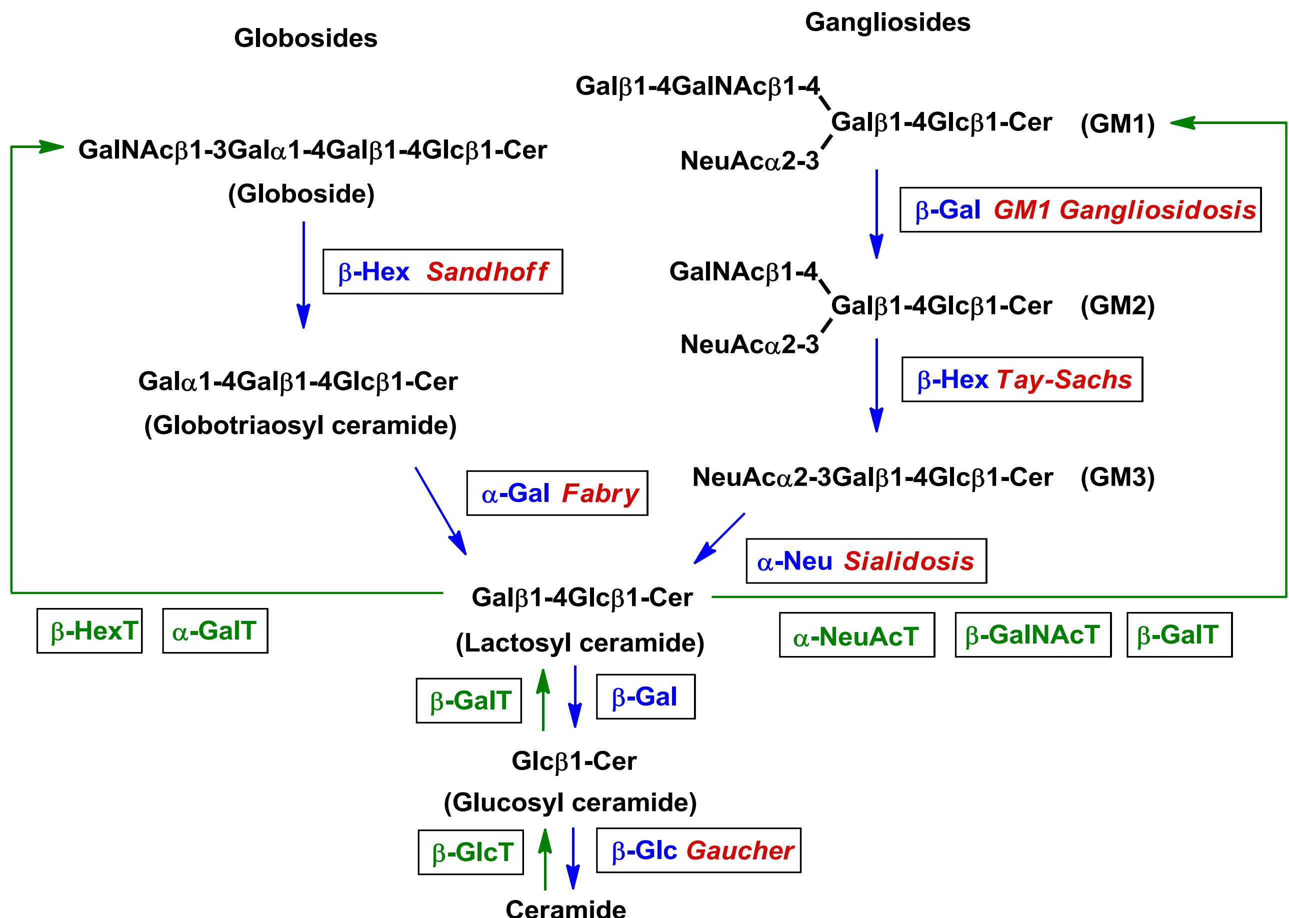
1.2.2. Type II Diabetes

In terms of therapeutic applications for iminosugars, type II diabetes is probably the most obvious. The disease is caused by insulin resistance, resulting in hyperglycaemia as the normal hormonal signal for glucose storage is ignored, with long term effects including increased risk of heart attacks, strokes and kidney failure. The disease is reaching epidemic proportions in the western world, and its rapid growth is largely attributed to poor diet, lack of exercise and rising childhood obesity. Among the various drugs available for the management of type II diabetes is the α-glucosidase inhibitor *N*-hydroxyethyl-DNJ, known as miglitol and marketed as Glyset.[32] Inhibition of gut glucosidases results in reduced uptake of glucose into the bloodstream, thus preventing hyperglycaemia and managing the symptoms of the disease.

1.2.3. Lysosomal Storage Disorders

Another major therapeutic target for iminosugars is lysosomal storage disorders, which are caused by defective lysosomal glycosidases; enzymes that play a crucial role in cell surface glycosylation. The surfaces of animal cells are heavily glycosylated, displaying a dense arrangement of glycans, the glycocalyx, which is essential for cell-cell interactions in the body as these glycans often function as recognition markers.[33] The presented glycans are the carbohydrate portions of membrane glycoproteins and glycolipids, in which the protein or lipid portion is embedded in the plasma membrane bilayer with the glycan extending from the cell surface. The glycolipids fall into two categories: the glycosphingolipids [GSLs], which are involved in cell-cell interactions, and the glycophospholipids, which are involved in anchoring proteins to the cell surface. The lysosomal glycosphingolipidoses are a group of rare autosomal recessive disorders associated with reduced

activity of glycosidases as a consequence of their misfolding.[34] GSLs are catabolised to ceramide by a series of glycosidase-catalysed trimming reactions in the lysosome, and are biosynthesised from ceramide in the Golgi apparatus by a series of glycosyltransferases (**Figure 1.4**). This cycle of GSL synthesis and degradation, which maintains appropriate levels of GSLs in the body, is ubiquitous in eukaryotic cells



Blue arrows represent the catabolic pathway in the lysosome, mutations of glycosidases (blue) result in lysosomal storage disorders (red); Green arrows represent biosynthesis facilitated by glycosyl transferases (green)

Figure 1.4: Catabolism and biosynthesis of glycoshingolipids

An inefficient step in the catabolic portion of the cycle leads to the accumulation of intermediates in the lysosome, causing swelling of the cell, which in turn results in the pathological effects of the disorder.[35] The symptoms of excessive lysosomal storage vary depending on the extent and site of storage, but include neurological degeneration, bone destruction, cardiovascular disease, kidney

failure and premature death. Two iminosugar based therapies have emerged, both of which rely on a reduced, as opposed to completely removed, activity of the mutated glycosidase.[36]

Assuming some residual catalytic activity is still associated with the enzyme, the bottleneck in the biosynthetic/catabolic cycle can be cleared by substrate reduction therapy [SRT]. By inhibiting GSL biosynthesis, the substrate for the affected glycosidase is reduced to a level that can be effectively managed by the defective enzyme, thus preventing lysosomal storage. In Gaucher's disease β-glucocerebrosidase is compromised, leading to a build up of glucosylceramide in the lysosome. It is the commonest lysosomal storage disorder, especially in Askenazi Jewish communities where the frequency of the disorder is around 3%.[36] Symptoms of non-neuropathic (type I) Gaucher's disease commonly include enlargement of the liver and spleen, bone destruction, anaemia and a reduced white blood cell and platelet counts. Types II and III are neuropathic, progressive and more severe, leading to premature death; while type III may not manifest until adulthood, type II is characterised by early onset and children with this condition usually die by the age of two. *N*-Butyl-DNJ [*N*B-DNJ], marketed as Zavesca, is a current clinical treatment for type I Gaucher's disease; its mode of action is inhibition of glucose ceramidetransferase, thus slowing the rate of GSL biosynthesis to a tolerable level. There have been indications that *N*B-DNJ can cross the blood-brain barrier and may therefore be beneficial for sufferers of neuropathic forms of Gaucher's disease. SRT is a general therapy that should be applicable to all lysosomal storage disorders as its success is independent of the defective enzyme's identity.

In addition to the lowered catalytic performance of the distorted active site of a misfolded enzyme, such proteins are inefficiently transported to the lysosome as quality control mechanisms in the endoplasmic reticulum [ER] actively detect and destroy misfolded proteins before transportation.[25] A second approach to the treatment of lysosomal storage disorders that addresses this aspect of

defective glycosidases is chaperone-mediated therapy [CMT], which specifically targets the defective glycosidase responsible for the disease. The success of CMT also relies on some residual activity of the defective glycosidase, and works by increasing the concentration of this enzyme in the lysosome, thus boosting the dangerously low rate of glycoside hydrolysis. CMT aims to increase the concentration of a defective enzyme to a level that is functional despite its deficiencies, whereas SRT aims to reduce the workload of the compromised enzyme to a manageable level.

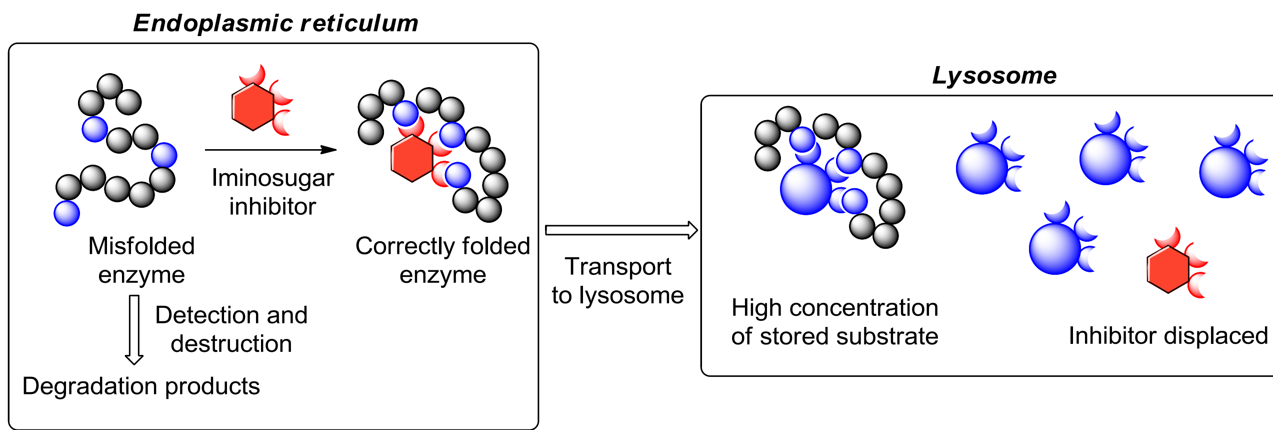


Figure 1.5: The mechanism of chaperone-mediated therapy for lysosomal storage disorders

In glycosphingolipidosis patients, where genetic mutation results in an abnormally high glycosidase misfolding frequency, the ER quality control pathway has a deleterious effect as the incidence of correct folding and transport is too infrequent to maintain functional levels of the mutant enzyme in the lysosome. Small molecules that act as potent reversible glycosidase inhibitors, such as iminosugars, can also act as pharmacological chaperones for the same enzymes. This observation is intuitive; if an iminosugar can bind strongly to a given glycosidase by virtue of highly favourable interactions with the active site, it follows that the same molecule can function as a template for the unfolded enzyme and direct correct folding through the very same favourable interactions. The presence of an iminosugar thus increases the likelihood of correct folding and the rate of transport of the enzyme-inhibitor complex from the ER to the lysosome (**Figure 1.5**). Less intuitive however is

the observation that the catalytic activity of the enzyme in the lysosome is enhanced by this process, as the inhibitor is cotransported with the enzyme, CMT might be expected to lower the efficiency of the glycosidase and exacerbate lysosomal storage. The key point here is that the inhibitor is competitive and reversible, and in cases of excessive lysosomal storage the concentration of the native substrate is usually very high. Once transported to the lysosome, the inhibitor can not effectively compete with the native substrate and is displaced because the native substrate is present in an unusually high concentration. As a result the increase in lysosomal enzyme concentration associated with the increased trafficking efficiency is the dominant factor; the activity of the competitive inhibitor at low concentration loses significance in the presence of a high native substrate concentration and the net effect is positive. DGJ **1.11** is a very potent and specific α-galactosidase inhibitor and has been successfully employed as a pharmacological chaperone. Marketed as Amigal, DGJ **1.11** is currently in phase III clinical trials for the treatment of Fabry's disease, which is caused by defective α-galactosidase, by means of CMT. As a potent and selective β-glucosidase inhibitor, IFG **1.12** is also in development for CMT of Gaucher's disease, targeting defective β-glucocerebrosidase.

1.2.4. Cancer

There are several approaches to the treatment of cancer with glycosidase inhibitors. Of them, the most widely studied is the inhibition of glucosidases and mannosidases involved in the assembly of glycoconjugates.[37] Overexpression of unusual *N*-linked glycans of the surface of cancer cells is well known and may contribute to a cancer's ability to grow uncontrollably and metastasise. Changes in glycosylation may modulate the cell's response to growth factors, and may cause changes in cell adhesion properties that allow the detachment of a leading edge cell from the primary tumor and subsequent tissue invasion at new sites, leading to secondary tumors. It is these metastases rather

than the primary tumor that are the cause of death in 90% of cancer patients, therefore the inhibition of metastasis would slow the progression of disease and present a wider window of opportunity for its treatment by combined therapies.[38] The process by which glycoconjugates are assembled involves the stepwise trimming of an oligosaccharide precursor by α-glucosidases and α-mannosidases to create a core structure that is then further elaborated. It is these enzymes that are targeted in this strategy with the aim of preventing adequate glycosylation for efficient metastasis, or by changing glycosylation in such a way as to illicit an immune response.[23] The same pathways are present and essential in normal cells, but the hyperactivity and reduced quality control associated with cancer cells makes them more susceptible to any disruptions in the pathway. α-Glucosidase inhibitors DNJ **1.3** and castanospermine **1.7** have both been investigated for this purpose and exhibited some anticancer activity. The α-mannosidase inhibitor swainsonine **1.8** has been extensively studied as an anticancer agent up to phase II clinical trials[39] where unfortunately it showed no anticancer activity despite its previously reported potential.[26]

Two alternative approaches involving hexosaminidase inhibitors also have the potential to develop into viable cancer therapies. In order for a cancer to metastasise, a leading edge cell must penetrate the basement membrane of the cancerous tissue, degrade and cross the extracellular matrix [ECM], and penetrate the basement membrane of a blood vessel, a process known as intravasation. Once the cell has entered the bloodstream it is transported to a new location, and the process of extravasation, the reverse of intravasation, leads to tissue invasion and colonisation (**Figure 1.6**).[37] The ECM is composed of fibrous proteins, proteoglycans and other polysaccharides and the crossing of basement membranes and the ECM is facilitated by a number of degradative proteinases, including matrix metalloproteinases, and glycosidases. The carbohydrate elements of the ECM include hyaluronic acid, a polymer of alternating glucuronic acid and *N*-acetyl-glucosamine residues, and the

glycosaminoglycans, which are the glycans of the densely glycosylated proteoglycans and similar in composition to hyaluronic acid. The ECM is therefore rich in *N*-acetyl-glucosamine; experiments have shown that cancer cells release this sugar at an especially rapid rate in their degradation of the ECM,[40] and in cases of advanced malignant disease elevated levels of β-*N*-acetyl-glucosaminidase are almost always present.[41]

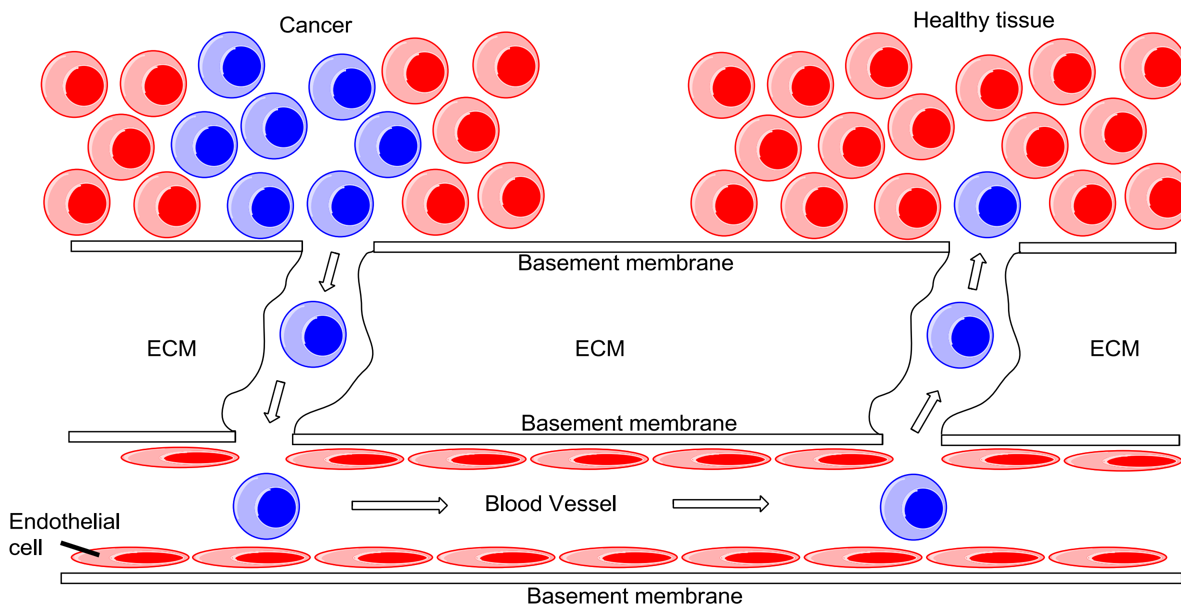


Figure 1.6: Tumor metastasis

The application of a suitable β-*N*-acetyl-glucosaminidase inhibitor could therefore be a successful approach for the suppression of metastasis by limiting the mobility of the leading edge cells responsible for metastasis, providing a therapeutic role complimentary to treatments that aim to destroy the original tumor.

α-*N*-Acetyl-galactosaminidase has also been implicated in the progression of cancer, and acts as an immunosuppressant. Cancerous cells would normally be destroyed by macrophages, which in turn are activated by the signalling molecule macrophage activating factor [MAF] (**Figure 1.7**). MAF is produced by trimming of a trisaccharide glycan of serum vitamin D_3-binding protein [Gc protein]; a β-linked galactose residue is first cleaved by a β-galactosidase secreted by the immune system's B

cells, followed by cleavage of an α-linked *N*-acetyl-neuraminic acid residue by an α-*N*-acetyl-neuraminidase secreted by the T cells. The resultant glycoprotein, monoglycosylated with α-linked *N*-acetyl-galactosamine, is the MAF that promotes a normal immune response.

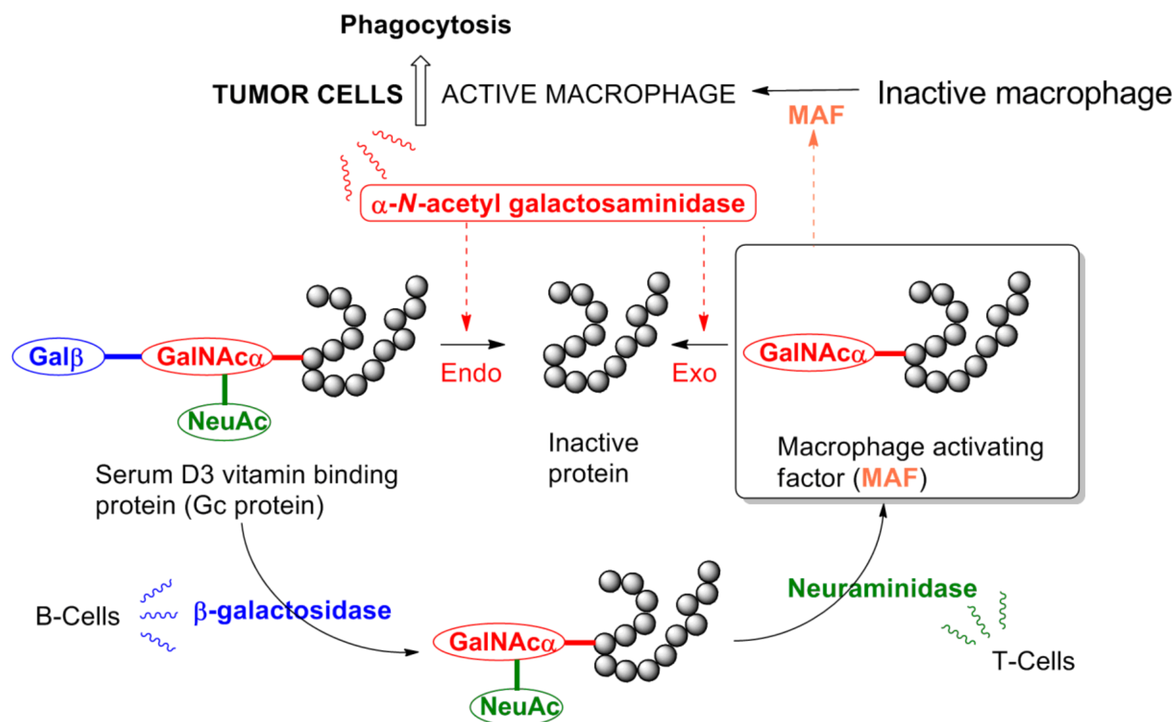


Figure 1.7: Immunosuppression by α-*N*-acetyl-galactosaminidase

It has been shown that melanomas secrete endo-α-*N*-acetyl-galactosaminidases that completely remove the trisaccharide from the Gc protein,[42] and exo-α-*N*-acetyl-galactosaminidases that deglycosylate MAF itself.[43] The exo/endo specificity of these glycosidases appears to vary with species and potentially also between cancer cell lines, but the net result is the same: destruction of the biosynthetic precursor to MAF thereby suppressing the immune response to the cancer and allowing progression of the disease. One can therefore envisage a strategy for the treatment of cancer by rescuing the production of MAF with the application of a suitable α-*N*-acetyl-galactosaminidase inhibitor, thereby restoring the immune response and facilitating the natural clearance of the cancer cells.[44] Hexosaminidase-inhibiting iminosugars may play an important role

in future cancer therapy and the synthesis of designed hexosaminidase inhibitors is discussed in this thesis.

1.2.5. Cystic Fibrosis

Iminosugars have also shown potential for the treatment of cystic fibrosis [CF]. The cause of the disease is mutation in the cysic fibrosis transmembrane conductance regulator [CFTR] gene, most commonly the delF508 point mutation. CFTR is a chloride ion channel protein that plays crucial roles in the production and regulation of sweat, digestive juices and mucus. The consequence of the delF508 mutation is that CFTR is not properly trafficked to the plasma membrane, resulting in abnormal water and ion movements. The knock-on effects of this inefficiency manifest throughout the body and can result in poor digestion, infertility, and pulmonary infections caused by the build-up of hyperviscous mucus. Thick pancreatic mucus traps digestive enzymes, leading to malnutrition and irreversible pancreatic damage that may prevent insulin production and lead to cystic fibrosis related diabetes. Almost all men with CF are infertile as a consequence of the failure of the vas deferens to form properly during embryonic development, and women can also experience fertility issues as a result of thickened cervical mucus or failure to ovulate caused by malnutrition. The most serious consequence of CF is hyperviscous mucus in the lungs, which is prone to bacterial infection, resulting in inflammation and damage to the lung tissue. This causes breathing difficulties and hypoxia, as well as pulmonary hypertension and even heart failure; the life expectancy of CF patients is around forty in the USA.[45] Current treatments for the disease target the symptoms as opposed to the cause, commonly employing courses of antibiotics to stave off bacterial infections and various mechanical methods for the dislodging of bronchial mucus; there is therefore a very real need for therapies that tackle the underlying cause of CF, defective CFTR.

The problems with the mutant CFTR protein are associated with protein misfolding; the primary consequence is not a reduced functional capacity of the protein, but inefficient transportation from the ER to the plasma membrane.

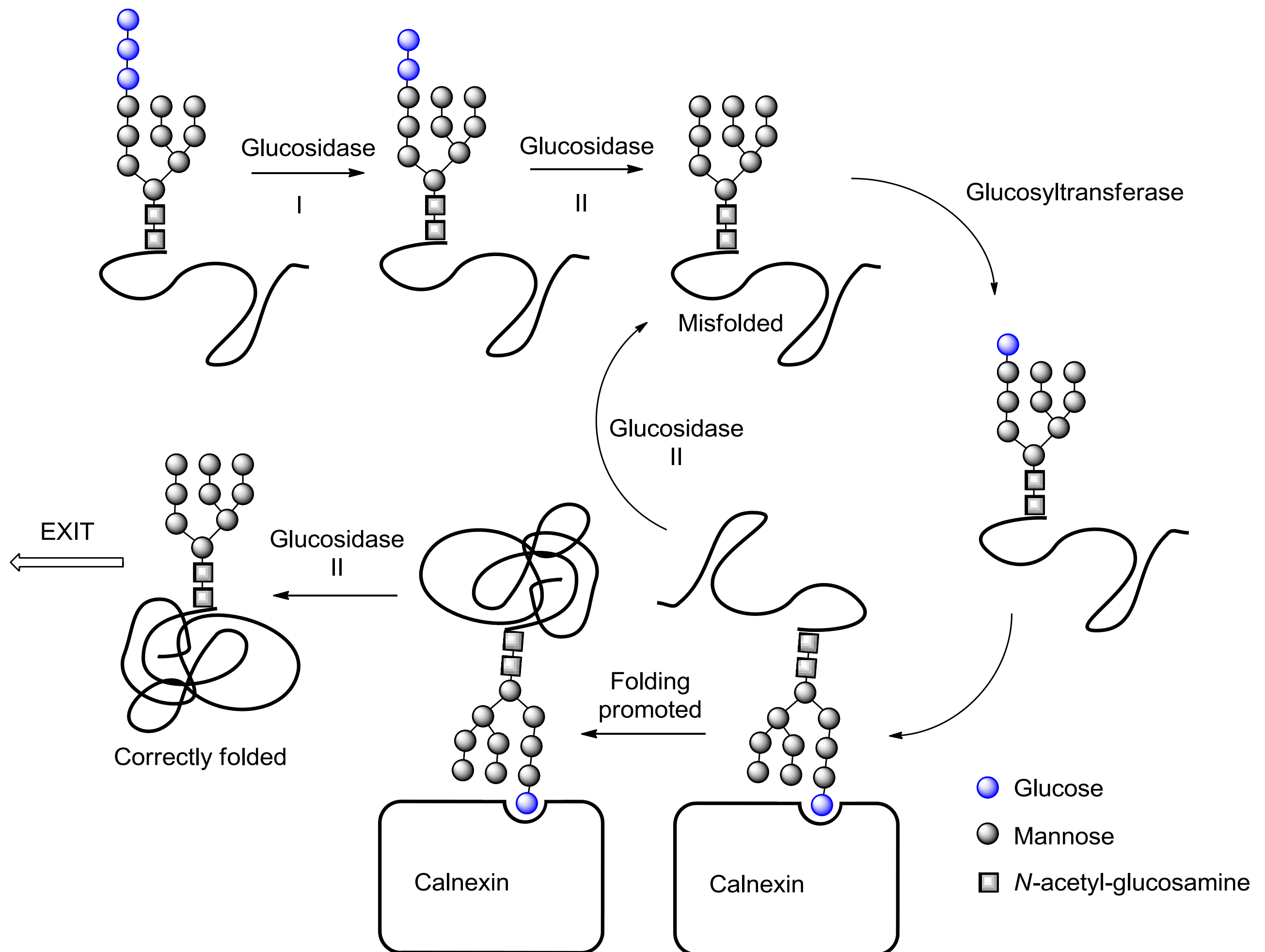


Figure 1.8: The ER quality control mechanism

Following *N*-linked glycosylation, ER glucosidases I and II are responsible for removal of the three terminal glucose residues of the glycan, to give a high mannose structure on the surface of unfolded CFTR (**Figure 1.8**). Correct folding is then normally induced by glucosylation by luminal glucosyltransferase, giving a monoglucosylated glycan that is a ligand for the chaperone calnexin. Association with calnexin then promotes correct folding and CFTR bearing the high mannose glycan is released by the action of ER glucosidase II. If the protein is still misfolded, it is again

recognised by glucosyltransferase and the cycle repeats, correctly folded CFTR is then transported out of the ER and ultimately reaches the plasma membrane. In the case of delF508-CFTR correct folding cannot be achieved and it is perpetually resubmitted to this cycle. It also associates with calnexin for an abnormally long time and is thus trapped in the ER; despite having the potential to function as an ion channel protein, delF508-CFTR fails the ER quality control and is ultimately destined for degradation. Extended association of delF508-CFTR with calnexin is a major cause of retention of the protein in the ER, and it has been shown that cells expressing a mutant calnexin can partially avoid this trapping.[46] Partial restoration of delF508-CFTR by the action of iminosugars has been attributed to disruption of interactions between delF508-CFTR and calnexin.[47] The theory of this approach was to disrupt the ER quality control pathway by targeting glucosidase I/II with *N*B-DNJ **1.13**, by doing so a higher proportion of delF508-CFTR may retain two or all three glucose units of the initial *N*-linked glycan. These glucosylated species are not effective ligands for calnexin and do not enter the quality control cycle. By reducing the rate at which delF508-CFTR enters the quality control cycle, the opportunity for it to escape the ER and reach the plasma membrane by alternative transport pathways independent of the quality control pathway is increased, partially restoring the function of defective CFTR. Whether defective CFTR rescue by *N*B-DNJ **1.13** is a consequence of glycosidase inhibition has not been firmly established; protein folding is a highly complex event that can follow several pathways, it is possible that disruption of the pathway shown in **Figure 1.8** is not the dominant cause of CFTR rescue by *N*B-DNJ **1.13**. With further investigation this strategy may translate to a clinical treatment of CF that targets the underlying cause of the disease.

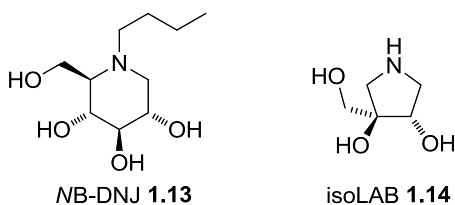


Figure 1.9: Iminosugars that rescue CFTR

A very interesting recent development[48] in CF research with iminosugars comes in the form of the branched pyrrolidine isoLAB **1.14** (**Figure 1.9**), which despite showing no inhibition of any glycosidases, including those resident in the ER, rescues the function of CFTR to a similar extent as *N*B-DNJ **1.13**. This observation suggests the presence of a non-inhibitory pathway for the rescue of CFTR which may ultimately allow a more specifically targeted treatment that avoids side effects associated with off-target glycosidase inhibition. The precise nature of this pathway is unknown, though it is possible that pyrrolidine **1.14** acts as a pharmacological chaperone for CFTR. The array of chaperones, regulatory proteins and enzymes involved in the ER quality control processes provide a host of potential corrector targets; the precise mode of chaperone activity in the rescue of inefficiently trafficked proteins is not well understood.[49] The synthesis and evaluation of compounds like pyrrolidine **1.14** may give further insights regarding the complex nature of protein folding disorders and may one day lead to highly targeted therapeutic agents.

The growing interest in and success of iminosugars as pharmaceuticals makes them highly attractive targets for organic synthesis. The broad range of potential medical applications for the iminosugars as a whole is coupled with the possibility of highly specific biological activity by modification of stereochemical configuration, ring size and substitution. Further investigation of iminosugars through their synthesis and biological evaluation may uncover new biological pathways, and help unravel some of the less well understood glycobiological processes.

1.3. Synthetic methods

The importance of stereoselectivity in organic synthesis cannot be overstated. Stereocontrolled reactions are at the heart of the efficient synthesis of complex organic molecules and a vast amount of research is dedicated to the development of both diastereoselective and enantioselective techniques. There are two approaches: asymmetric synthesis and chiral pool. The former involves the creation of new asymmetric centres by the use of chiral catalysts or chiral auxiliaries to impart stereochemical information to the new centre, and the latter involves the use of optically pure chiral starting materials rich in stereochemistry that can be modified in a controlled fashion. The most extensive source of optically pure and stereochemically complex starting materials is the carbohydrates,[50] and though their use in this context is primarily associated with the synthesis of carbohydrate derivatives, such as iminosugars, their application to the synthesis of complex targets with little carbohydrate character has also been investigated. Carbohydrates are most suitable for targets bearing several contiguous asymmetric centres, particularly hydroxyl groups, but not well-suited to systems containing more isolated stereocentres where a controlled and often lengthy stepwise destruction of stereochemical information would be required to furnish the final product. Iminosugars fall squarely in the former category, and monosaccharides are ideal starting materials for their synthesis.

Only a small proportion of monosaccharides are cheaply available from commercial suppliers: six of the twenty-four hexoses, D-glucose, -mannose, -galactose, -fructose, -tagatose and L-sorbose; and four of the twelve pentoses, D-arabinose, -ribose, -xylose and L-arabinose. Although many of the less available monosaccharides are easily produced on laboratory scale by short chemical sequences, there is clearly a gap in the market for rare sugars. In addition to their potential as carbohydrate building blocks, rare sugars are an important class for the food industry; they are generally not

metabolised and still taste sweet. The industrial production of D-tagatose, which is the main ingredient in the low calorie sweetener Tagatesse, has recently increased its availability.

The Izumori group at the Rare Sugar Institue, Kagawa, Japan have developed a general approach to the interconversion of all the monosaccharides that is approaching commercial scale (**Figure 1.10**). Using a series of biotechnological interconversions, the group have demonstrated access to rare sugars previously unobtainable quantities.[51,52]

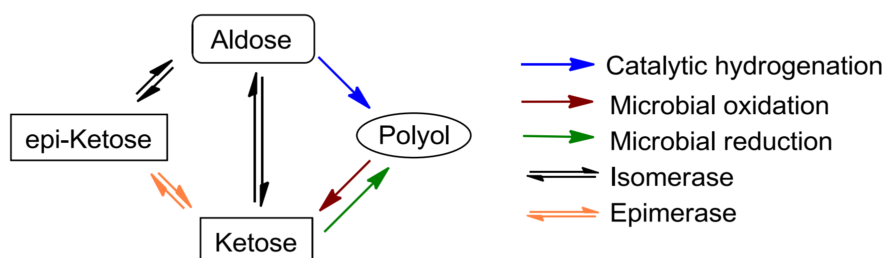


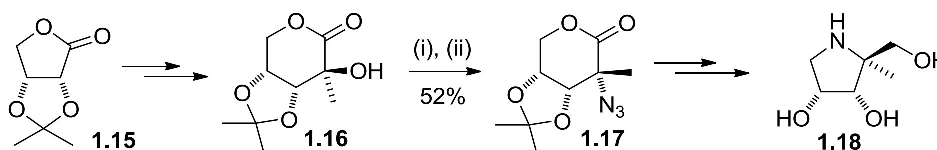
Figure 1.10: Chemoenzymatic interconversion of monosaccharides

Aldoses are equilibrated with their keto forms by isomerases, or can be reduced by catalytic hydrogenation to their corresponding polyols. These polyols can be selectively oxidised at either end to give a single ketose by microbial oxidation, and ketoses are enzymatically epimerised to give new epimeric ketoses and the cycle continues. This approach has been used for the interconversion of all tetroses, pentoses and hexoses and has allowed the use of rare sugars, such as D-psicose, as starting materials. This approach is now being applied to the interconversion of sugars bearing unnatural functionality, and is discussed in further detail in **Chapter 4**.

Elaboration of a carbohydrate scaffold for the synthesis of an iminosugar requires a number of synthetic modifications to furnish the target. Clearly nitrogen must be introduced and cyclisation must be induced to access an iminocyclitol, but further modification of the carbon backbone is often required. The controlled inversion of stereocentres is a very important tool, either specifically by S_N2, or selectively by reduction of prochiral ketones with asymmetric induction. In either case it is the stereochemical information present in the starting material that dictates the stereoisomer

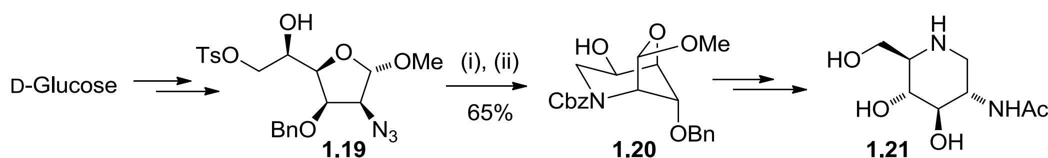
produced. Furthermore, chain extension techniques are common, as well as chain truncation reactions and there are several methods for the introduction of branches; that is addition of carbon substituents to a non-terminal position in the starting monosaccharide backbone. Examples of some synthetic methods for the synthesis of iminosugars are given below.

The most prevalent method for introduction of nitrogen is nucleophilic displacement, with common nitrogen nucleophiles including hydrazine, ammonia, benzylamine, pthalimide and azide. Of these the most successful is probably azide as it avoids nitrogen overalkylation associated with amines and hydrazine, and it is a very powerful nucleophile that can even be introduced at a neopentyl or tertiary positions given a leaving group of appropriate reactivity.[53] Furthermore, once installed the azido group is unreactive under a broad range of reaction conditions and is easily reduced to amine functionality when required. While halides are generally appropriate leaving groups for primary positions, sulfonates are generally employed for more hindered alcohols with tosylates and mesylates often being sufficiently reactive for S_N2 at secondary positions. Imidazoylsulfonates and trifluoromethanesulfonates [triflates] are even more reactive; azide has been shown to displace tertiary triflates with complete inversion of configuration, the *ribo*-azidolactone **1.17** is the major product of displacement of the triflate from alcohol **1.16** (**Scheme 1.1**).[53] Side products were formed by elimination of triflic acid, but no *arabino*-configured analogue was isolated; the displacement presumably proceeds by a highly selective S_N1 reaction, the stereoselectivity of which is controlled by the boat conformation of the carbocation.



Reagents: (i) Tf_2O, Py; (ii) NaN_3
Scheme 1.1: Azide displacement of a tertiary triflate

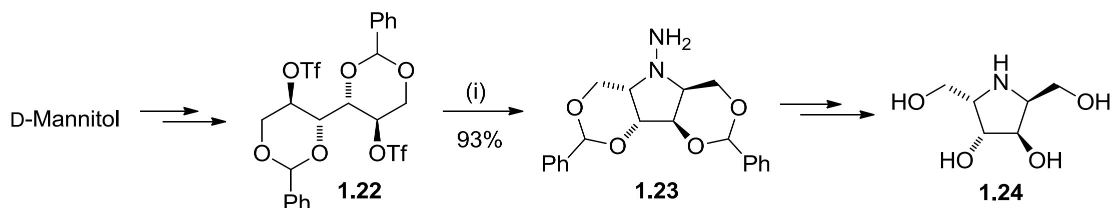
The ring opening of epoxides is a closely related approach that has also been utilised successfully for the introduction of azide and other nucleophiles. Formation of an epoxide by intramolecular S_N2 followed by ring opening allows the introduction of a nucleophile with net retention of configuration, which is a very useful tool in iminosugar synthesis.[54] Epimeric α-azido lactones can be equilibrated under mildly basic conditions to give the thermodynamically favoured azide,[55] and in certain cases can allow very efficient inversion of configuration following S_N2 introduction of azide, giving net retention. An alternative method of nitrogen introduction is reductive amination of a sugar's carbonyl functionality, commonly achieved by condensation with hydroxylamine or benzylamine and reduction of the resultant oxime/imine, which can be performed in one pot. The main complication with this approach is diastereomeric mixtures produced in the reductive amination of ketoses, where the imine is prochiral, but these reactions can be very selective owing to asymmetric induction by the carbohydrate scaffold. Free amines generated in this way normally require protection as amides or carbamates due to their reactivity and, in many cases, poor mobility on silica gel. With nitrogen incorporated into the carbohydrate backbone, cyclisation can then be achieved using the same techniques; intramolecular substitution or intramolecular reductive amination. Azido-sulfonates, for example, can cyclise to iminocyclitols by reduction of the azide functionality and intramolecular S_N2 as demonstrated by the cyclisation of azido-tosylate **1.19** (**Scheme 1.2**).[56,57]



Reagents: (i) H_2, Pd; (ii) $ClCO_2Bn$
Scheme 1.2: Intramolecular S_N2 ring closure

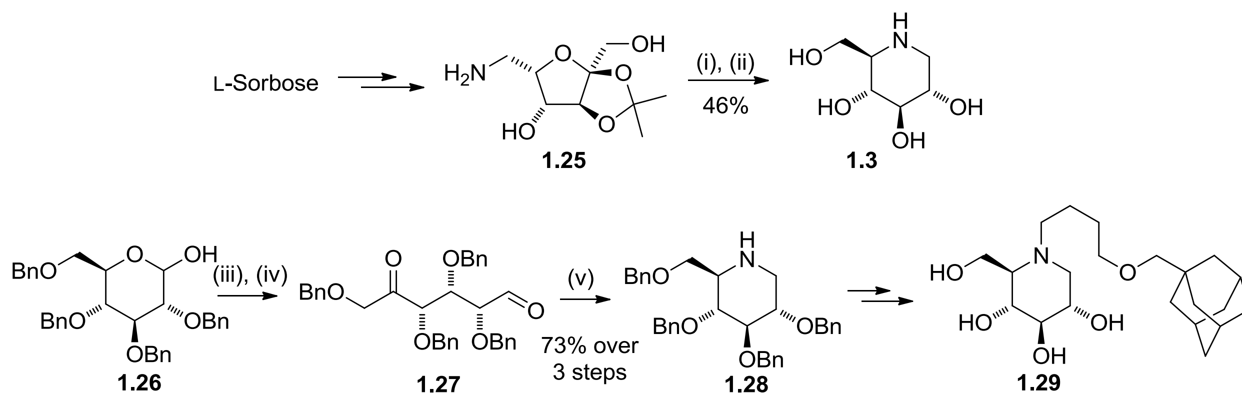
Iminocyclitols may also be constructed by nucleophilic double displacements. In these reactions disulfonates are treated with primary amines or ammonia, introducing nitrogen and effecting

cyclisation in a single step. This approach is often taken by dimesylation of a 1,4 diol followed by a benzylamine double displacement,[58] but a particularly neat example is the double displacement of ditriflate **1.22** with hydrazine;[59] the key step in a highly efficient synthesis of pyrrolidine **1.24** (**Scheme 1.3**). This reaction is one of the very few successful double displacements of ditriflates reported in the literature.



Reagents: (i) NH_2NH_2
Scheme 1.3: Double displacement of a ditriflate

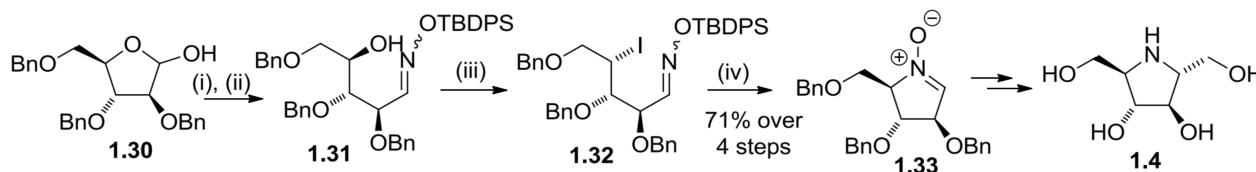
Intramolecular reductive amination is a very common approach to cyclisation that has great historical significance in the field of the iminosugars, being utilised for the first syntheses of DNJ **1.3** and DMDP **1.4**. If an amino sugar is submitted to reductive conditions, iminocyclitols are obtained by imine formation and reductive amination in one pot. In this way protected 6-amino-L-sorbose **1.25** was converted to DNJ **1.3** in a highly stereoselective intramolecular reductive amination in the first synthesis of DNJ **1.3** by Paulsen (**Scheme 1.4**).[11]



Reagents: (i) HCl, H_2O; (ii) H_2, PtO_2; (iii) $LiAlH_4$; (iv) DMSO, $(ClCO)_2$, then TEA; (v) $NaBH_3CN$, NH_4HCO_2
Scheme 1.4: Reductive amination as a tool for nitrogen introduction and ring closure

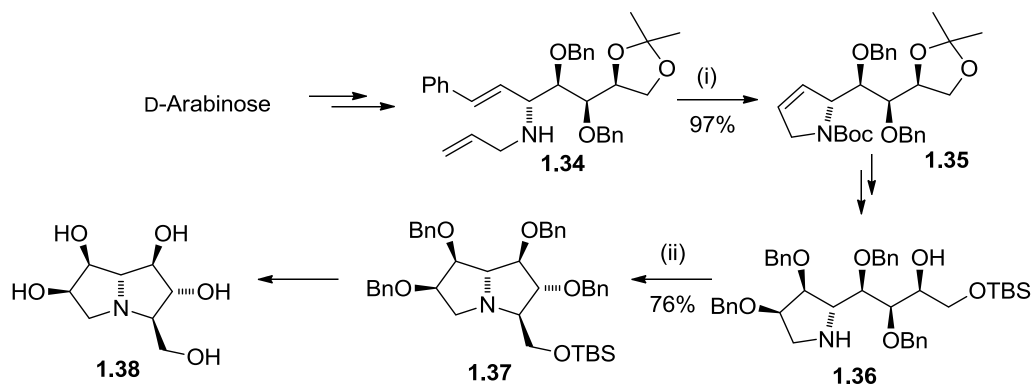
Double reductive amination reactions have also been reported; the diketone **1.27** was effectively cyclised in this way to produce the *N*-substituted DNJ **1.29**, an extremely potent β-glucocerebrosidase inhibitor.[60]

Oximes also make effective internal nucleophiles, cyclising by S_N2 displacement to give cyclic nitrones. A recent demonstration of this versatile approach provided routes to a library of 2,5-imino-hexitols, such as DMDP **1.4**.[61] The D-arabinose derived protected oxime **1.31** was cyclised to the corresponding nitrone **1.33** (**Scheme 1.5**), the carbon scaffold of which was successfully extended with high diastereoselectivity to ultimately give DMDP **1.4**. Cyclisation was achieved with overall retention of configuration by two sequential S_N2 inversions; an Appel reaction installed an appropriate leaving group with inversion, followed by its displacement by the oxime. Alternatively, treatment of protected oxime **1.31** with mesyl chloride allowed cyclisation with inversion of configuration. All eight stereoisomers of nitrone **1.33** were produced from the D-pentoses, demonstrating the generality of the approach.



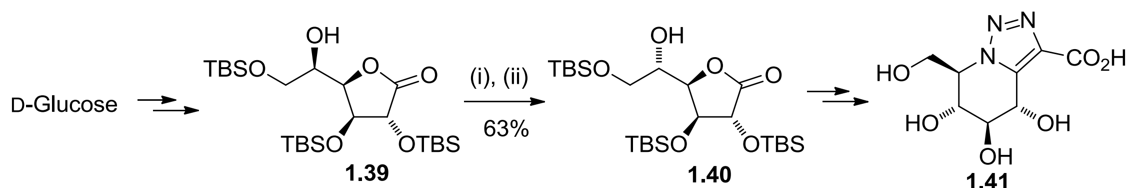
Reagents: (i) NH_2OH; (ii) TBDPSCl, imidazole; (iii) I_2, PPh_3, imidazole; (iv) TBAF
Scheme 1.5: Iminosugars from cyclic nitrones

A recent strategy for the synthesis of several members of the pyrrolizidine group utilised Grubbs ring closing metathesis and the Mitsunobu reaction for the construction of the two fused five-membered rings (**Scheme 1.6**).[62] Following construction of the first pyrrolidine ring by Grubbs metathesis of diene **1.34**, the alkene **1.35** was ultimately converted to uniflorine A **1.38** by Upjohn dihydroxylation followed by an intramolecular S_N2 ring closure under Mitsunobu conditions.



Reagents: (i) Grubbs I catalyst; (ii) DIAD, PPh_3
Scheme 1.6: Ring closure by Grubbs metathesis and Mitsunobu reactions

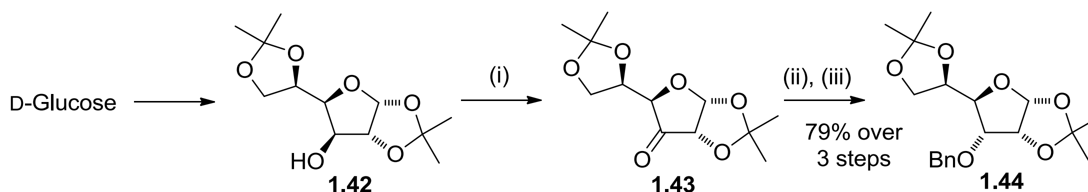
Alternative stereochemical configurations and substitutions were achieved by epoxidation of alkene **1.35**, followed by epoxide ring opening with hydride or hydrogen sulfate, and Misunobu reactions with *p*-nitrobenzoic acid allowed inversion of individual hydroxyls, although only in moderate yield. The epimerisation of alcohols is often required in the synthesis of iminosugars, and there are three predominant methods for these transformations: S_N2 displacement with trifluoroacetate, oxidation and diastereoselective reduction *via* prochiral ketones, and ring opening of epoxides.



Reagents: (i) Tf_2O, Py; (ii) CF_3CO_2Cs, then K_2CO_3
Scheme 1.7: Alcohol inversion by displacement of triflates with trifluoroacetate

Displacement of triflates with trifluoroacetate often provides access to inverted alcohols more efficiently than Mitsunobu inversion, and the intermediate trifluoroacetylated alcohol is readily exchanged in the presence of aqueous or methanolic base to give the free hydroxyl. There are examples of efficient inversion of alcohols with sodium trifluoroacetate,[63] but superior yields are generally reported with a caesium counterion.[64] For example, formation of the *gluco*-configured

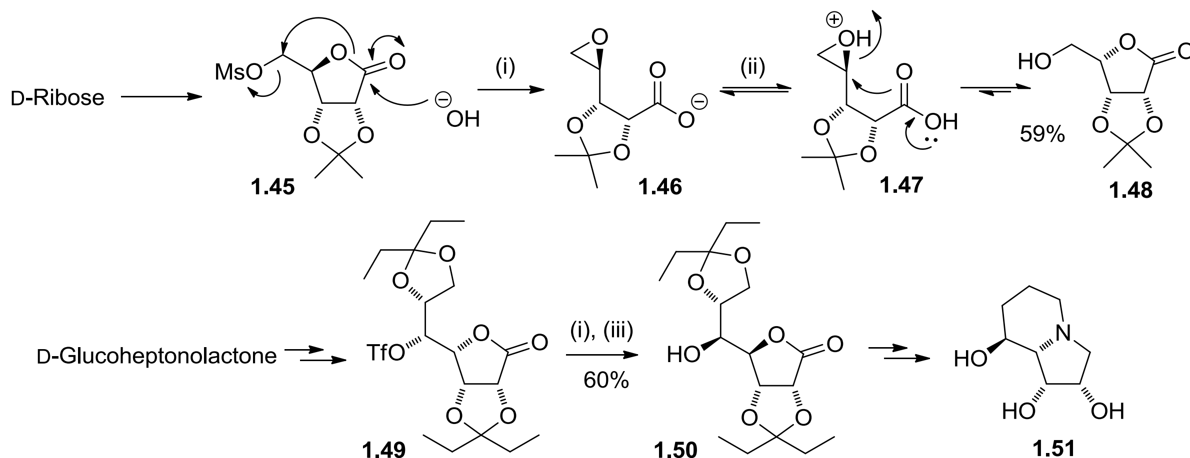
triazole **1.41** (**Scheme 1.7**) from D-glucose required introduction of azide at C5 with overall retention, achieved by trifluoroacetate inversion of **1.39** at C5 followed by S_N2 azide displacement.[65]



Reagents: (i) PCC; (ii) $NaBH_4$; (iii) BnBr, NaH
Scheme 1.8: Inversion of configuration by reduction of a prochiral ketone

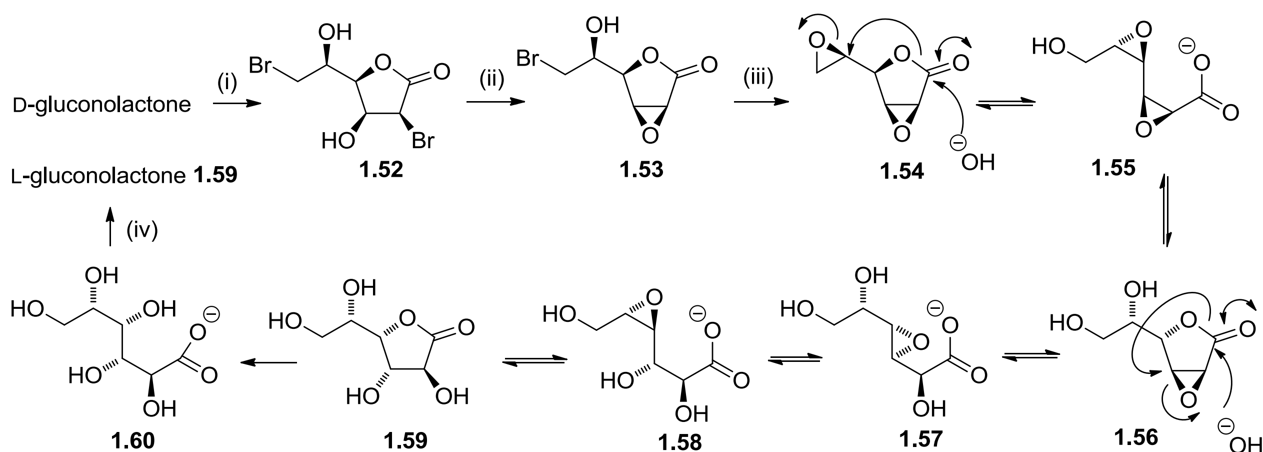
Inversion of configuration by an oxidation-reduction sequence can lead to clean inversion of configuration under the right circumstances. This classic transformation is best exemplified by the inversion of diacetone glucose **1.42** at C3 (**Scheme 1.8**);[66] the *Re* face of intermediate ketone **1.43** is sterically hindered by the 1,2-*O*-isopropylidene, leading to clean addition of hydride to the *Si* face to give the *allo* scaffold **1.44**. The major limitation with this strategy is the requirement of considerable steric bulk for effective asymmetric induction in the reduction step, but it is nevertheless an extremely useful tool.

Another valuable transformation relating to alcohol inversion can be realised on treatment of a suitably activated carbohydrate lactone with base, followed by acidic workup, as exemplified in the synthesis of L-lyxonolactone **1.48** from D-ribose (**Scheme 1.9**).[67] The outcome of this reaction is not intutitive; the mechanism proceeds by base-catalysed hydrolysis of the lactone, ejecting an alkoxide which displaces the adjacent mesylate to form an intermediate *ribo*-configured epoxide **1.46**. Upon acidification, intramolecular epoxide opening in a 5-exo-tet fashion proceeds with inversion of configuration to give the *lyxo* scaffold **1.48**. If a secondary mesylate is used, two stereocentres are inverted concurrently by the same mechanism,[68] as exemplified in the synthesis of L-swainsonine **1.51** (**Scheme 1.9**).[69]



Reagents: (i) KOH; (ii) HCl; (iii) Amberlyst 15 (H^+ resin)
Scheme 1.9: Inversion of alcohols *via* a epoxides

A truly remarkable example of epoxide mediated inversion is the synthesis of L-gluconolactone from D-gluconolactone, a transformation involving inversion of every stereocentre of the starting material (**Scheme 1.10**). The dibromo mannonolactone **1.52**, obtained by treatment of D-gluconolactone with HBr/acetic acid, was then treated with potassium carbonate in acetone to give the *manno* 2,3-epoxide **1.53**.[70] This epoxidation proceeds by base catalysed epimerisation at C2, giving an intermediate *gluco* bromo-lactone with the appropriate *tran*-2,3 relationship required for epoxide formation.

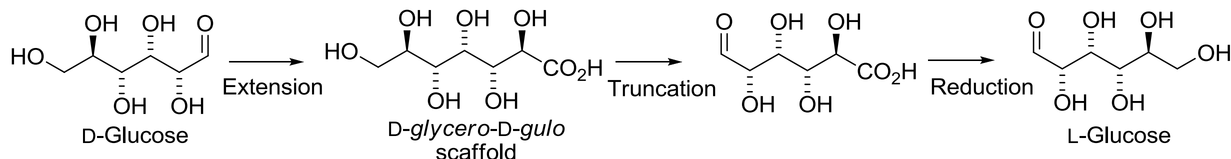


Reagents: (i) HBr; (ii) K_2CO_3; (iii) KOH; (iv) H^+
Scheme 1.10: Inversion of every sterocentre of gluconolactone

Treatment of the bromo-epoxide **1.53** with aqueous potassium hydroxide leads to the formation of the diepoxide **1.54** and initiates a series of epimerisation reactions involving Payne rearrangements.[71] The diepoxide **1.55** is presumably opened by intramolecular 5-exo-trig displacement by the carboxylate residue to give transient lactone **1.56**, which is then hydrolysed and undergoes a Payne rearrangement to *trans*-3,4-epoxide **1.57**, which is in equilibrium with *trans*-4,5-epoxide **1.58** by further Payne rearrangement. The *trans*-4,5-epoxide **1.58** is free to cyclise according to Baldwin's rules, giving L-gluconolactone **1.59**, which is irreversibly hydrolysed under the reaction conditions. Acidic workup then gives L-gluconolactone **1.59** in a very short and quite amazing sequence.

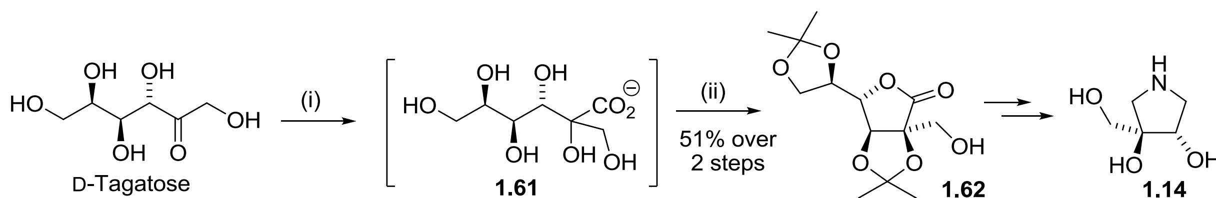
Chain extension of a monosaccharide's carbon backbone provides access to higher sugars that can provide very useful synthetic scaffolds. Fully protected lactones may be extended by various textbook carbon nucleophiles; Grignard reagents, organolithiums, enolates including Reformatskii reagents, and dithianates to name but a few. Aldoses on the other hand are almost always obtained in hemiacetal form, so the scope for chain extension is somewhat limited due to inevitable hydroxyl functionality. There are three particularly popular methods for this task, none of which are new, but all of which are still common practice in carbohydrate laboratories: Kiliani ascension involving addition of cyanide followed by cyanohydrin hydrolysis; Henry reactions proceeding by addition of nitromethanide followed by Nef degradation; and Wittig chain extensions followed by dihydroxylation. These extension techniques are particularly useful as the stereochemical configuration of the extended sugar can normally be predicted by the Felkin-Ahn model, or in the case of Wittig extensions is controllable by asymmetric dihydroxylation or epoxidation. Higher sugars can thus be produced that incorporate uncommon stereochemical motifs and controlled cleavage of the unwanted portion of the scaffold, commonly with periodate, provides a rare sugar building block. L-Glucose can be produced from D-glucose in this way; Kiliani chain extension at

the aldehydic terminus of D-glucose gives an extended D-*glycero*-D-*gulo* scaffold, and subsequent oxidative cleavage at the opposite terminus ultimately provides L-glucose. This requires a simple protecting group strategy that will be discussed in **Chapter 2**, the essential concept is depicted in **Scheme 1.11**.



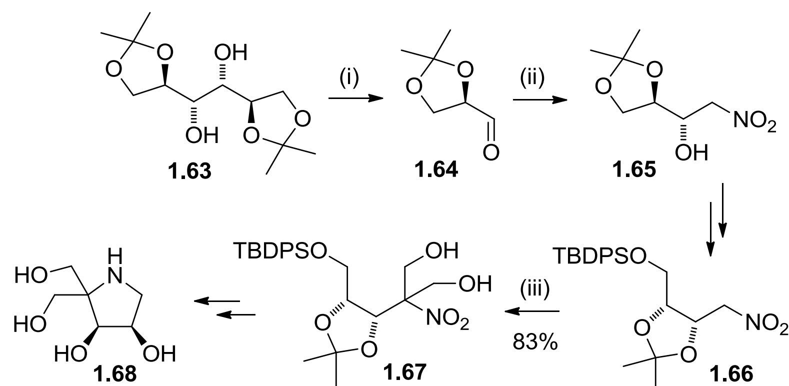
Scheme 1.11: Chain extension-truncation sequences can provide rare sugars

Branched chain iminosugars are uncommon in comparison to their unbranched counterparts, but can provide inhibitors with very high specificity and potency, as will be discussed in **Chapter 3**. Their synthesis can be achieved through the use of branched carbohydrate building blocks, which can be constructed by chain extension of ketoses.



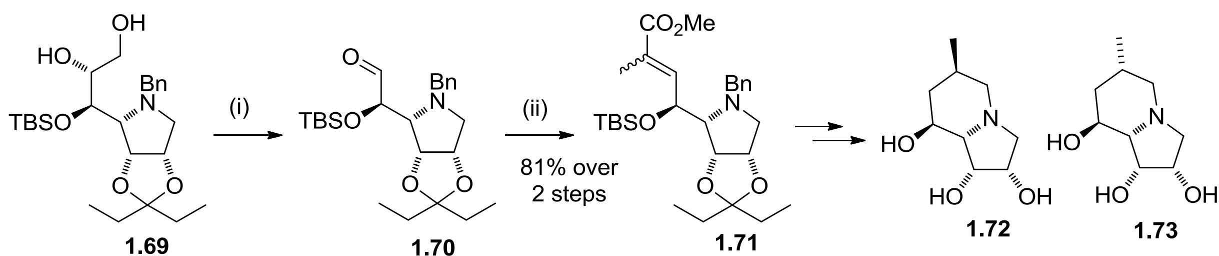
Reagents: (i) NaCN, H_2O; (ii) Me_2CO, H_2SO_4
Scheme 1.12: Branched chirons for iminosugar synthesis by Kiliani extension of ketoses

The major product obtained by Kiliani extension of D-tagatose followed by acetonation is the branched D-talonolactone **1.62** (**Scheme 1.12**),[72] which was successfully utilised as a carbohydrate chiron in the synthesis of potential anti-cystic fibrosis agent **1.14**.[48] Similar application of the Kiliani reaction to other D-fructose,[73] L-sorbose[73] and D-psicose[72] gave the corresponding branched *manno*, *gulo* and *allo*-configured lactones.



Reagents: (i) $NaIO_4$; (ii) $MeNO_2$, KF; (iii) $(HCHO)_n$, NaOAc
Scheme 1.13: Henry reactions in the synthesis of branched iminosugars

An interesting recent application of the Henry reaction to the synthesis of branched iminosugars is presented in **Scheme 1.13**. Starting from the D-mannitol derived diacetonide **1.63**, periodate cleavage gave the glyceraldehyde **1.64**, a suitable substrate for chain extension with nitromethane. Nitroalcohol **1.65** was then used as the carbon nucleophile in further Henry reactions with paraformaldehyde to give the hydroxymethyl-branched scaffold **1.67**. The nitro group ultimately provided an internal nitrogen nucleophile, allowing formation of the branched iminosugar **1.68**.[74] This strategy has also been extended to the synthesis of branched azepane iminosugars.[75]

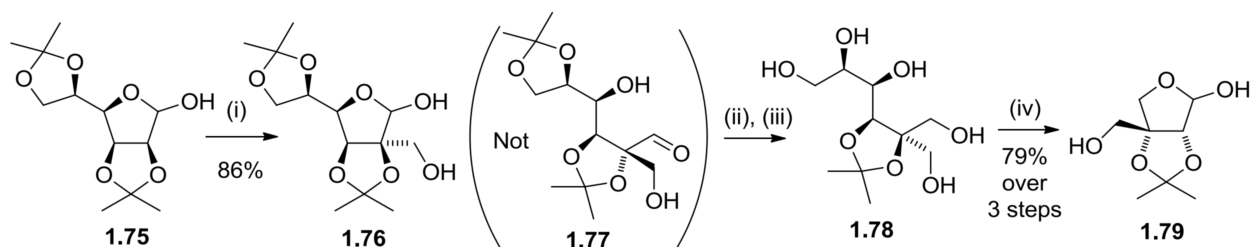


Reagents: (i) $NaIO_4$; (ii) $Ph_3P{=}C(Me)CO_2Me$ **1.74**
Scheme 1.14: Wittig chain extension leading to branched iminosugars

Appropriate Wittig reagents also allow access to branched scaffolds for iminosugar synthesis (**Scheme 1.14**). Phosphorous ylid **1.74** was utilised for chain extension and the introduction of the methyl branch in the synthesis of 6-*C*-methyl-L-swainsonines **1.72** and **1.73**.[68]

The diol in **1.69** functioned as a protected aldehyde, revealed on periodate cleavage. Wittig extension of aldehyde **1.70** gave the corresponding methyl-branched unsaturated esters **1.71** that were ultimately transformed into the two epimeric methyl-branched swainsonines **1.72** and **1.73**.

Hydroxymethyl branches are readily introduced at the C2 position of aldoses by the Ho aldol reaction. On treatment with formaldehyde under basic conditions, aldoses such as diacetone mannose **1.75**, form adducts resulting in a hydroxymethyl-branched sugar (**Scheme 1.15**).

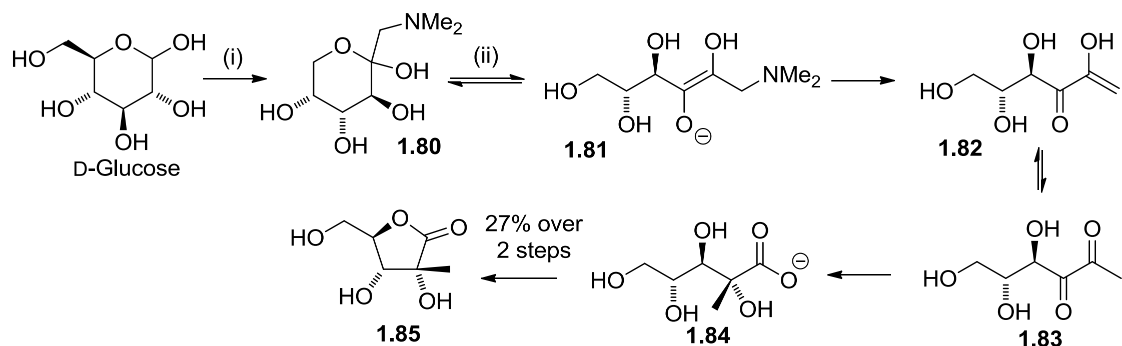


Reagents: (i) HCHO, K_2CO_3; (ii) dil. H_2SO_4; (iii) $NaBH_4$; (iv) $NaIO_4$
Scheme 1.15: Introduction of branches by Ho aldol addition

The 2,3-*O*-isopropylidene is required for direction of the stereochemical outcome of the Ho aldol reaction, in which the thermodynamically favoured furanose **1.76** is formed in preference to open-chain aldehyde **1.77**. Reduction of the lactol **1.76** and selective hydrolysis of the terminal acetonide gave tetraol **1.78**, which was readily converted to the acetonide of naturally occurring branched sugar D-apiose **1.79** by two successive periodate cleavages.[76]

Methyl branches may be introduced to aldoses by conversion to Amadori ketoses, followed by Amadori rearrangement in aqueous calcium oxide (**Scheme 1.16**).[77-79] The net result is a C2 branched lactone with a backbone shortened by one carbon, e.g. a branched pentonolactone from a hexose, bearing a 2,3-*erythro* diol as the major product. In the case of D-glucose, conversion to the Amadori amino-fructose **1.80** followed by calcium oxide treatment gave branched ribonolactone **1.85** without the formation of the *arabino* epimer. The mechanism of the Amadori rearrangement is complex, and was suggested to proceed by elimiation of dimethylamine *via* ene-diol **1.81**, giving the

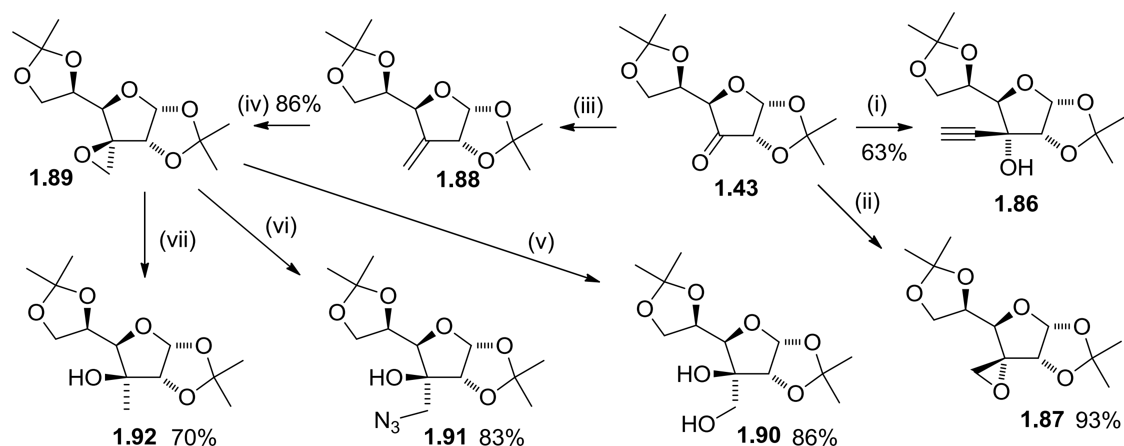
enol **1.82** which is in equilibrium with the the diketone **1.83**. The terminal methyl group is then transferred to the adjacent ketone by a benzilic acid rearrangement in a chelated-Felkin-Ahn fashion, presumably a result of the presence of calcium ions, giving **1.84**. Lactonisation during acidic workup gives **1.85**.



Reagents: (i) $NHMe_2$; (ii) CaO
Scheme 1.16: Amadori rearrangement of Amadori ketoses gives facile access to methyl-branched lactones

Although the overall yield is low for a two step synthesis, it is a complicated transformation of a very cheap starting material that would otherwise require protecting group strategies and chromatography; the transformation is amenable to scale-up and has been performed on kilo scale.[79] Synthetic manipulation of prochiral non-anomeric ketones can provide a range of branched derivatives. This approach has been extensively applied to the readily accessed D-glucose derived ketone **1.43**. The stereoselectivity of direct addition to ketone **1.43** is highly selective owing to asymmetric induction by the 1,2-*O*-isopropylidene as shown previously for hydride addition (**Scheme 1.8**); carbon nucleophiles, such as acetylenylmagnesium bromide,[80] which provides branched derivative **1.86**, can also be applied with similar selectivity (**Scheme 1.17**). Insertion of methylene to the less hindered *Si* face of the CO double bond can be achieved by addition of dichloromethanide, followed by dechlorination of the resultant chloro-epoxide with tributyltin hydride, to give the *allo*-configured epoxide **1.87**.[81] The epimeric *gluco*-configured epoxide **1.89** can

be accessed *via* alkene **1.88**, formed by a Wittig chain extension reaction. Epoxidation of the less hindered face of alkene **1.88** with *m*-chloroperbenzoic acid gave epoxide **1.89** in excellent yield.[82]



Reagents: (i) HCCLi; (ii) $CHCl_2Li$, then Bu_3SnH, AIBN; (iii) $Ph_3P{=}CH_2$; (iv) mCPBA; (v) NaOH; (vi) NaN_3; (vii) $LiAlH_4$
Scheme 1.17: Branched carbohydrated by addition of carbon nucleophiles to prochiral ketones

Epoxides such as **1.87** and **1.89** are highly versatile synthetic intermediates as they may be opened with a variety of nucleophiles; epoxide ring opening with hydride provides methyl-branched sugars (e.g. **1.92**),[83] treatment with hydroxide provides access to hydroxymethyl-branched sugars (e.g. **1.90**),[82] and azide displacement gives the corresponding azidomethyl-branched derivative (e.g. **1.91**).[84] The approach is highly versatile in the case of glucose, where a ketone can be readily accessed and the degree of asymmetric induction is high. Facial discrimination is not always as straightforward, and the approach is therefore not general to large number of carbohydrate scaffolds.

In this chapter, motivation for the synthesis of iminosugars has been identified. These small molecule inhibitors can be highly potent glycosidase inhibitors, leading to a vast number of potential therapeutic applications. DNJ derivatives are currently seeing clinical use, with other iminosugars following at various stages of clinical trials and development. Iminosugars can also show biological activity that is unrelated to glycosidase inhibition, and these more elusive modes of action require dissemination; the synthesis of novel iminosugars and the study of their biological profiles may aid

the understanding of the less well understood pharmacological pathways. The potential of these compounds is enormous, and their chemical synthesis challenging. The tools and strategies available to organic chemists in the carbohydrate field, examples of which have been briefly introduced, are numerous, ingenious and highly valuable. In the following two chapters, strategies for the synthesis of biologically active iminosugars will be presented that have allowed easy access to both important natural products and novel iminocyclitols. In the final chapter, the synthesis of deoxy- and fluoro-hexitols and their applicability to biotechnological isomerisation strategies will be discussed.

1.4. References

1. Zechel, D. L.; Withers, S. G. *Accounts Chem Res* **2000,** *33*, 11-18.
2. Heightman, T. D.; Vasella, A. T. *Angew Chem Int Ed* **1999,** *38*, 750-770.
3. D'Alonzo, D.; Guaragna, A.; Palumbo, G. *Curr Med Chem* **2009,** *16*, 473-505.
4. Horne, G.; Wilson, F. X.; Tinsley, J.; Williams, D. H.; Storer, R. *Drug Discovery Today* **2010,** *In Press, Corrected Proof*.
5. Davis, B. G. *Tetrahedron: Asymmetry* **2009,** *20*, 652-671.
6. Lillelund, V. H.; Jensen, H. H.; Liang, X. F.; Bols, M. *Chem Rev* **2002,** *102*, 515-553.
7. Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000,** *11*, 1645-1680.
8. Inouye, S.; Tsuruoka, T.; Niida, T. *J Antibiot* **1966,** *19*, 288-292.
9. Koyama, M.; Sakamura, S. *Agr Biol Chem Tokyo* **1974,** *38*, 1111-1112.
10. Yagi, M.; Kouno, T.; Aoyagi, Y.; Murai, H. *J Agr Chem Soc Jpn* **1976,** *50*, 571-572.
11. Paulsen, H. *Angew Chem Int Ed* **1966,** *5*, 495-510.
12. Schmidt, D. D.; Frommer, W.; Muller, L.; Truscheit, E. *Naturwissenschaften* **1979,** *66*, 584-585.
13. Welter, A.; Jadot, J.; Dardenne, G.; Marlier, M.; Casimir, J. *Phytochemistry* **1976,** *15*, 747-749.
14. Colegate, S. M.; Dorling, P. R.; Huxtable, C. R. *Aust J Chem* **1979,** *32*, 2257-2264.
15. Hohenschutz, L. D.; Bell, E. A.; Jewess, P. J.; Leworthy, D. P.; Pryce, R. J.; Arnold, E.; Clardy, J. *Phytochemistry* **1981,** *20*, 811-814.
16. Nash, R. J.; Fellows, L. E.; Dring, J. V.; Fleet, G. W. J.; Derome, A. E.; Hamor, T. A.; Scofield, A. M.; Watkin, D. J. *Tetrahedron Letters* **1988,** *29*, 2487-2490.
17. Molyneux, R. J.; Benson, M.; Wong, R. Y.; Tropea, J. E.; Elbein, A. D. *J Nat Prod* **1988,** *51*, 1198-1206.
18. Tepfer, D.; Goldmann, A.; Pamboukdjian, N.; Maille, M.; Lepingle, A.; Chevalier, D.; Denarie, J.; Rosenberg, C. *J Bacteriol* **1988,** *170*, 1153-1161.
19. Nash, R. J.; Rothschild, M.; Porter, E. A.; Watson, A. A.; Waigh, R. D.; Waterman, P. G. *Phytochemistry* **1993,** *34*, 1281-1283.
20. Asano, N.; Yamauchi, T.; Kagamifuchi, K.; Shimizu, N.; Takahashi, S.; Takatsuka, H.; Ikeda, K.; Kizu, H.; Chuakul, W.; Kettawan, A.; Okamoto, T. *J Nat Prod* **2005,** *68*, 1238-1242.

21. Asano, N. *Mech Ageing Dev* **2000,** *116*, 155-156.
22. Kite, G. C.; Horn, J. M.; Romeo, J. T.; Fellows, L. E.; Lees, D. C.; Scofield, A. M.; Smith, N. G. *Phytochemistry* **1990,** *29*, 103-105.
23. Nash, R. J. World Patent WO 06/067419. **2006** Jun 29.
24. Watson, A. A.; Nash, R. J.; Wormald, M. R.; Harvey, D. J.; Dealler, S.; Lees, E.; Asano, N.; Kizu, H.; Kato, A.; Griffiths, R. C.; Cairns, A. J.; Fleet, G. W. J. *Phytochemistry* **1997,** *46*, 255-259.
25. Winchester, B. G. *Tetrahedron: Asymmetry* **2009,** *20*, 645-651.
26. Compain, P.; Martin, O. R.; Editors *Iminosugars: From Synthesis Therapeutic Applications*; John Wiley & Sons Ltd., 2007.
27. Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J Med Chem* **2005,** *48*, 2036-2044.
28. Jespersen, T. M.; Dong, W. L.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew Chem Int Ed* **1994,** *33*, 1778-1779.
29. Jespersen, T. M.; Bols, M.; Sierks, M. R.; Skrydstrup, T. *Tetrahedron* **1994,** *50*, 13449-13460.
30. Jensen, H. H.; Bols, M. *J Chem Soc Perk T 1* **2001**, 905-909.
31. Liu, H. Z.; Liang, X. F.; Sohoel, H.; Bulow, A.; Bols, M. *J Am Chem Soc* **2001,** *123*, 5116-5117.
32. Sels, J.-P. J. E.; Huijberts, M. S. P.; Wolffenbuttel, B. H. R. *Expert Opin. Pharmacother.* **1999,** *1*, 149-156.
33. Taylor, M.; Drickamer, K. *Introduction to Glycobiology*; Oxford University Press, 2003.
34. Vellodi, A. *Brit J Haematol* **2005,** *128*, 413-431.
35. Kolter, T.; Sandhoff, K. *Brain Pathol* **1998,** *8*, 79-100.
36. Butters, T. D.; Dwek, R. A.; Platt, F. M. *Glycobiology* **2005,** *15*, R43-R52.
37. Dimitroff, C. J.; Sharma, A.; Bernacki, R. J. *Cancer Invest* **1998,** *16*, 279-290.
38. Hanahan, D.; Weinberg, R. A. *Cell* **2000,** *100*, 57-70.
39. Shaheen, P. E.; Stadler, W.; Elson, P.; Knox, J.; Winquist, E.; Bukowski, R. M. *Invest New Drugs* **2005,** *23*, 577-81.
40. Niedbala, M. J.; Madiyalakan, R.; Matta, K.; Crickard, K.; Sharma, M.; Bernacki, R. J. *Cancer Res* **1987,** *47*, 4634-4641.
41. Plucinsky, M. C.; Prorok, J. J.; Alhadeff, J. A. *Cancer-Am Cancer Soc* **1986,** *58*, 1484-1487.
42. Yamamoto, N.; Naraparaju, V. R.; Asbell, S. O. *Cancer Res* **1996,** *56*, 2827-2831.
43. Mohamad, S. B.; Nagasawa, H.; Uto, Y.; Hori, H. *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology* **2002,** *132*, 1-8.
44. Greco, M.; De Mitri, M.; Chiriaco, F.; Leo, G.; Brienza, E.; Maffia, M. *Cancer Lett* **2009,** *283*, 222-229.
45. *Cystic Fibrosis Foundation Patient Registry: Annual Data Report* **2008**.
46. Okiyoneda, T.; Wada, I.; Jono, H.; Shuto, T.; Yoshitake, K.; Nakano, N.; Nagayama, S.; Harada, K.; Isohama, Y.; Miyata, T.; Kai, H. *Febs Lett* **2002,** *526*, 87-92.
47. Norez, C.; Noel, S.; Wilke, M.; Bijvelds, M.; Jorna, H.; Melin, P.; DeJonge, H.; Becq, F. *Febs Lett* **2006,** *580*, 2081-2086.
48. Best, D.; Jenkinson, S. F.; Saville, A. W.; Alonzi, D. S.; Wormald, M. R.; Butters, T. D.; Norez, C.; Becq, F.; Bleriot, Y.; Adachi, I.; Kato, A.; Fleet, G. W. J. *Tetrahedron Letters* **2010,** *51*, 4170-4174.
49. Gong, Q. M.; Jones, M. A.; Zhou, Z. F. *J Biol Chem* **2006,** *281*, 4069-4074.
50. Bols, M. *Carbohydrate Building Blocks*; Wiley, 1995.
51. Izumori, K. *J Biotechnol* **2006,** *124*, 717-722.

52. Granstrom, T. B.; Takata, G.; Tokuda, M.; Izumori, K. *J Biosci Bioeng* **2004,** *97*, 89-94.
53. da Cruz, F. P.; Horne, G.; Fleet, G. W. J. *Tetrahedron Letters* **2008,** *49*, 6812-6815.
54. Fleet, G. W. J.; Smith, P. W. *Tetrahedron Letters* **1985,** *26*, 1469-1472.
55. Krulle, T. M.; Davis, B.; Ardron, H.; Long, D. D.; Hindle, N. A.; Smith, C.; Brown, D.; Lane, A. L.; Watkin, D. J.; Marquess, D. G.; Fleet, G. W. J. *Chem Commun* **1996**, 1271-1272.
56. Fleet, G. W. J.; Smith, P. W. *Tetrahedron* **1987,** *43*, 971-978.
57. Fleet, G. W. J.; Fellows, L. E.; Smith, P. W. *Tetrahedron* **1987,** *43*, 979-990.
58. Fleet, G. W. J.; Son, J. C.; Green, D. S.; Dibello, I. C.; Winchester, B. *Tetrahedron* **1988,** *44*, 2649-2655.
59. Shing, T. K. M. *Tetrahedron* **1988,** *44*, 7261-7264.
60. Wennekes, T.; Lang, B.; Leeman, M.; van der Marel, G. A.; Smits, E.; Weber, M.; van Wiltenburg, J.; Wolberg, M.; Aerts, J. M. F. G.; Overkleeft, H. S. *Org Process Res Dev* **2008,** *12*, 414-423.
61. Tsou, E.-L.; Yeh, Y.-T.; Liang, P.-H.; Cheng, W.-C. *Tetrahedron* **2009,** *65*, 93-100.
62. Ritthiwigrom, T.; Willis, A. C.; Pyne, S. G. *J Org Chem* **2009,** *75*, 815-824.
63. Csuk, R.; Honig, H.; Nimpf, J.; Weidmann, H. *Tetrahedron Letters* **1980,** *21*, 2135-2136.
64. Bell, A. A.; Pickering, L.; Finn, M.; delaFuente, C.; Krulle, T. M.; Davis, B. G.; Fleet, G. W. J. *Synlett* **1997**, 1077-1078.
65. Krülle, T. M.; de la Fuente, C.; Pickering, L.; Aplin, R. T.; Tsitsanou, K. E.; Zographos, S. E.; Oikonomakos, N. G.; Nash, R. J.; Griffiths, R. C.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **1997,** *8*, 3807-3820.
66. Fleet, G. W. J.; Ramsden, N. G.; Dwek, R. A.; Rademacher, T. W.; Fellows, L. E.; Nash, R. J.; Green, D. S.; Winchester, B. *J Chem Soc Chem Commun* **1988**, 483-485.
67. Batra, H.; Moriarty, R. M.; Penmasta, R.; Sharma, V.; Stanciuc, G.; Staszewski, J. P.; Tuladhar, S. M.; Walsh, D. A. *Org Process Res Dev* **2006,** *10*, 484-486.
68. Hakansson, A. E.; van Ameijde, J.; Guglielmini, L.; Horne, G.; Nash, R. J.; Evinson, E. L.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2007,** *18*, 282-289.
69. Håkansson, A. E.; van Ameijde, J.; Horne, G.; Nash, R. J.; Wormald, M. R.; Kato, A.; Besra, G. S.; Gurcha, S.; Fleet, G. W. J. *Tetrahedron Letters* **2008,** *49*, 179-184.
70. Lundt, I.; Pedersen, C. *Synthesis* **1992**, 669-672.
71. Lundt, I.; Madsen, R. *Top Curr Chem* **2001,** *215*, 177-191.
72. Soengas, R.; Izumori, K.; Simone, M. I.; Watkin, D. J.; Skytte, U. P.; Soetaert, W.; Fleet, G. W. J. *Tetrahedron Letters* **2005,** *46*, 5755-5759.
73. Hotchkiss, D.; Soengas, R.; Simone, M. I.; van Ameijde, J.; Hunter, S.; Cowley, A. R.; Fleet, G. W. J. *Tetrahedron Letters* **2004,** *45*, 9461-9464.
74. Otero, J. M.; Soengas, R. G.; Estévez, J. C.; Estévez, R. J.; Watkin, D. J.; Evinson, E. L.; Nash, R. J.; Fleet, G. W. J. *Org Lett* **2007,** *9*, 623-626.
75. Otero, J. M.; Estévez, A. M.; Soengas, R. G.; Estévez, J. C.; Nash, R. J.; Fleet, G. W. J.; Estévez, R. J. *Tetrahedron: Asymmetry* **2008,** *19*, 2443-2446.
76. Ho, P. T. *Can J Chem* **1979,** *57*, 381-383.
77. Hotchkiss, D. J.; Jenkinson, S. F.; Storer, R.; Heinz, T.; Fleet, G. W. J. *Tetrahedron Letters* **2006,** *47*, 315-318.
78. Hotchkiss, D. J.; Soengas, R.; Booth, K. I. V.; Weymouth-Wilson, A. C.; Eastwick-Field, V.; Fleet, G. W. J. *Tetrahedron Letters* **2007,** *48*, 517-520.
79. Booth, K. V.; da Cruz, F. P.; Hotchkiss, D. J.; Jenkinson, S. F.; Jones, N. A.; Weymouth-Wilson, A. C.; Clarkson, R.; Heinz, T.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2008,** *19*, 2417-2424.

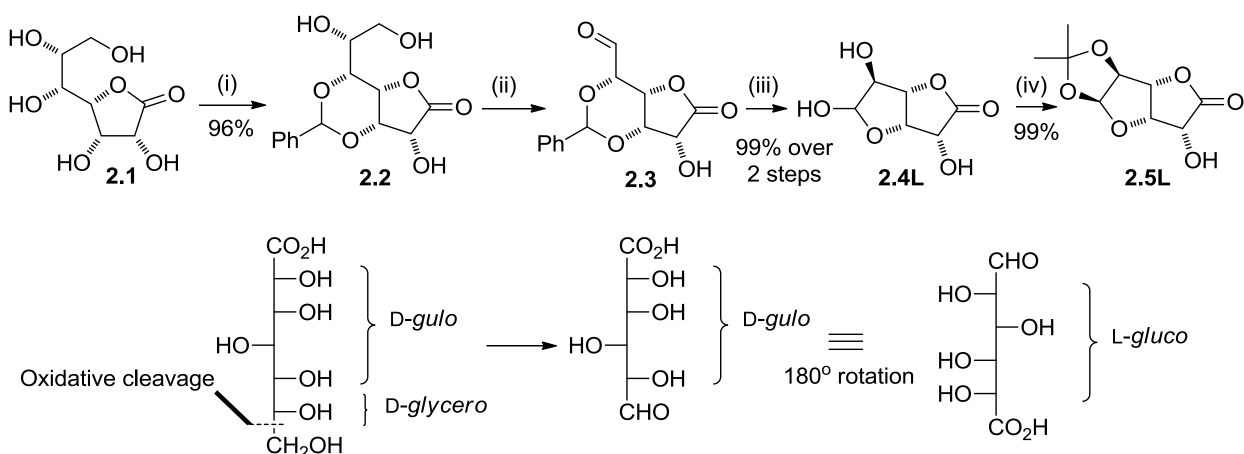
80. Ramana, C. V.; Mallik, R.; Gonnade, R. G. *Tetrahedron* **2008,** *64*, 219-233.
81. Sato, K.; Suzuki, K.; Ueda, M.; Katayama, M.; Kajihara, Y. *Chem Lett* **1991**, 1469-1472.
82. Sato, K. I.; Akai, S.; Shoji, H.; Sugita, N.; Yoshida, S.; Nagai, Y.; Suzuki, K.; Nakamura, Y.; Kajihara, Y.; Funabashi, M.; Yoshimura, J. *J Org Chem* **2008,** *73*, 1234-1242.
83. Sahabuddin, S.; Ghosh, R.; Achari, B.; Mandal, S. B. *Org Biomol Chem* **2006,** *4*, 551-557.
84. Tardy, S.; Vicente, J. L.; Tatibouet, A.; Dujardin, G.; Rollin, P. *Synthesis* **2008**, 3108-3120.

2. Iminosugars from Glucuronolactone

2.1. Introduction

This chapter provides an example of the great utility of carbohydrate starting materials in iminosugar synthesis, and aims to demonstrate the flexibility of such chirons as the starting point for iminosugar libraries. The principal goal of this project was the efficient synthesis of specific iminosugar targets with the development of methodology allowing access to a diverse range of iminosugars varying in stereochemical configuration, ring size and substitution from a single readily available starting material. Glucuronolactone was chosen as a starting material for two primary reasons: a) Both enantiomers are readily available and enantiopure; b) It represents a highly versatile scaffold from which a variety of iminosugars can be generated. D-Glucuronolactone is commercially available, inexpensive, and is readily protected as the 1,2-acetonide **2.5D**[1] that, as will be demonstrated in this chapter, serves as an excellent carbohydrate chiron.

D-Glucoheptonolactone **2.1** [or less ambiguously D-*glycero*-D-*gulo*-heptono-1,4-lactone], which is also commercially available, can be readily transformed into the L-glucuronolactone acetonide **2.5L** by a short, scalable and very efficient sequence (**Scheme 2.1**) that does not require chromatography.[2]

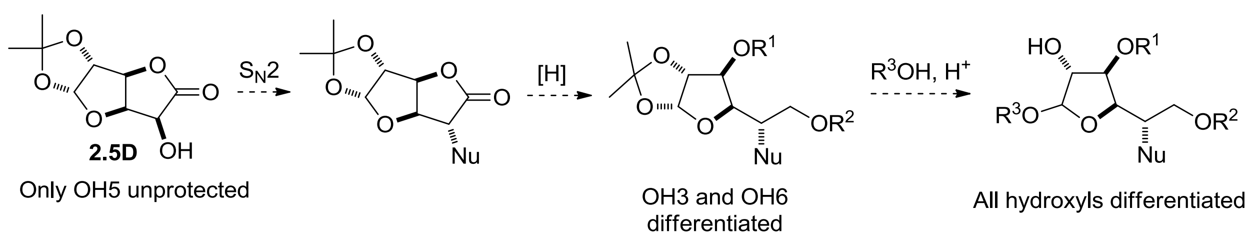


Reagents: (i) PhCHO, HCl; (ii) $NaIO_4$; (iii) TFA(aq); (iv) Me_2CO, H_2SO_4

Scheme 2.1: Synthesis of L-glucuronolactone acetonide **2.5L**

Conceptually the approach is straightforward (**Scheme 2.1**): Bearing in mind that L-*gluco* = D-*gulo*, a D-*glycero*-D-*gulo*-heptonic acid scaffold is an ideal precursor to an L-*gluco*-hexuronic acid scaffold; all that is required is truncation of the carbon backbone by one carbon atom (C7), and oxidation at C6 to the aldehyde level, ie. oxidative cleavage of the C6-C7 bond. In the specific case of D-*glycero*-D-*gulo*-heptono-1,4-lactone, such an oxidative cleavage requires a protecting group pattern in which the only diol present is terminal, which is readily achieved by formation of the 3,5-benylidene acetal **2.2**.[3] Oxidative cleavage of the C6-C7 bond with sodium periodate followed by hydrolysis of the benzylidene acetal and re-protection with acetone gives the L-glucuronolactone derivative **2.5L**.[4] As will be discussed, the availability of **2.5L** has allowed the synthesis and biological evaluation of both enantiomers of several iminosugars, for conciseness only syntheses from the D-glucuronolactone acetonide **2.5D** will be discussed in detail, the enantiomeric procedures being identical.

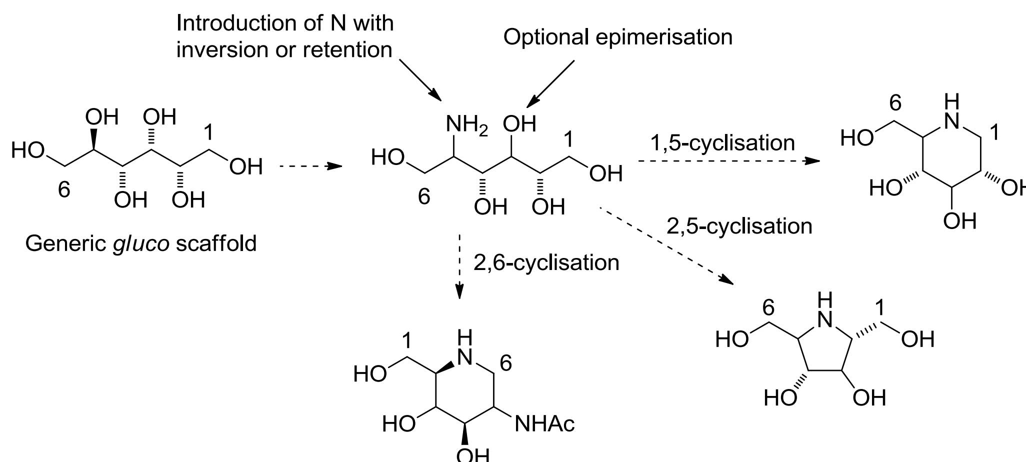
The opportunity to access several stereochemical configurations and different ring sizes from a single starting material, coupled with the availability of both enantiomers of that starting material, allows the synthesis of extensive iminosugar libraries. In addition, synthetic strategies developed for one configuration are, generally speaking, transferable to diastereoisomeric configurations with minimal redesign or refinement necessary. The great advantage of glucuronolactone is that it is particularly amenable to various protecting group strategies; indeed the glucuronolactone acetonide **2.5D** is more versatile than it may first appear (**Scheme 2.2**).



Scheme 2.2: A general example of the versatility of glucuronolactone

For example, only OH5 is unprotected and can be modified specifically, such as by activation and displacement with a nucleophile; the 3,6-lactone can be considered a protected diol revealed

on reduction, and OH3 and OH6 are easily distinguished by exploitation of their very different steric environments; the 1,2-acetonide can be removed by alcoholysis, giving a glycoside, allowing convenient differentiation of OH1 and OH2. This possibility to introduce orthogonal protecting groups at many positions is key to the versatility of glucuronolactone.



Scheme 2.3: General concepts for iminosugar synthesis from a *gluco* scaffold

Diversification of the *gluco* scaffold provided by glucuronolactone is achieved by epimerisation reactions (**Scheme 2.3**). Nitrogen can be introduced stereospecifically at C5 with retention or inversion of configuration as desired and the configuration at C3 can be independently epimerised. This leads to four theoretical scaffolds which may be closed to form iminosugars in various different modes; this thesis will deal with examples of three of those modes: a) 1,5-cyclisation *via* the nitrogen at C5, giving DNJ-type piperidines; b) 2,5-cyclisation by internal S_N2 *via* the nitrogen at C5, giving DMDP-type pyrrolidines; c) 2,6-cyclisation by double S_N2 displacement with a primary amine, ultimately giving acetamido-piperidines.

Potential targets accessible from the 1,5-cyclisation strategy (**Scheme 2.3**) are of the DNJ subset of the piperidine class. The isomers accessed by this 1,5-cyclisation strategy would be of *gluco*, *ido*, *allo* and *talo* configuration. Five examples of the DNJ subclass have been isolated from natural sources: DNJ,[5] 1,5-dideoxy-1,5-imino-D-mannitol or 1-deoxymannojirimycin [DMJ],[6] 1,5-dideoxy-1,5-imino-D-altritol [*altro*-DNJ], 1,5-dideoxy-1,5-imino-D-gulitol [*gulo*-DNJ][7] and 1,5-dideoxy-1,5-imino-D-allitol [*allo*-DNJ].[8] All of the sixteen isomers of DNJ have been

obtained synthetically,[8-11] and all but one (1,5-dideoxy-1,5-imino-D-talitol [DTJ]) have been examined to varying degrees as glycosidase inhibitors.[8,11] Much of the synthetic effort in the field has been devoted to direct piperidine analogues of the more common natural monosaccharides, i.e. DNJ **2.6**, DMJ and DGJ **2.9**.[12] These have been fruitful avenues of exploration; the *N*-butyl and *N*-(2-hydroxyethyl) alkylated derivatives of DNJ, known as miglustat (Zavesca) **2.7** and miglitol (Glyset) **2.8** respectively, are marketed drugs for the treatment of type I Gaucher's disease and type II diabetes, respectively. DGJ **2.9**, also known as migalastat (Amigal) is in phase III clinical trials for the treatment of Fabry's disease (**Figure 2.1**, see also **Chapter 1**).

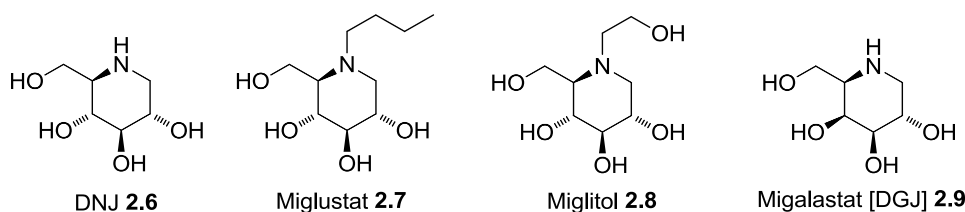
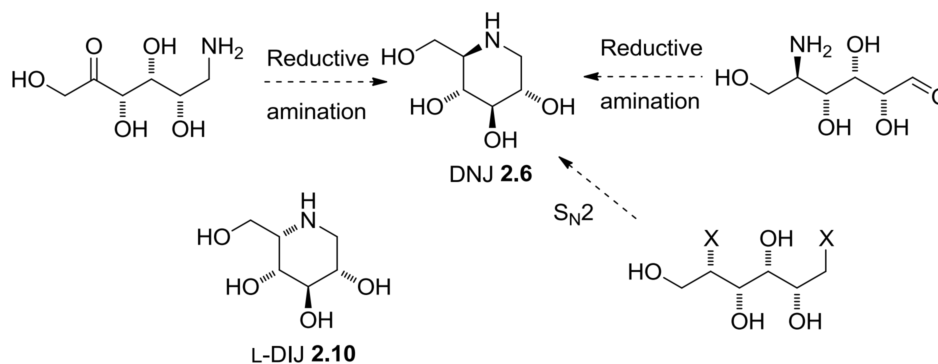


Figure 2.1: Medically relevant piperidines

The therapeutic utility of DNJ **2.6** is largely, although not exclusively, due to its very potent competitive inhibition of α-glucosidases (e.g. IC_{50} 0.03 μM from rice), and similarly DGJ is a very potent competitive inhibitor of α-glucosidases (e.g. IC_{50} 0.003 μM from coffee bean).[8] Of the lesser known DNJ-type piperidines bearing unusual configurations, few come close to this level of potency with IC_{50}s typically exceeding 30 μM,[8,11] with the very interesting exceptions of L-DNJ and L-DGJ – the enantiomers of the aforementioned examples. L-DNJ was found to be a potent inhibitor of α-glucosidases (e.g. IC_{50} 4.3 μM from rice),[8] a seemingly paradoxical result considering its completely inverted configuration relative to DNJ. It was subsequently found that the inhibition mode in this case is non-competitive. Similarly, L-DGJ is a non-competitive α-galactosidase inhibitor (e.g. IC_{50} 13 μM from coffee bean).[8] These results may imply allosteric binding sites that can be targeted for glycosidase inhibition, and highlights the importance of the synthesis and screening of iminosugars with unnatural configurations bearing little

stereochemical resemblance to the enzymes' natural substrates. Thus the availability of both enantiomers of glucuronolactone makes it a powerful starting material for the investigation of glycosidase inhibitors.

Due to its high academic and commercial relevance, DNJ **2.6** was chosen as a target for the demonstration of the utility of glucuronolactone with the development of a scalable, atom efficient and high yielding synthesis in collaboration with Dextra Laboratories. The first synthesis of DNJ **2.6** was reported by Paulsen in 1966,[13,14] predating its first natural isolation[5] by a decade. This iconic synthesis utilised the readily available ketohexose L-sorbose as a starting material, with a general strategy involving introduction of nitrogen at C6 followed by intramolecular reductive amination of the ketone at C2 (**Scheme 2.4**). An enormous number of syntheses have been developed since, which have been comprehensively reviewed,[12] including many applications of Paulsen's original strategy, with a notable example being the large scale variant reported by Behling *et al.*[15] One particular problem with these sorbose variants is a selective acetonide hydrolysis that is problematic on large scale,[16] and another is the formation of traces of 1,5-dideoxy-1,5-imino-L-iditol [L-DIJ] **2.10**, the C5 epimer of DNJ, in the reductive amination step. Although highly stereoselective in this case, the production of mixtures from the reductive amination of prochiral ketones is a general problem associated with the transformation and can complicate the clean isolation of the target, which is of particular concern in a commercial context where rigorous quality control criteria must be met with minimum expenditure. Intramolecular reductive amination of ketones is nevertheless still a popular strategy for the synthesis of DNJ **2.6**, with several examples reported in the last five years including routes from D-glucose,[17,18] and L-sorbose.[19] Circumvention of the issue of diastereoselectivity with specific control of the stereochemistry at C5 by S_N2 introduction of nitrogen has also received much attention over the years; recent examples have been reported from D-glucose[20,21] and L-gulonolactone.[22]



Scheme 2.4: Some general strategies for the synthesis of DNJ

D-Glucuronolactone has previously been used as a starting material for competitive syntheses of DNJ; one approach requires an extended high temperature treatment with sodium azide for the introduction of nitrogen,[23] and the other a highly stereoselective but moderate yielding reductive amination.[24] These procedures have not been demonstrated as scalable, and are less efficient than the sequence presented in this chapter. Chemoenzymatic routes to DNJ, such as the highly efficient method reported by Kinast *et al*,[25] have also been developed and this continues to be an active area of research.[26]

The importance of "unnatural enantiomers"[27] has also been observed in pyrrolidine systems. 1,4-Dideoxy-1,4-imino-D-arabinitol [DAB] **2.11D** is a natural product and potent competitive α-glucosidase inhibitor (e.g. IC_{50} 5.8 μM rat intestinal isomaltase), whereas its unnatural enantiomer LAB **2.11L** is a non-competitive inhibitor of the same enzyme with increased potency (IC_{50} 0.08 μM).[28] Of the 2,5-imino-hexitol subclass of pyrrolidines, all ten stereoisomers (there are six degenerate pairs: *galacto* and *allo* are *meso*; D-*altro* = L-*talo* and *vice versa*; D-*gluco* = L-*gulo* and *vice versa*) have been previously synthesised[29-32] and seven of them (*altro*, *gluco*, *manno*, and *galacto*) are theoretically accessible by the 2,5-cyclisation strategy developed in this project (**Scheme 2.3**). Three isomers of the 2,5-imino-hexitol subclass have been isolated from natural sources: 2,5-dideoxy-2,5-imino-D-mannitol [DMDP] **2.12D**;[33] 2,5-dideoxy-2,5-imino-D-glucitol [DGDP] **2.13**;[34] and more recently 2,5-dideoxy-2,5-imino-D-altritol [DALDP] **2.14**[35] (**Figure 2.2**). In terms of biological activity, these natural products are the most potent

glycosidase inhibitors of the subclass, with the exception of the unnaturally configured L-DMDP **2.12L** and *meso*-2,5-dideoxy-2,5-imino-galactitol [DGLDP] **2.15**.

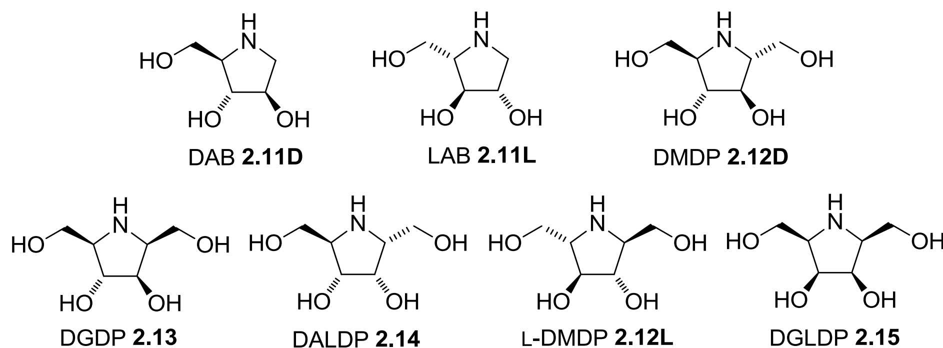
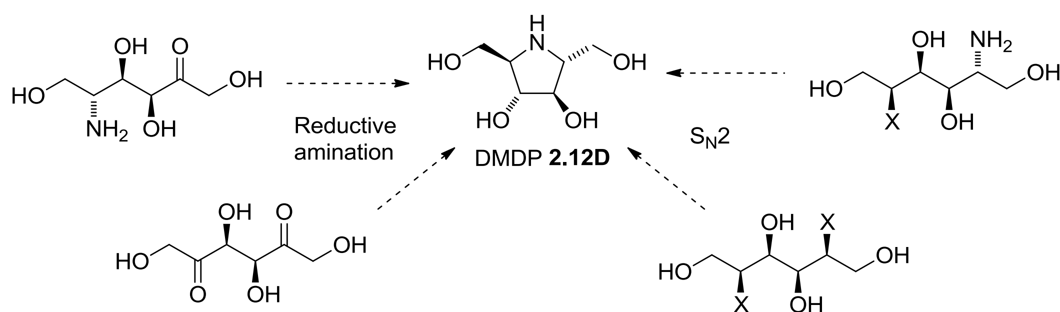


Figure 2.2: Biologically active 2,5-imino-hexitols

DMDP **2.12D** is a potent β-glucosidase (e.g. IC_{50} 9.7 μM from bovine liver) and β-galactosidase inhibitor (e.g. 3.3 μM from bovine liver);[36] DGDP **2.13** is a potent α-glucosidase inhibitor (e.g. IC_{50} 2.6 μM from *Bacillus stearothermophilus*);[37] DALDP **2.14** is a potent α-galactosidase inhibitor (e.g. IC_{50} 0.69 μM from human lysosome);[35] compared to its natural enantiomer, L-DMDP **2.12L** is a more potent and specific inhibitor of α-glucosidase (e.g. IC_{50} 0.62 μM rat intestinal sucrase);[36] DGLDP **2.15** is an α-galactosidase inhibitor (K_i 50 nM from coffee bean).[38] The importance of both enantiomers of DMDP **2.12** made it an attractive target for the investigation of the proposed 2,5-cyclisation strategy. Synthesis of the natural product has received considerable interest since the first reported example by Card in 1985 from L-sorbose.[39] This method arguably remains the simplest and most practical ever reported and involves a highly stereoselective intramolecular reductive amination of 5-azido-D-fructose, which can also be accessed chemoenzymatically (**Scheme 2.5**).[40,41] Similar approaches from D-fructose have been reported,[42,43] as well as less stereoselective double reductive aminations of diketones.[44] Another popular approach is chain extension of pentoses such as D-arabinose[45] and L-xylose,[29,46] and although the efficiency of these approaches is often compromised to some extent by diastereoselective reactions, an efficient and general chain extension strategy has been recently developed.[37]



Scheme 2.5: Some general strategies for the synthesis of DMDP

Stereospecific syntheses of DMDP **2.12D** involving S_N2 displacement have been shown previously from D-glucose[47] and particularly effectively from D-gluconolactone.[48] Ring contraction of piperidines has also been reported,[49,50] as well as a recent *de novo* approach involving palladium catalysed asymmetric allylic alkylation.[51] Whilst DMDP **2.12D** has attracted a good deal of attention, its enantiomer L-DMDP **2.12L** is far more elusive in the synthetic literature. Four preparations have been reported, the first in 1998 from D-tartate[52] which was superseded in 2004 with a synthesis from D-gulonolactone[53] and more recently a very effective method involving chain extension of a D-xylose derived cyclic nitrone.[37] There has also been one synthetic article reporting L-DMDP **2.12L** as the minor component of a mixture in a chemoenzymatic sequence.[54] New methods for the synthesis of L-DMDP **2.12L** will facilitate a more extensive biological evaluation of this important pyrrolidine.

Acetamido-substituted piperidines have received very little synthetic attention in comparison to the iminosugar subclasses discussed thus far. Of the sixteen possible isomers, only four had been described prior to this work and none have been isolated from natural sources. Two potential strategies for the treatment of cancer were introduced in **Chapter 1** with the key enzyme targets being α-*N*-acetyl-galactosaminidase and β-*N*-acetyl-glucosaminidase, and therefore *N*-acetyl-galactosamine [GalNAc] and *N*-acetyl-glucosamine [GlcNAc] analogues 2-acetamido-1,2,5-trideoxy-1,5-imino-D-galactitol [DGJNAc] **2.16D** and 2-acetamido-1,2,5-trideoxy-1,5-imino-D-gluctitol [DNJNAc] **2.17D** respectively, were chosen as initial targets based on a structural resemblance analogous to the relationship between D-glucose and DNJ **2.6** (**Figure 2.3**).

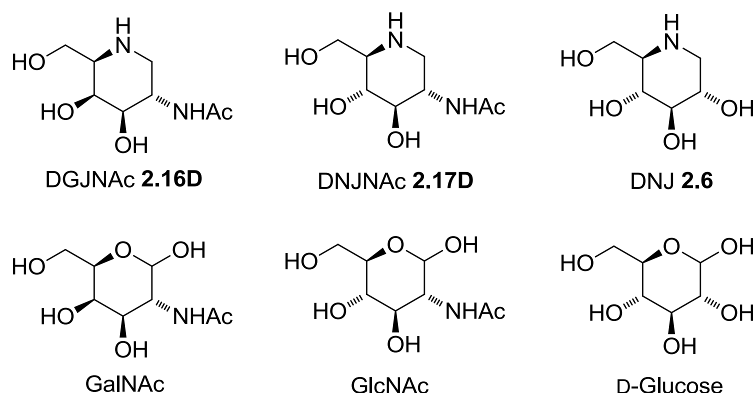


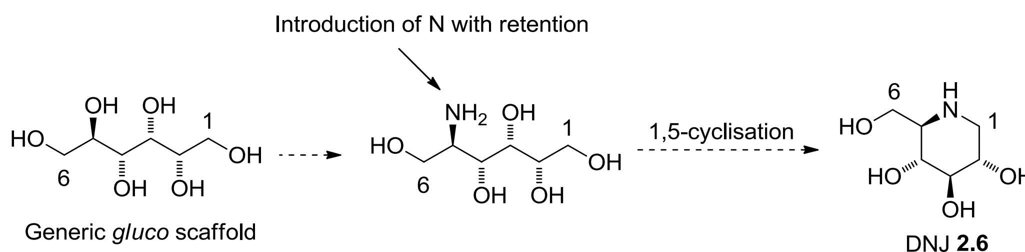
Figure 2.3: Iminosugar inhibitors with close structural resemblance to natural sugars

Racemic DGJNAc **2.16** has been prepared from D-ribose,[55] but the only two homochiral syntheses of this iminosugar both utilised DNJ **2.6**[56,57] as a starting material in similar sequences with overall yields below 5%; an impractical option for the majority of synthetic laboratories owing to the high price of DNJ **2.6**. No biological evaluation of DGJNAc **2.16D** was reported; in fact α-*N*-Acetyl-galactosaminidase inhibitors are particularly sparse in the literature;[58-60] the synthesis and biological evaluation of DGJNAc **2.16D** undertaken as part of this project constitutes one of the very few examples of such a report.[61] As a known β-*N*-acetyl-glucosaminidase inhibitor (e.g. IC_{50} 6.0 μM from human placenta), DNJNAc **2.17D** has received more interest, with a handful of syntheses reported. Since the first synthesis from D-glucose and biological evaluation by Fleet *et al* in 1986,[62,63] routes from DNJ **2.6**,[64,57] GlcNAc[65,66] and sucrose[67] have been reported, as well as a chemoenzymatic approach.[68] The most efficient approaches are currently from GlcNAc, with Furneaux's[69] and Vasella's[70] syntheses being the highest yielding at 10-12%. 2-Acetamido-1,2,5-trideoxy-1,5-imino-D-mannitol [DMJNAc] is also known and has been prepared from D-glucose,[63,62] DNJ **2.6**,[57] chemoenzymatically[68] and by a *de novo* approach.[71] DMJNAc was not found to be an inhibitor of glycosidases,[62] but weak inhibition of UDP-GlcNAc-epimerase (IC_{50} 0.5 mM)[71] has been reported. The 3-acetamido-1,5-imino-D-mannitol and the 2- and 3-acetamido-1,5-imino-D-altritols have also been prepared from

DNJ **2.6**.[57] There is a clear need for the development of efficient strategies towards this class of understudied piperidines.

2.2. 1-Deoxynojirimycin (1,5-Cyclisation)

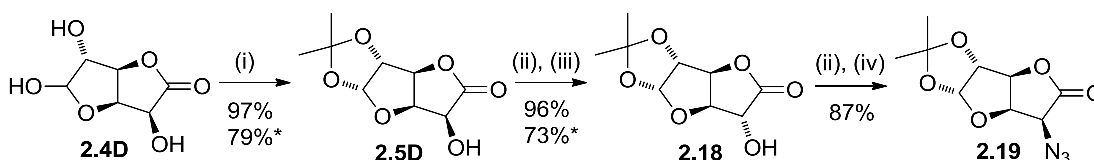
A worthwhile target for the demonstration of the 1,5-cyclisation strategy was identified as DNJ **2.6**. As was shown in **Chapter 1**, this naturally occurring piperidine iminosugar is the best known, most widely synthesised example of its class; it is a potent α-glucosidase inhibitor and the parent structure of the commercial drugs miglustat and miglitol. Despite the abundance of chemical syntheses of DNJ **2.6** reported, relatively few are practically scaleable and the development of a new, efficient, scaleable synthesis of DNJ **2.6** was undertaken in collaboration with Dextra Laboratories. Conceptually, DNJ **2.6** should be accessible by introduction of nitrogen at C5 of a *gluco* scaffold with retention of configuration, followed by 1,5-cyclisation (**Scheme 2.6**).



Scheme 2.6: General concepts for the synthesis of DNJ from a *gluco* scaffold

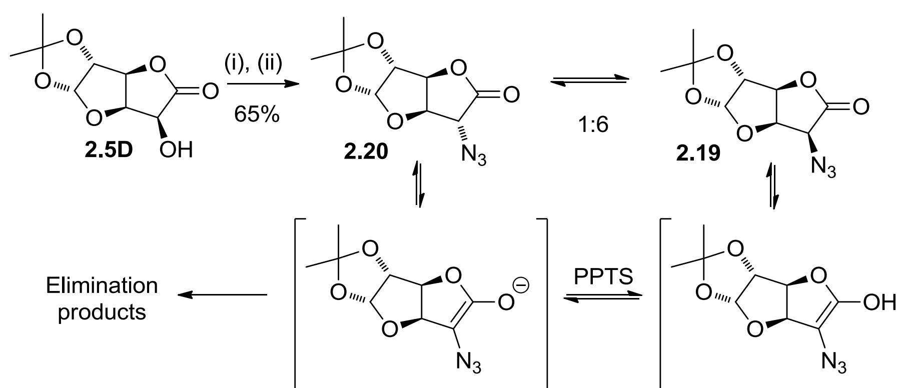
Following sulfuric acid catalysed protection of D-glucuronolactone **2.4D** with acetone, the first task in the synthesis of DNJ **2.6** was introduction of azide at C5 of glucuronolactone acetonide **2.5D**, achieved by a series of two sequential S_N2 displacements giving net retention (**Scheme 2.7**). OH5 was first activated as a trifluoromethanesulfonic ester by treatment with triflic anhydride in the presence of pyridine (triflation) and the resulting triflate was then treated with sodium trifluoroacetate in DMF.[72] Rapid S_N2 displacement of triflate by trifluoroacetate gave, after aqueous workup, the epimeric iduronolactone **2.18** in 73% yield isolated by recrystallisation on 250 g scale, on smaller scale (10 g) this product could be isolated in 96% by

chromatography. The corresponding trifluoroacetyl ester was not observed, presumably hydrolysing rapidly on workup. Triflation of iduronolactone **2.18** followed by displacement with sodium azide in DMF at -30°C gave the azido-lactone **2.19**, with the desired *gluco* configuration, in 87% yield.[73]



Reagents and conditions: (i) Me_2CO, H_2SO_4, RT, 5 h; (ii) Tf_2O, Py, DCM, -30°C, 1 h; (iii) CF_3CO_2Na, DMF, RT, 1 h; (iv) NaN_3, DMF, -20°C, 1 h; *indicates recrystallised yield on 250 g scale
Scheme 2.7: Introduction of nitrogen at C5 with retention of configuration.

The reactivity of α-azido-lactones such as **2.19** is interesting and worthy of further discussion. Their sensitivity to basic conditions, attributed to the high acidity at C2 and therefore their ease of enolate formation, has been documented. When sugar lactones bearing an α-triflate are treated with sodium azide in DMF at higher temperatures with longer reaction times, elimination and epimerisation reactions may proceed *via* enolates. Undesired elimination reactions are suppressed by addition of pyridinium *p*-toluenesulfonate [PPTS], which lowers the basicity of the reaction mixture, allowing these systems to readily undergo base catalysed epimerisation at C2, resulting in an equilibrium mixture of C2-epimeric azides (**Scheme 2.8**).[74]

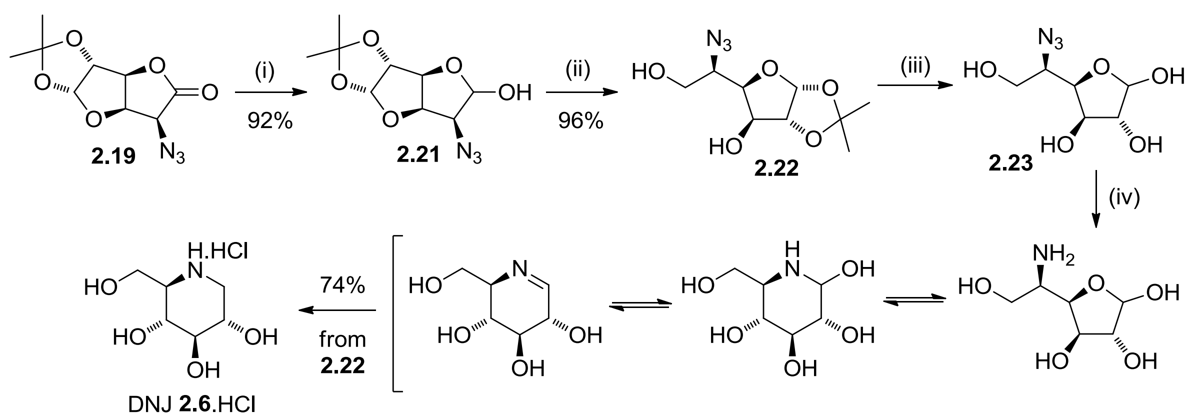


Reagents: (i) Tf_2O, Py; (ii) NaN_3, PPTS, DMF
Scheme 2.8: Introduction of nitrogen with retention *via* base catalysed epimerisation

If, following triflation, glucuronolactone acetonide **2.5D** is treated directly with sodium azide and PPTS in DMF and allowed to equilibrate in this fashion, *gluco* azido-lactone **2.19** is in fact

the thermodynamic product obtained, rendering the need for two sequential S_N2 displacements questionable. Whilst introduction of azide with retention by base catalysed equilibration is an undeniably powerful synthetic tool, in this particular case the product distribution is 6:1 in an overall yield of 65%;[74] this complicates the clean isolation of the desired epimer **2.19**, especially on a large scale due to the fact that both epimers **2.19** and **2.20** are highly crystalline. Consequently the sequential S_N2 approach was developed as it allows clean generation of azido-lactone **2.19** at low temperature in good yield despite elongating the synthesis.

With azido-lactone **2.19** in hand, attention was turned to the reduction of the 3,6-lactone (**Scheme 2.9**). Owing to the base sensitivity of this system, a delicate approach was required; direct treatment with sodium or lithium borohydride gave complex mixtures, presumably arising from epimerisation and elimination[75,76] reactions associated with the basic nature of the reagents. Diisobutylaluminium hydride, being Lewis acidic, proved effective in reducing the azido-lactone **2.19** to the corresponding azido-lactol **2.21** at -78°C in 92% yield. Treatment of this lactol **2.21** with sodium borohydride in methanol at a carefully controlled internal temperature of up to -10°C gave the desired azido-diol **2.22** cleanly in 96% yield.



Reagents and conditions: (i) DIBAL, DCM, -78°C, 1 h; (ii) $NaBH_4$, MeOH, -10°C, 45 min; (iii) Dowex (H^+), H_2O, 70°C, 3 h; (iv) H_2, Pd (10% on C), H_2O, RT, 50 psi, 18 h, then HCl(aq)

Scheme 2.9: The synthesis of DNJ **2.6** by 1,5-cyclisation

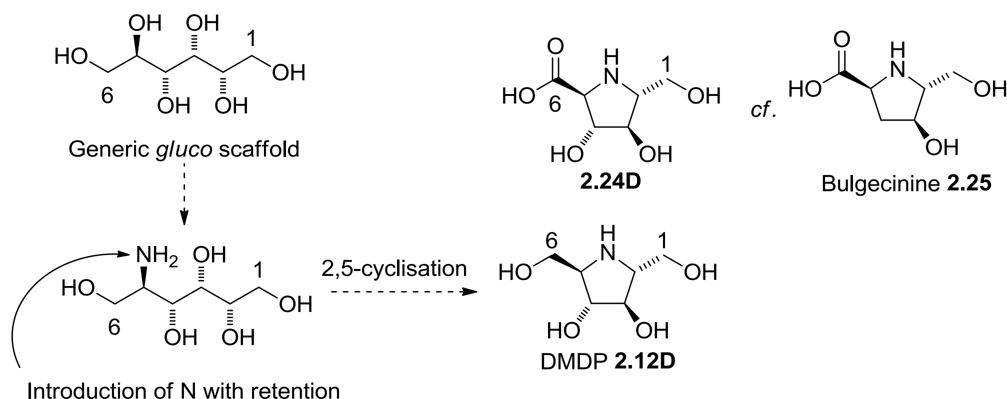
Subsequent Dowex resin catalysed hydrolysis of the 1,2-acetonide gave the free azido-glucose **2.23**, which was treated with hydrogen in the presence of a palladium catalyst (10% on carbon), inducing reduction of the azide at C5 to the corresponding amine and intramolecular reductive

amination at C1. Thus a successful 1,5-cyclisation gave DNJ **2.6**, isolated as its hydrochloride salt by recrystallisation in up to 74% yield. The overall yield of DNJ **2.6**.HCl from D-glucuronolactone **2.4D** was up to 54% over nine steps; this makes the synthesis one of the most efficient reported to date, with excellent atom efficiency associated with the use of an acetonide as the only protecting group. In terms of scalability, the crystalline nature of the intermediates has allowed the demonstration of multigram scale preparation of DNJ **2.6** with limited chromatography in 33% overall yield, which still constitutes a highly competitive process. Implementation of this strategy at even larger scales with no chromatography at Dextra Laboratories has met with similar success and is used for the production of DNJ as a commercial product.[77]

2.3. DMDP and Sugar Amino Acids (2,5-Cyclisation)

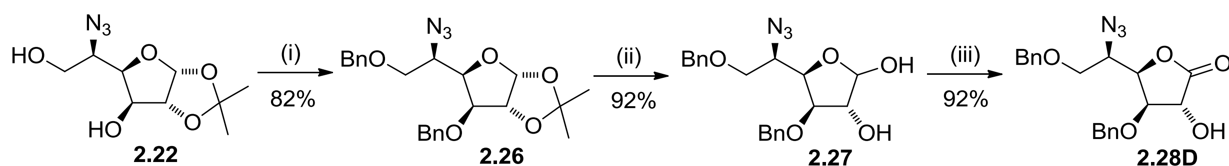
Of the pyrrolidine class of iminosugars, natural product 2,5-dideoxy-2,5-imino-D-mannitol [DMDP] **2.12D** is one of the best known examples and displays potent β-gluco- and β-galactosidase inhibition. Compared to DNJ **2.6** there are relatively few syntheses of this iminosugar reported, and fewer still of its synthetic enantiomer L-DMDP **2.12L** – a more potent and specific α-glucosidase inhibitor than the natural product. In addition, a strategy of this type was also expected to allow access to novel sugar amino acids such as **2.24D** with structural resemblance to the natural product bulgecinine **2.25**.

It was proposed that DMDP **2.12D** would be accessible from intermediates utilised in the synthesis of DNJ **2.6** by employing a 2,5-cyclisation strategy in place of 1,5-cyclisation. Following introduction of nitrogen with retention, as in the DNJ **2.6** synthesis, ring closure by intramolecular S_N2 at C2 would lead to the *manno* configuration of DMDP **2.12D** (**Scheme 2.10**).



Scheme 2.10: Conceptual strategy for the synthesis of DMDP

This approach required a system in which only OH2 was unmasked, available for activation and intramolecular displacement (**Scheme 2.11**). Diol **2.22**, an intermediate from the DNJ **2.6** synthesis, was first treated with excess sodium hydride and benzyl bromide in DMF to block the alcohol functionality at C3 and C6, giving dibenzyl ether **2.26** in 82% yield. Attention was then turned to unmasking OH2, achieved by hydrolysis of the acetonide followed by selective protection at C1; treatment of fully protected **2.26** with *p*TSA in aqueous 1,4-dioxane at 90°C gave the lactol **2.27** in 92% yield, which was then protected as the corresponding lactone **2.28D** by oxidation with iodine in 92% yield.



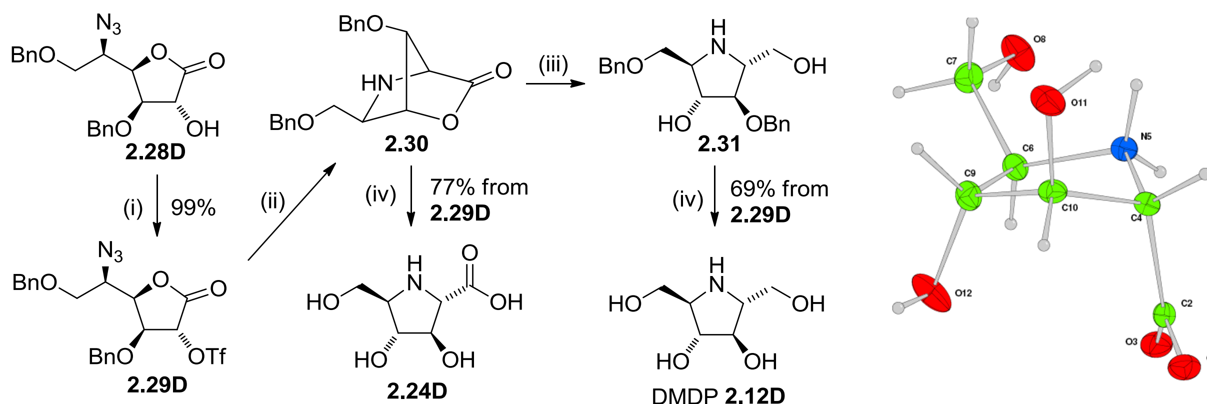
Reagents and conditions: (i) BnBr, NaH, DMF, 0°C to RT, 90 min; (ii) *p*TSA, H_2O, 1,4-dioxane, 90°C, 2.5 h; (iii) I_2, K_2CO_3, ^{t}BuOH, reflux, 4 h

Scheme 2.11: Synthesis of lactone **2.28D**, a key precursor to 2,5-cyclisation

Anomeric oxidations of this type are more commonly achieved with basic bromine water,[78-80] and these conditions were initially applied to lactol **2.27** but were found to be less than ideal. The standard reaction conditions required modification; when performed in aqueous media such oxidations are usually buffered with a carbonate, commonly barium carbonate, however as the lactol **2.27** is not water-soluble at room temperature the presence of an organic solvent is required - this reduces the solubility, and hence efficacy, of the usual buffering agents. Whilst

successful bromine oxidation under these conditions has been reported,[81] successful optimisation of solvent/buffer combinations to grant reliable oxidation of lactol **2.27** with bromine was not trivial, and the transformation was further hampered by the observation of competitive side reactions - benzyl ether cleavage, plausibly by hydrobromic acid or indeed oxidative cleavage by bromine,[82] and bromination of the benzene rings. Iodine oxidation in refluxing *tert*-butanol with potassium carbonate[83] proved a cleaner and more reliable alternative that also avoids the toxicity and handling issues associated with liquid bromine. The choice of a lactone as a C1 protecting group also deserves comment, since alcoholysis of acetonide **2.26** would give the corresponding glycoside bearing the desired protecting group pattern in a single step. The ultimate fate of the anomeric centre C1 of acetonide **2.26** is a hydroxymethyl group in DMDP **2.12D**, so whilst a glycoside can be introduced in a single step, it would require a subsequent two step modification (hydrolysis and reduction) to furnish the target DMDP **2.12D**. A lactone requires two steps to introduce, but only a single reduction step to achieve the same transformation; overall there is no loss of efficiency and lactone functionality grants easy access to sugar amino acids on hydrolysis as opposed to reduction. A glycoside would also be further complicated by anomeric mixtures, increasing the difficulty of product identification and complicating the reactivity at C2 in subsequent S_N2 displacement reactions, the success of which are very much depenent on the anomeric configuration. The homogeneity of a lactone is preferable to an anomeric mixture of glycosides, and in a synthetic strategy involving S_N2 displacement at C2 a lactone offers not only protection at C1 but also activates C2 to S_N2 displacement, promoting the desired 2,5-cyclisation.

Triflation of OH2 of lactone **2.28D** was straightforward, furnishing chromatographically stable triflate **2.29D** in 99% yield (**Scheme 2.12**). The system was now ideally configured for 2,5-cyclisation; a triflate at C2, further activated by the adjacent lactone, with an azide at C5 poised for pyrrolidine formation on reduction of the azide **2.29D**.



Reagents and conditions: (i) Tf_2O, Py, DCM, -30°C, 1 h; (ii) H_2, Pd (10% on C), EtOH, RT, 1 h; (iii) $NaBH_4$, EtOH, RT, 16 h; (iv) H_2, Pd (10% on C), EtOH (for DMDP **2.12D**) or 1,4-dioxane(aq) (for **2.24D**), HCl, RT, 16 h

Scheme 2.12: The synthesis of DMDP and related sugar amino acid by 2,5-cyclisation; Right: X-ray crystal structure of sugar amino acid **2.24D**

Treatment of an ethanolic solution of azido-triflate **2.29D** with hydrogen in the presence of palladium (10% on carbon) caused reduction of the azide to the corresponding amine accompanied by instantaneous intramolecular S_N2 displacement of the triflate to give the [2.2.1] bicyclic lactone **2.30**. No appreciable rate of benzyl hydrogenolysis was observed under these conditions, even with extended reaction times, presumably due to the catalyst poisoning effect of amine functionality.[84,85] Bicyclic lactone **2.30** is highly strained (ν_{max} 1824 cm^{-1}), and attempts to purify the crude material met with decomposition. Following filtration the reaction mixture was treated directly with excess sodium borohydride to give the dibenzylated DMDP **2.31**, which was found to be unsuitable for chromatography on silica and not purified further but simply acidified with aqueous hydrochloric acid and stirred with palladium (10% on carbon) under an atmosphere of hydrogen. Acidification in this manner overcomes the catalyst poisoning effect of the amine,[86] allowing hydrogenolysis of the benzyl ether, furnishing DMDP **2.12D** in 69% yield in three steps from azido-triflate **2.29D** (81% for the enantiomer **2.12L** from **2.28L**), with an overall yield of 34% from glucuronolactone acetonide **2.5D** over thirteen steps. Access to the hydroxy-bulgecinine **2.24D** was achieved by a similar sequence under aqueous conditions with omission of the borohydride reduction step, providing the target in 77% yield from azido-triflate **2.29D** (86% for the enantiomer **2.24L** from **2.28L**) and an overall yield of 42% from glucuronolactone

acetonide **2.5D** over twelve steps, the structure of which was unequivocally confirmed by X-ray crystallography.[87]

The biological profiles of DMDPs **2.12D**, **2.12L** and hydroxy-bulgecinines **2.24D**, **2.24L** were examined in collaboration with Prof. Atsushi Kato (Univ. Toyama, Japan) (**Table 2.1**).[36] The potency and specificity of the enantiomeric DMDPs **2.12D**, **2.12L** were confirmed – DMDP **2.12D** is a potent β-gluco- and β-galactosidase inhibitor, whereas L-DMDP **2.12L** is a more potent and specific α-glucosidase inhibitor.

	IC_{50} (μM)			
Enzyme	**2.12D**	**2.12L**	**2.24D**	**2.24L**
α-Glucosidase				
Rice	214	5.8	290	NI (43.4%)
Yeast	NI[a]	NI (34.9%)[b]	144	NI (7.1%)
Rat intestinal maltase	290	1.2	204	205
Rat intestinal isomaltase	91	0.77	NI (38.2%)	188
Rat intestinal sucrase	40.0	0.62	470	124
β-Glucosidase				
Almond	10	NI (12.1%)	NI (48.6%)	NI (16.0%)
Bovine liver	9.7	NI (4.6%)	NI (36.7%)	NI (0.8%)
Rat intestinal cellobiase	24	NI (16.1%)	NI (23.6%)	NI (7.2%)
β-Galactosidase				
Bovine liver	3.3	NI (0%)	NI (46.2%)	NI (2.6%)
Rat intestinal lactase	7.9	NI (19.3%)	663	NI (0%)
β-Mannosidase				
Snail	721	NI (12.6%)	NI (6.4%)	NI (0.2%)
Trehalase				
Porcine kidney	200	48	NI (5.2%)	NI (0.1%)
Rat intestinal	626	665	NI (16.4%)	NI (0%)
Glycogen Phosphorylase	932	NI (0%)	664	NI (0%

[a] NI : No inhibition (less than 50% inhibition at 1000 μM).

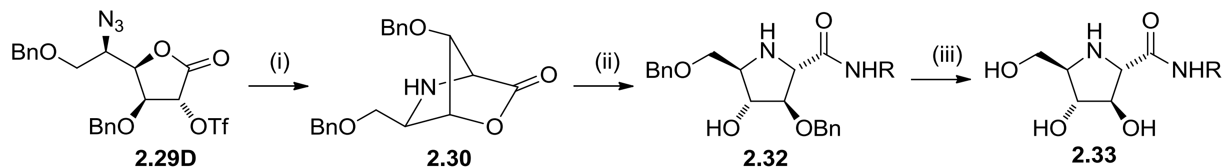
[b] ( ) : inhibition % at 1000 μM

NI was observed in each case for: α-galactosidase (coffee bean); α-mannosidase (Jack bean); α-rhamnosidase (*P. decumbens*); α-fucosidase (bovine epididymis); β-glucuronidase (*E. coli*, bovine liver), β-*N*-acetyl-glucosaminidase (Jack bean, bovine kidney, human placenta); α-*N*-acetyl-galactosaminidase (chicken liver).

Table 2.1: Concentration of iminosugars giving 50% inhibition of various glycosidases and glycogen phosphorylase

Unfortunately, neither of the hydroxy-bulgecinine amino acids **2.24** showed any appreciable glycosidase inhibition, both showing only weak α-glucosidase activity. However, in a very

recent extension of this project,[88] it has been shown that corresponding amino amides, generated by aminolysis of the [2.2.1] bicyclic lactone **2.30** (**Scheme 2.13**) have particularly interesting biological profiles.



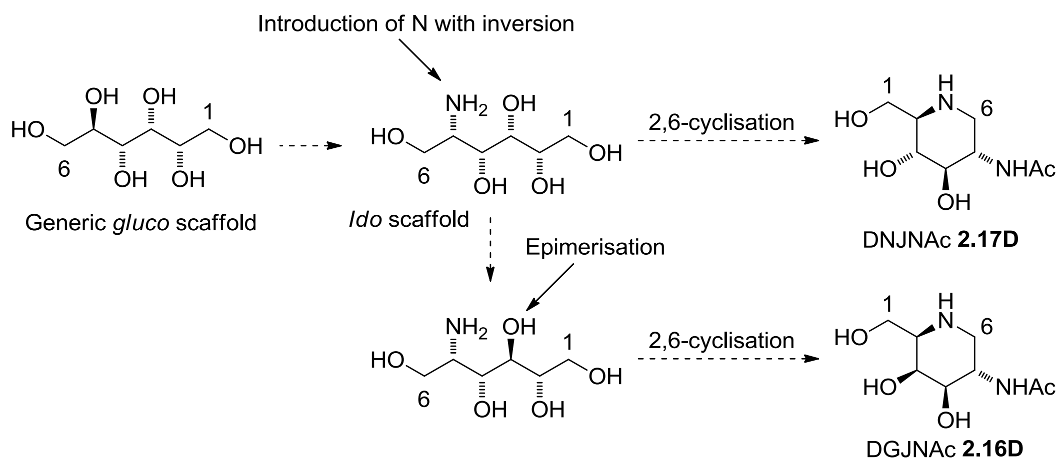
Reagents: (i)) H_2, Pd (10% on C); (ii) RNH_2; (iii)) H_2, Pd (10% on C), HCl
Scheme 2.13: The synthesis of sugar amino amides, potent hexosaminidase inhibitors

In particular amide **2.33** (R=Me) is a very potent inhibitor of β-*N*-acetyl-glucosaminidase, with IC_{50} 0.033 μM against the enzyme from Jack bean, which may prove an important discovery for cancer research, as was discussed in **Chapter 1**. This chemistry further exemplifies the applicability of the methodology developed here for library synthesis, generating new potential leads for therapeutic agents as well facilitating efficient natural product synthesis.

2.4. Hexosamine Analogues (2,6-Cyclisation)

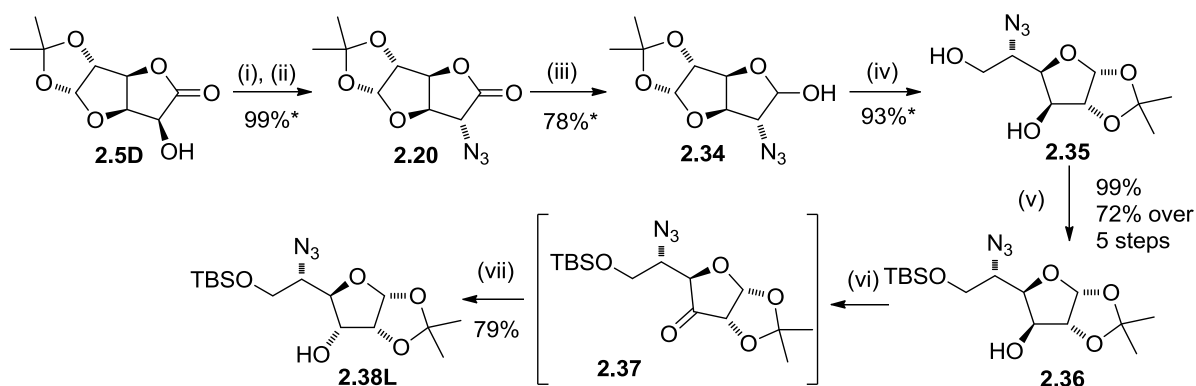
The potential for hexosaminidase inhibitors in the treatment of cancer was discussed in **Chapter 1**, which highlights the reasons for our interest in hexosamine analogues.

Working from glucuronolactone's *gluco* scaffold, conceptual access to the target glucosamine analogue DNJNAc **2.17D** and the galactosamine analogue DGJNAc **2.16D** is provided by a 2,6-cyclisation strategy (**Scheme 2.14**). Introduction of nitrogen to the *gluco* scaffold at C5 with inversion of configuration ultimately provides the desired acetamido substituent, and 2,6-cyclisation by S_N2 double displacement with an amine provides access to a 2,6-imino-L-gulitol = 1,5-imino-D-glucitol DNJNAc **2.17D**. Additional inversion of configuration of the intermediate *ido* scaffold at C3, followed by S_N2 double displacement, ultimately gives a 2,6-imino-L-galactitol = 1,5-imino-D-galactitol DGJNAc **2.16D**, the synthesis of which will be discussed first.



Scheme 2.14: Conceptual approach to acetamido-piperidines

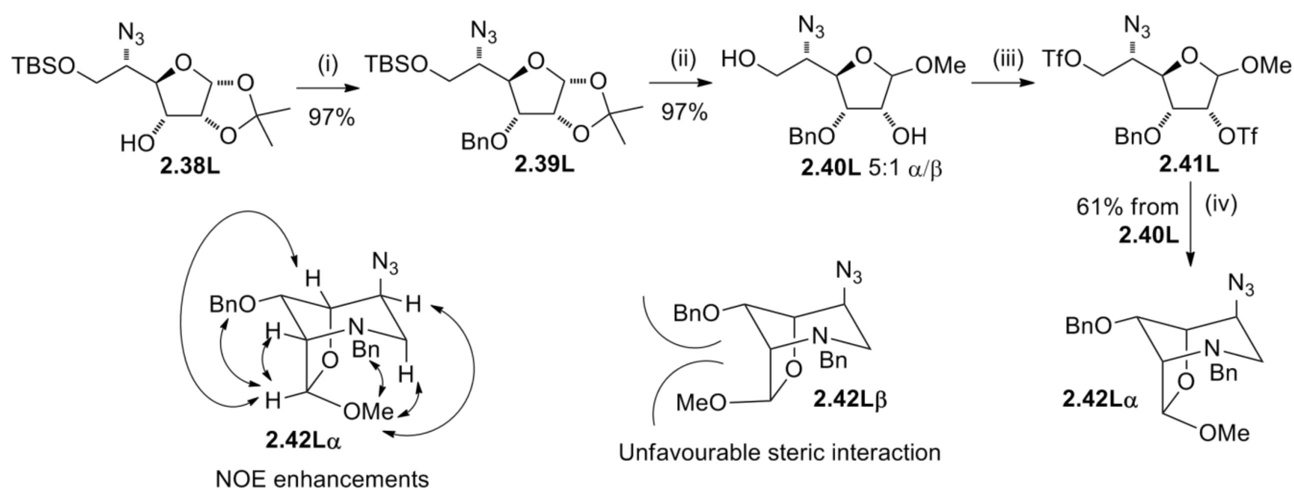
Nitrogen was introduced to glucuronolactone acetonide **2.5D** by triflation followed by azide displacement at low temperature,[74,73] giving the *ido* azido-lactone **2.20** with no observation of epimerisation (**Scheme 2.15**). The lactone **2.20** was then reduced to the corresponding diol **2.35** in a two step reduction; sequential treatment with diisobutylaluminium hydride at -78°C followed by sodium borohydride in methanol below -10°C as was employed in the synthesis of DNJ **2.6**. The azido-diol **2.35** was then selectively protected as a silyl ether by treatment with *tert*-butyldimethylsilyl chloride in pyridine, giving azido-alcohol **2.36** in 72% yield over five steps with a single final chromatographic purification.



Reagents and conditions: (i) Tf_2O, Py, DCM, -30°C, 1 h; (ii) NaN_3, DMF, -30°C, 1 h; (iii) DIBAL, DCM, -78°C, 1 h; (iv) $NaBH_4$, MeOH, -10°C, 1 h; (v) TBSCl, Py, RT, 7 h; (vi) PCC, DCM, RT, 16 h; (vii) $NaBH_4$, EtOH, H_2O, 0°C to RT, 2 h; * Crude yield
Scheme 2.15: Introduction of nitrogen with inversion at C5 and epimerisation at C3

This system **2.36**, in which only OH3 is unmasked, was then epimerised at C3 by an oxidation-reduction sequence. Pyridinium chlorochromate oxidation gave the intermediate ketone **2.37**,

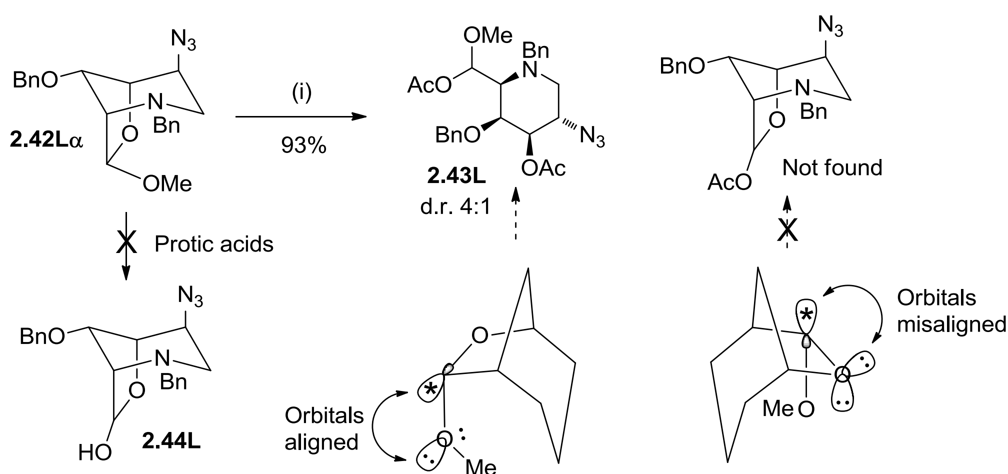
which was treated with sodium borohydride; delivery of hydride from the least hindered face gave the *talo* configured azide **2.38L** as the only observed product in 79% yield over two steps. The protecting group pattern was then rearranged in preparation for 2,6-cyclisation (**Scheme 2.16**); OH3 was first benzylated in 97% yield with sodium hydride and benzyl bromide in DMF, then OH2 and OH6 were unmasked by methanolysis of the silyl ether and acetonide in **2.39L**, giving an inseparable mixture of talofuranosides **2.40L** (5:1 α/β) in 97% yield.



Reagents and conditions: (i) BnBr, NaH, DMF, 0°C to RT, 1 h; (ii) AcCl, MeOH, 50°C, 3 h; (iii) Tf_2O, Py, DCM, -30°C, 2 h; (iv) $BnNH_2$, THF, -30°C to 60°C, 18 h
Scheme 2.16: Synthesis of bicyclic glycoside **2.42L** by 2,6-cyclisation

2,6-Cyclisation was achieved by formation of ditriflate **2.41L** in the usual manner, followed by S_N2 double displacement with benzylamine in THF to give the [3.2.1] bicyclic 2,6-imino-galactofuranoside **2.42Lα** in 61% yield as a single anomer, as determined by NOE analysis. Displacement of the primary triflate was essentially instantaneous at even -30°C, the second intramolecular displacement was induced by heating to 50°C overnight. The apparent reluctance of the β-anomer **2.42Lβ** to cyclise in this reaction can be explained by the 1,3 steric clashing between the benzyl and methoxy groups increasing the already considerable demands associated with this transformation. Examples of successful double displacements of ditriflates are rare,[89,90] a yield of 61% for the cyclisation was therefore deemed respectable.

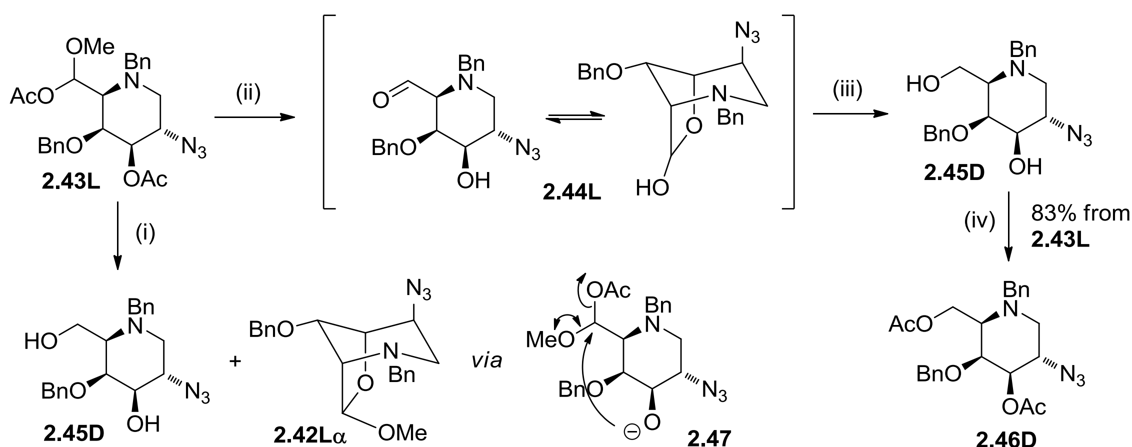
With the key 2,6-cyclisation step complete, and every required stereocentre in place, the next task was hydrolysis and reduction of the acetal functionality in galactofuranoside **2.42Lα** – a process that proved far more complicated than originally anticipated (**Scheme 2.17**). Hydrolysis was initially attempted with catalysis by strong protic acids in the presence of water, but these reactions proved difficult to monitor and extremely slow due to ammonium ion formation. Lewis acid catalysed acetolysis proved much more effective; in the presence of boron trifluoride diethyl etherate in acetic anhydride the bicyclic glycoside was cleaved to give the monocyclic mixed acetal **2.43L** in 93% yield, which bears a more productive base-labile acetyl group. Excess boron trifluoride is required for an appreciable reaction rate, presumably due to coordination of the first equivalent to the tertiary amine's lone pair.



Reagents and conditions: (i) $BF_3.Et_2O$, Ac_2O, -30°C to RT, 3h
Scheme 2.17: Acetolysis of bicyclic glycoside **2.42L**

It is interesting to note the observation of endocyclic C-O bond cleavage, as opposed to exocyclic C-O bond cleavage as is normally observed.[91,92] This observation can be rationalised by consideration of the stereoelectronic effects imparted by the restricted geometry of bicyclic glycoside **2.42Lα**. The endocyclic oxygen atom does not possess a lone pair with the orientation required for effective overlap with the adjacent C-O σ* orbital, whereas the endocyclic oxygen has rotational freedom and an overlapping orientation can be achieved.

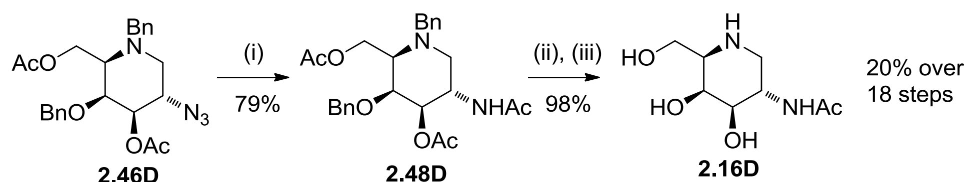
Base induced deprotection and subsequent reduction of mixed acetal **2.43L** was expected by a one-pot treatment with sodium borohydride in methanol, with acetyl cleavage proceeding by $B_{AC}2$ tranesterification with methanol and subsequent borohydride reduction giving **2.45D** (**Scheme 2.18**). Whilst this approach was marginally successful, the desired diol **2.45D** was detected but not amenable to chromatography, regeneration of the bicyclic glycoside **2.42Lα**, isolated in up to 35% yield, was observed as a side reaction.



Reagents and conditions: (i) $NaBH_4$, MeOH, 0°C to RT, 16 h; (ii) DIBAL, DCM, -78°C, 45 min; (iii) $NaBH_4$, MeOH, RT, 1 h; (iv) Ac_2O, Py, RT, 16 h

Scheme 2.18: Reduction of mixed acetal **2.43L**

This side reaction was also observed in methanolic sodium methoxide and sodium hydroxide in aqueous 1,4-dioxane. An explanation for this curious observation could be that the alkoxide **2.47**, a plausible intermediate under these conditions, can undergo an intramolecular displacement of acetate, presumably assisted by the lone pair of the methoxy substituent. A solution to this problem was found in reductive, rather than base catalysed, cleavage of the acetyl groups with excess diisobutylaluminium hydride followed by reduction of the resultant aldehyde/lactol **2.44L** with sodium borohydride to give the desired diol **2.45D**. This diol **2.45D** was then reacetylated for the purposes of purification giving the desired piperidine **2.46D** in a respectable 83% yield over three steps.



Reagents and conditions: (i) Zn, $CuSO_4$(aq), Ac_2O, AcOH, THF, RT, 20 min;
(ii) NaOMe, MeOH, RT, 16 h; (iii) H_2, Pd (10% on C), HCl, H_2O, 1,4-dioxane, 16 h
Scheme 2.19: The final steps in the synthesis of DGJNAc

The azido substituent in **2.46D** was then converted to an acetamido group by rapid copper mediated zinc reduction in the presence of acetic anhydride,[93,94] generating the protected DGJNAc **2.48D** in 79% yield (**Scheme 2.19**). Zinc was found to be a preferable reducing agent compared to hydrogen, giving cleaner and more efficient conversion. Finally, global deprotection gave the target DGJNAc **2.16D** in 98% yield over a two step procedure involving base catalysed methanolysis of the acetates followed by hydrogenolysis of the benzyl groups under acidic conditions. The overall yield of DNJNAc **2.16D** was 20% over eighteen steps..

Enzyme	IC50 (μM) 2.16D	IC50 (μM) 2.16L
α-Galactosidase		
Coffee beans	64	NI[a] (17.3%)[b]
β-*N*-Acetyl-glucosaminidase		
Jack bean	1.8	NI (46.3%)
Bovine kidney	4.2	NI (46.8%)
Human placenta	8.3	830
α-*N*-Acetyl-galactosaminidase		
Chicken liver	0.32	NI (22.6%)

[a] NI : No inhibition (less than 50% inhibition at 1000 μM).
[b] ( ) : inhibition % at 1000 μM

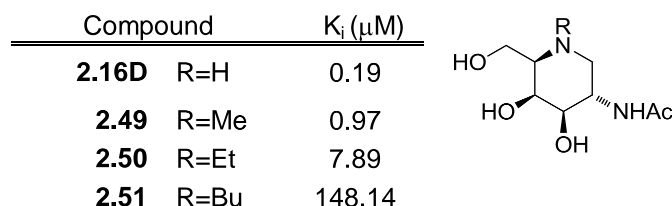
NI was observed in each case for: α-glucosidase (rice, yeast); β-glucosidase (almond, bovine liver); β-galactosidase (bovine liver); α-mannosidase (Jack bean); β-mannosidase (snail); α-rhamnosidase (*P. decumbens*); α-fucosidase (bovine epididymis); β-glucuronidase (*E. coli*, bovine liver).

Table 2.2: Concentration of iminosugars giving 50% inhibition of various glycosidases

Biological screening of both enantiomers of DGJNAc **2.16** (Atsushi Kato, Univ. Toyama) revealed that DGJNAc **2.16D** is a potent competitive inhibitor of α-*N*-acetyl-galactosaminidases (K_i 0.08 μM from chicken liver) and β-*N*-acetyl-glucosaminidases (K_i 2.2 μM from human placenta), its enantiomer **2.16L** is a very weak non-competitive inhibitor of the latter (K_i 1100

μM), a more extensive collection of IC_{50} values is given in **Table 2.2**. DGJNAc **2.16D** was identified as a target in the quest for a specific α-*N*-acetyl-galactosaminidase inhibitor. Whilst the specificity of DGJNAc **2.16D** for hexosaminidases is only around one order of magnitude in favour of α-*N*-acetyl-galactosaminidase, this is nevertheless an extremely encouraging result given the rarity of molecules that exhibit such potency and specificity. Iminosugars of this nature may develop into interesting agents for the treatment of cancer (**Chapter 1**), DGJNAc **2.16D** analogues may provide compounds with increased potency and specificity.

The *N*-alkylated derivatives of DGJNAc **2.49-2.51**, prepared by reductive amination of the relevant aldehydes with DGJNAc by Andreas Glawar, were also screened for hexosaminidase activity against α-*N*-acetyl-galactosaminidase (*C. lampas*) (**Table 2.3**).[95]



Compound		K_i (μM)
2.16D	R=H	0.19
2.49	R=Me	0.97
2.50	R=Et	7.89
2.51	R=Bu	148.14

Table 2.3: K_i measurements for the inhibition of α-*N*-acetyl-galactosaminidase from *Charonia lampas* by DGJNAc and some *N*-alkylated analogues

A very clear trend is observed whereby the inhibition drops by an order of magnitude for each additional carbon atom added to the endocyclic nitrogen. This suggests that that the amine functionality is buried deep within the active site, with no suitable lipophilic pocket for accommodation of the alkyl chain. The crystal structure (**Figure 2.4**) of DGJNAc **2.16D** bound to the active site of human α-*N*-acetyl-galactosaminidase, obtained in collaboration with Dr. Scott Garman (UMass Amherst) appears to support this hypothesis. Following complete refinement of the crystal structure, this information should allow a directed approach to analogue design.

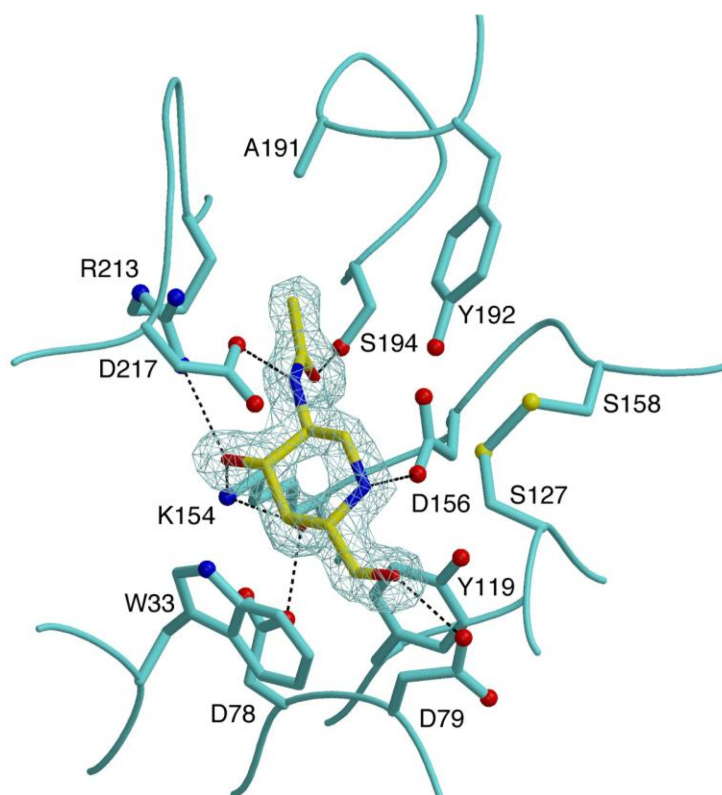
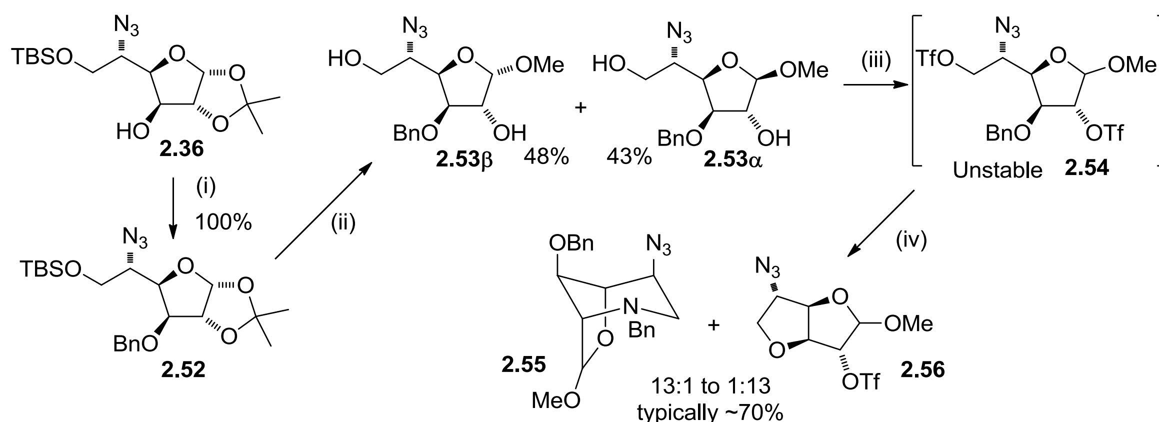


Figure 2.4: DGJNAc crystallised in the active site of human α-*N*-acetyl-galactosaminidase

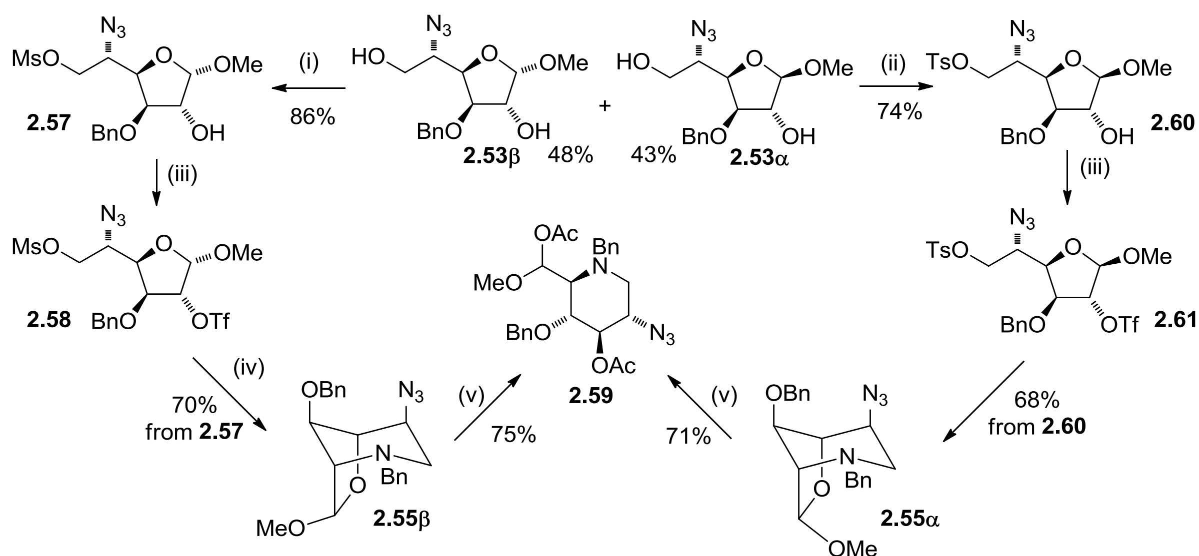
The epimeric target DNJNAc **2.17D** was obtained by a similar strategy, but with the omission of the pyridinium chlorochromate mediated epimerisation. As with the DGJNAc **2.17D** synthesis, the *ido* configured alcohol **2.36** was first benzylated (100%) and the silyl ether and acetonide removed by methanolysis to give a mixture of separable glycosides **2.53** (4:5 α/β, assigned by NOE) in 91% combined yield (**Scheme 2.20**).



Reagents and conditions: (i) BnBr, NaH, DMF, 0°C to RT, 1 h; (ii) AcCl, MeOH, 50°C, 1 h; (iii) Tf_2O, Py, DCM, -30°C, 2 h; (iv) $BnNH_2$, THF, -30°C to 60°C, 18 h

Scheme 2.20: Formation of unwanted THF side products

2,6-Cyclisation was then attempted as previously developed, however it was soon apparent that the ditriflates **2.54** derived from the idofuranosides **2.53** were unstable. This shift to instability in comparison with the previously investigated *talo* configured system **2.38** is entirely attributable to the epimeric benzyl ether at C3; the triflate at the primary position of ditriflate **2.54** is spontaneously displaced by the benzyl ether and accompanying debenzylation gives the tetrahydrofuran (THF) **2.56**. Treatment of the crude ditriflates **2.54** with benzylamine did provide the desired bicyclic guloside **2.55**, but THF **2.56** was always found as a significant inseperable contaminant (5-65%). This side reaction could be limited by a one-pot triflation/benzylamine displacement procedure, omitting intermediate aqueous workup, but not wholly suppressed and an alternative procedure was devised. The employment of less reactive sulfonic acid esters was found to completely suppress the formation of unwanted THF **2.56**. Treatment of idofuranoside **2.53β** with methanesulfonyl chloride in the presence of 2,4,6-collidine allowed selective mesylation of the primary hydroxyl OH6, giving a stable mesylate **2.57** in 86% yield with no detectable THF formation (**Scheme 2.21**).

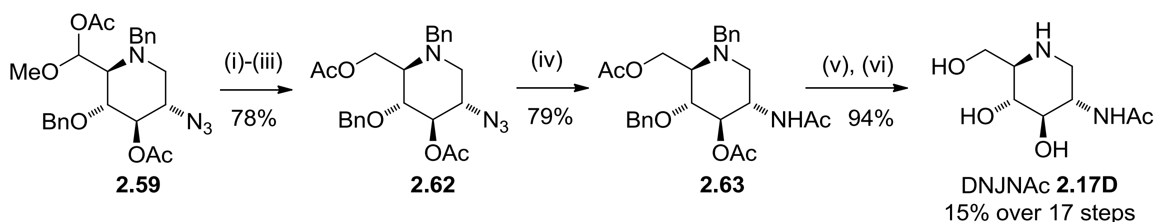


Reagents and conditions: (i) MsCl, 2,4,6-collidine, DCM, 0°C, 3 h; (ii) TsCl, 2,4,6-collidine, DCM, RT, 24 h; (iii) Tf_2O, Py, DCM, -30°C, 1 h; (iv) $BnNH_2$, RT to 100°C, 16 h; (v) $BF_3.Et_2O$, Ac_2O, -30°C to RT, 36 h from **2.55β** or 3 h from **2.55α**

Scheme 2.21: 2,6-Cyclisation *via* mixed disulfonates to avoid THF formation

The secondary hydroxyl OH2 was then activated by triflation to afford the mixed disulfonate **2.58**, which could be successfully cyclised with benzylamine to give the desired bicyclic gulofuranoside **2.55β** in 70% yield over two steps. Compared to the cyclisation of ditriflate **2.41L**, much harsher conditions were required for displacement; no appreciable reaction was observed below 50°C, but complete conversion was observed in an acceptable timeframe at 100°C in neat benzylamine. It is unclear whether the initial intermolecular displacement is of the primary mesylate or the secondary triflate as no intermediate amino-sulfonate was detected. This implies that the intramolecular displacement proceeds at a much faster rate than the intermolecular displacement, which is not altogether surprising. It should be noted that subjection of the corresponding dimesylate to benzylamine at elevated temperature does not result in ring closure, only single displacement at the primary position; the high reactivity of a triflate is required at C2 for ring closure, but is counterproductive at C6 as it leads to THF formation. In the case of the anomeric idofuranoside **2.53α**, attempted selective mesylation proceeded with poor selectivity. This is presumably a steric effect; in the case of **2.53β** the methoxy substituent is *cis* to OH2, imparting an additional degree of steric hinderance in comparison to **2.53α** in which OH2 is the sole substituent on the back face of the furanose ring. The bulkier *p*-toluenesulfonyl chloride in the presence of 2,4,6-collidine proved an effective alternative, allowing the formation of tosylate **2.60** in 74% yield, and a familiar triflation and benzylamine double displacement gave the desired bicyclic guloside **2.55α** in 68% yield *via* mixed disulfonate **2.61**. A sequence analogous to that used in the final stages of the synthesis of DGJNAc **2.16D** was employed to furnish the target DNJNAc **2.17D**. As was observed in the epimeric series, gulofuranosides **2.55** were found to be exceedingly stable to acid catalysed hydrolysis. Acetolysis was once again employed to generate the mixed acetal **2.59** from either anomer of gulofuranoside **2.55** in similar yields up to 75%. Treatment of the mixed acetal **2.59** with base once again lead to the observation of unwanted side products, and the sequence of

reductive deacetylation, reduction and reacetylation was applied to give the azido-piperidine **2.62** in 78% yield (**Scheme 2.22**).



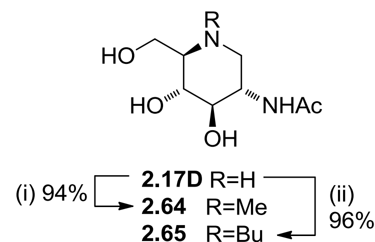
Reagents and conditions: (i) DIBAL, DCM, -78°C, 40 min; (ii) $NaBH_4$, MeOH, -10°C, 2 h; (iii) Ac_2O, Py, RT, 16 h; (iv) Zn, $CuSO_4$(aq), Ac_2O, AcOH, THF, RT, 20 min; (v) NaOMe, MeOH, RT, 16 h;(vi) H_2, Pd (10% on C), HCl, H_2O, 1,4-dioxane, 24 h
Scheme 2.22: The final steps in the synthesis of DNJNAc

Zinc was once again employed for reductive amidation, giving protected DNJNAc **2.63** in 79% yield, followed by global deprotection to give the target DNJNAc **2.17D** in 94% yield (overall 15% over seventeen steps from glucuronolactone acetonide **2.5D**).

DNJNAc **2.17D** was also converted into *N*-methyl and -butyl analogues **2.64** and **2.65**, by palladium catalysed reductive amination of formaldehyde (94% yield) and butyraldehyde (96% yield) respectively (**Scheme 2.23**), and their activity against hexosaminidases probed (**Table 2.4**).

Enzyme	IC50 (μM) 2.17D	2.64	2.65
β-*N*-Acetyl-glucosaminidase			
Human placenta	7.0	18	10
Bovine kidney	7.4	12	8.1
Aspergillus oryzae	891	225	478
Jack bean	2.9	17	24
β-*N*-Acetyl-galactosaminidase			
Aspergillus oryzae	998	167	381

In all cases <50% inhibition was observed for α-*N*-acetyl-galactosaminidase (chicken liver) at 1 mM
Table 2.4: Hexosaminidase inhibition by DNJNAc and alkylated analogues



Reagents and conditions: (i) HCHO, H_2, Pd (10% on C), H_2O, RT, 24 h; (ii) HCHO, H_2, Pd (10% on C), H_2O, RT, 24 h
Scheme 2.23: Synthesis of *N*-alkyl DNJNAc for biological screening

DNJNAc **2.17D** was confirmed to be a potent inhibitor of β-*N*-acetyl-glucosaminidases, with the exception of the fungal enzyme from *A. oryzae*, a source from which neither β-hexosaminidase

was very strongly inhibited. Little can be inferred at this stage regarding *N*-alkylation as these modifications show no remarkable perturbations to the biological activity.

2.5. Conclusions and Future work

The versatility of glucuronolactone as a chiral pool starting material has been exemplified. Ease of independent epimerization at two asymmetric centres of the starting *gluco* scaffold provides access to a variety of iminosugars with differing stereochemical configurations; glucitol, mannitol and galactitol iminosugars have been successfully synthesised. Three different modes of cyclisation have been successfully employed to further expand the range of attainable target iminosugars by allowing construction of different ring sizes; both piperidines and pyrrolidines can be obtained by the strategies that have been developed. Functionality other than the conventional hydroxyl group can be introduced easily; acetamido substitution has been demonstrated as well as the incorporation of carboxyl functionality in the carbon backbone. The well known natural products DNJ **2.6** and DMDP **2.12D**, which have enormous importance in glycobiological research by virtue of their powerful glycosidase inhibition, have been synthesised with efficiency rivalling the best examples in the literature; the strategy developed herein for the production of DNJ **2.6** has been adopted in a commercial setting. L-DMDP **2.12L**, of which there are very few syntheses reported, is also an important iminosugar that can be readily obtained by the methods developed. A logical extension of the 1,5-cyclisation strategy is the synthesis of DTJ, the biological activity of which is completely unknown. Prior to the synthesis of DGJNAc **2.16D** reported in this chapter the biological activity of this GalNAc mimic had not been reported, and the sequence presented here is superior in efficiency to the homochiral syntheses in the literature and utilises a starting material that is more affordable by several orders of magnitude. The α-*N*-acetyl-galactosaminidase activity uncovered may prove useful in investigations towards a treatment for cancer by preservation of the macrophage activating factor. DNJNAc **2.17D** is a better known acetamido-piperidine, and the approach

demonstrated in this thesis is competitive with the methods divulged in the literature. As a specific β-*N*-acetyl-glucosaminidase inhibitor, the biological activity associated with DNJNAc **2.17D** may aid the development of metastasis suppression strategies involving protection of the extracellular matrix. There are still eleven isomers of DNJNAc that have never been synthesised or biologically screened; the 2,6-cycisation approach to these acetamido-piperidines should provide convenient access to four of these potentially useful compounds: those with D-*allo*, L-*allo*, L-*gluco* and L-*manno* configuration. Additionally, whilst neither enantiomer of the hydroxy-bulgecinine displayed appreciable glycosidase inhibition, the recent discovery of the remarkably potent β-*N*-acetyl-glucosaminidase activity associated with its amide analogues is a major lead that could pave the way for a new line of hexosaminidase inhibitors with applications to cancer research. Further investigation of this class of proline-amides is vital.

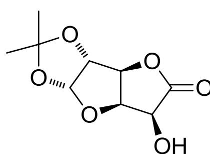
2.6. Experimental

All commercial reagents were used as supplied. DMF and pyridine were purchased dry from the Aldrich chemical company in sure-seal bottles over molecular sieves. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Thin layer chromatography [TLC] was performed on aluminium sheets coated with 60 F254 silica. Sheets were visualised using a spray of 0.2% w/v cerium (IV) sulphate and 5% w/v ammonium molybdate in 2 M sulphuric acid; 1% w/v potassium permanganate and 6.3% w/v potassium carbonate in 0.02 M sodium hydroxide; or 10% v/v sulphuric acid in ethanol. Flash chromatography was performed on Sorbsil C60 40/60 silica. Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g 100 mL^{-1}. Infrared spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform spectrophotometer using thin films on NaCl plates unless otherwise stated. Characteristic peaks are quoted in units of cm^{-1}. Low resolution mass spectra [LRMS] were recorded on VG MassLab 20–250, Micromass BIOQ-II,

Micromass Platform 1, Micromass TofSpec 2E, or Micromass Autospec 500 OAT spectrometers and high resolution mass spectra [HRMS] on a Micromass Autospec 500 OAT spectrometer. The technique used was electrospray ionisation [ESI]. Nuclear magnetic resonance [NMR] spectra were recorded on Bruker AMX 500 (1H: 500 MHz and 13C: 125.7 MHz) and Bruker DPX 400 and DQX 400 spectrometers (1H: 400 MHz and 13C: 100.6 MHz) in the deuterated solvent stated. All chemical shifts [δ] are quoted in ppm and coupling constants [*J*] in Hz, signals for mixtures of anomers/diastereoisomers are labelled ^{A} and ^{B} to indicate the major and minor components, respectively, and signals for diastereotopic protons are labelled "a" and "b" in order of chemical shift (upfield to downfield). Residual signals from the solvents were used as an internal reference, MeCN was added as an internal reference for samples in D_2O.[96]

2.6.1. Synthesis of DNJ

1,2-*O*-Isopropylidene-α-D-glucurono-3,6-lactone, 2.5D

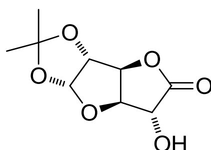


Glucurono-3,6-lactone **2.4D** (250 g, 1.42 mol) was suspended in acetone (6 L), concentrated sulfuric acid (200 mL) added and stirred at RT. TLC analysis (EtOAc) after 5 h showed the complete consumption of starting material (R_f 0.05) and the formation of a major product (R_f 0.75). The reaction mixture was neutralised with $NaHCO_3$, filtered and concentrated under reduced pressure. The resultant yellow crystalline crude was dissolved in DCM, filtered and concentrated under reduced pressure to remove any residual starting material. Recrystallisation from hot PhMe followed by treatment with decolourising charcoal afforded the title compound **2.5D** (241 g, 79%) as a white crystalline solid.

HRMS (ESI +ve): $C_9H_{12}NaO_6$ found 239.0527; (M+Na$^+$) requires 239.0526; $[\alpha]_D^{25}$ +54.9 (*c* 1.95, $CHCl_3$); mp 118-120°C; ν_{max} (thin film): 3442 (br, m, OH), 1780 (s, CO); δ_H ($CDCl_3$, 400

MHz): 1.35, 1.52 (2 x 3H, s, Me), 2.92 (1H, d, OH, $J_{OH,5}$ 9.3), 4.52 (1H, dd, H5, $J_{5,OH}$ 9.3, $J_{5,4}$ 4.5), 4.82-4.84 (2H, m, H2, H3), 5.04-5.05 (1H, m, H4), 5.99 (1H, d, H1, $J_{1,2}$ 3.5); δ_C ($CDCl_3$, 100 MHz): 26.5, 26.9 (Me), 70.6 (C5), 78.1 (C4), 81.3, 82.9 (C2, C3), 106.6 (C1), 113.7 (<u>C</u>Me_2), 173.7 (C6); LRMS (ESI +ve): 280 (83%, M+MeCN+Na^+), 455 (100%, 2M+Na^+); [Lit.[73] m.p. 120.5-121.5°C; $[\alpha]_D^{20}$ +52.5 (*c* 1.95, $CHCl_3$)].

1,2-*O*-Isopropylidene-β-L-idurono-3,6-lactone, 2.18

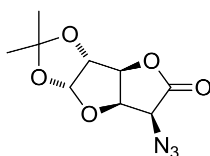


Glucuronolactone acetonide **2.5D** (242 g, 1.12 mol) was dissolved in DCM (2 L) and pyridine (270 mL, 3.34 mol) added. The solution was cooled to -30°C and trifluoromethanesulfonic anhydride (210 mL, 1.25 mol) was added over a period of 1 h, after which the reaction mixture was stirred at -30°C. TLC analysis (1:2 EtOAc/hexane) after a further 1 h showed complete consumption of starting material (R_f 0.10) and formation of a major product (R_f 0.80). The reaction mixture was washed with 2 M HCl(aq) (3 x 2 L) and the organic fraction concentrated under reduced pressure to afford the crude triflate derivative. The crude triflate was dissolved in DMF (1 L) and sodium trifluoroacetate (305 g, 2.24 mol) added portionwise. The reaction mixture was stirred at RT for 1 h, after which TLC analysis (1:2 EtOAc/hexane) revealed the presence of a single product (R_f 0.45). The reaction mixture was diluted with sat. $NaHCO_3$(aq), extracted with EtOAc (3 x 2 L) and the combined organic fractions concentrated to approx. 200 mL under reduced pressure. The resultant brown solution was seeded with a small amount of crystalline lactone and refrigerated overnight. The resultant white crystals were collected by filtration to afford the title compound **2.18** (87 g, 36%). The mother liquor was then concentrated and recrystallisation from hot PhMe followed by treatment with decolourising charcoal gave a

second crop of the title compound **2.18** (89 g, 37%) as a white crystalline solid. Total yield: 176 g, 73%.

HRMS (ESI +ve): $C_{10}H_{16}NaO_7$ found 271.0785; (M+MeOH+Na^+) requires 271.0788; $[\alpha]_D^{25}$ +107.9 (*c* 1.23, $CHCl_3$); mp 133-135°C; ν_{max} (thin film): 3406 (br, m, OH), 1789 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.35, 1.52 (2 x 3H, s, Me), 3.17 (1H, d, OH, $J_{OH,5}$ 3.4), 4.33 (1H, d, H5, $J_{5,OH}$ 3.2), 4.79 (1H, d, H4, $J_{4,3}$ 3.2), 4.83 (1H, d, H2, $J_{2,1}$ 3.6), 5.06 (1H, d, H3 $J_{3,4}$ 3.2), 5.93 (1H, d, H1, $J_{1,2}$ 3.7); δ_C ($CDCl_3$, 100 MHz): 26.6, 27.0 (Me), 71.7 (C5), 82.0 (C2), 82.4 (C4), 85.1 (C3), 106.1 (C1), 113.2 (<u>C</u>Me_2), 174.7 (C6); LRMS (ESI +ve): 312 (100%, M+MeOH+MeCN+Na^+); [Lit.[97] m.p. 128-130°C; $[\alpha]_D^{20}$ +109.4 (*c* 0.8, $CHCl_3$)].

5-Azido-5-deoxy-1,2-*O*-isopropylidene-α-D-glucurono-3,6-lactone, 2.19

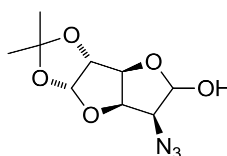


Lactone **2.18** (98.3 g, 0.46 mol) was dissolved in DCM (l L) and pyridine (110 mL, 1.36 mol) added. The solution was then cooled to -30°C and trifluoromethanesulfonic anhydride (84 mL, 0.50 mol) was added over a period of 1 h, after which the reaction mixture was stirred at -30°C. TLC analysis (1:2 EtOAc/hexane) after a further 1 h showed complete consumption of starting material (R_f 0.45) and formation of a single product (R_f 0.80). The reaction mixture was diluted with DCM (1 L) and washed with 2 M HCl(aq) (3 x 2 L) and the organic fraction concentrated under reduced pressure to afford the crude triflate derivative. The triflate was dissolved in DMF (1 L), cooled to -20°C and sodium azide (29.6 g, 0.46 mol) added. The reaction was stirred at -20°C for 1 h after which TLC analysis (1:2 EtOAc/hexane) showed the presence of a major product (R_f 0.75). The reaction mixture was diluted with 1:19 brine/water (1 L) and extracted with EtOAc (3 x 2 L). The combined organic fractions were concentrated under reduced pressure

and purification by medium pressure chromatography afforded the title compound **2.19** (96.0 g, 87%) as a white crystalline solid.

$[\alpha]_D^{19}$ +26.2 (*c* 1.02, $CHCl_3$); mp 82-84°C; ν_{max} (thin film): 2122 (s, N_3), 1795 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.35, 1.52 (2 x 3H, s, Me), 4.09 (1H, d, H5, $J_{5,4}$ 4.3), 4.84-4.85 (2H, m, H2, H3), 5.01 (1H, dd, H4, $J_{4,3}$ 2.8, $J_{4,5}$ 4.3), 6.02 (1H, d, H1, $J_{1,2}$ 3.5); δ_C ($CDCl_3$, 100 MHz): 26.5, 26.9 (Me), 60.5 (C5), 79.1 (C4), 82.4, 82.5 (C2, C3), 106.8 (C1), 113.6 ($\underline{C}Me_2$), 172.8 (C6); LRMS (ESI +ve): 291 (75%, M+MeOH+NH_4^+), 337 (100%, M+MeOH+MeCN+Na^+); [Lit.[74] m.p. 89°C; $[\alpha]_D^{21}$ +25.1 (*c* 0.56, $CHCl_3$)].

5-Azido-5-deoxy-1,2-*O*-isopropylidene-α-D-*gluco*-hexodialdo-1,4:3,6-difuranose, 2.21

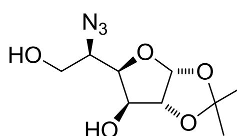


Azido-lactone **2.19** (20.0 g, 83 mmol) was dissolved in DCM (100 mL) and cooled to -78°C. DIBAL solution (1.0 M in hexanes, 95 mL, 95 mmol) was added over a period of 30 min and the reaction mixture stirred at -78°C. TLC analysis (1:2 EtOAc/hexane) after a further 30 min revealed the complete consumption of starting material (R_f 0.75) and the formation of a major product (R_f 0.50). The reaction mixture was diluted with DCM (600 mL), saturated aqueous potassium sodium tartrate solution (800 mL) added and stirred for 20 h at RT. The organic layer was collected and the aqueous extracted with DCM (5 x 600 mL), the combined organic fractions were dried over $MgSO_4$, filtered, and concentrated to approx. 100 mL under reduced pressure. PhMe (100 mL) was added and the solution concentrated to afford the title compound **2.21** (18.6 g, 92%) as a white crystalline solid.

HRMS (ESI +ve): $C_9H_{13}N_3NaO_5$ found 266.0745; (M+Na^+) requires 266.0747; $[\alpha]_D^{18}$ -7.2 (*c* 1.44, $CHCl_3$); mp 110-112°C; ν_{max} (thin film): 3420 (br, m, OH), 2114 (s, N_3); δ_H ($CDCl_3$, 400

MHz): 1.35, 1.50 (2 x 3H, s, Me), 3.44 (1H, t, H5, $J_{5,6} = J_{5,4}$ 4.8), 3.51 (1H, d, OH, $J_{OH,6}$ 6.8), 4.64 (1H, d, H3, $J_{3,4}$ 4.3), 4.75 (1H, d, H2, $J_{2,1}$ 3.5), 4.99 (1H, t, H4, $J_{4,5} = J_{4,3}$ 4.6), 5.40 (1H, dd, H6, $J_{6,OH}$ 6.8 $J_{6,5}$ 4.8), 6.09 (1H, d, H1, $J_{1,2}$ 3.5); δ_C ($CDCl_3$, 100 MHz): 26.9, 27.4 (Me), 62.6 (C5), 81.8 (C4), 85.4 (C3), 85.8 (C2), 97.8 (C6), 107.2 (C1), 113.3 ($\underline{C}Me_2$); LRMS (ESI +ve): 307 (84%, M+MeCN+Na^+), 339 (63%, M+MeCN+MeOH+Na^+), 541 (82%, 2M+MeOH+Na^+), 573 (62%, 2M+2MeOH+Na^+).

5-Azido-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose, 2.22

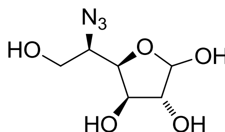


Azido-lactol **2.21** (18.6 g, 76.5 mmol) was dissolved in methanol (200 mL) and cooled to -20°C. Sodium borohydride (525 mg, 19.8 mmol) was added portionwise, and the reaction mixture stirred at -10°C for 1 h. TLC analysis (1:1 EtOAc/hexane) revealed the presence of starting material (R_f 0.75) and a major product (R_f 0.50). The reaction mixture was cooled to -20°C, a further portion of sodium borohydride (525 mg, 19.8 mmol) added, and stirred for a further 45 min at -10°C, after which time TLC analysis revealed complete consumption of starting material. The reaction mixture was neutralized with glacial acetic acid and concentrated under reduced pressure. Purification by flash column chromatography afforded the title compound **2.22** (18.0 g, 96%) as a colourless oil, which crystallised on standing.

HRMS (ESI +ve): $C_9H_{15}NaO_5$ found 268.0900; (M+Na^+) requires 268.0904; $[\alpha]_D^{25}$ -14.5 (*c* 1.10, $CHCl_3$); mp 63-65°C; ν_{max} (thin film): 3423 (br, s, OH), 2104 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.31, 1.49 (2 x 3H, s, Me), 2.63 (1H, br, s, OH6), 2.84 (1H, a-s, OH3), 3.80 (1H, dd, H6a, J_{gem} 11.1, $J_{6a,5}$ 5.8), 3.84-3.88 (1H, m, H5), 3.98 (1H, dd, H6b, J_{gem} 11.1, $J_{6b,5}$ 2.8), 4.12 (1H, dd, H4, $J_{4,5}$ 8.6, $J_{4,3}$ 2.8), 4.32 (1H, br, s, H3), 4.53 (1H, d, H2, $J_{2,1}$ 3.5), 5.93 (1H, d, H1, $J_{1,2}$ 3.5); δ_C ($CDCl_3$, 100 MHz): 26.1, 26.7 (Me), 60.8 (C5), 63.0 (C6), 74.8 (C3), 79.2 (C4), 85.0 (C2), 104.8

(C1), 112.1 ($\underline{C}Me_2$); LRMS (ESI +ve): 263 (74%, $M+NH_4^+$), 309 (100%, $M+MeCN+Na^+$), 513 (62%, $2M+Na^+$); [Lit.[98] oil; $[\alpha]_D^{25}$ -10.55 (*c* 2.18, $CHCl_3$)].

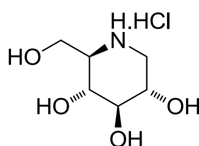
5-Azido-5-deoxy-D-glucofuranose, 2.23



Diol **2.22** (31.8 g, 130 mmol) was dissolved in water (300 mL), Dowex (50W-X2, H^+) (65 g) added, and stirred at 60°C. TLC analysis (EtOAc) after 24 h showed the complete consumption of starting material (R_f 0.75) and the formation of a single product (R_f 0.30). The reaction mixture was then filtered and concentrated under reduced pressure to afford the title compound **2.23** (26.0 g, 98%) as a pale yellow oil in a 1:1 ratio of anomers.

HRMS (ESI +ve): $C_6H_{11}NaO_5$ found 228.0588; ($M+Na^+$) requires 228.0591; $[\alpha]_D^{18}$ -19.4 (*c* 1.63, H_2O); ν_{max} (thin film, Ge): 3385 (br, OH), 2114 (s, N_3); δ_H (D_2O, 400 MHz): 3.69-3.78, 3.87-4.01 (2 x 3H, m, H5, H6), 4.08-4.14 (4H, m, H2, H4), 4.24 (1H, d, H3, *J* 4.5), 4.30 (1H, t, H3, *J* 3.3), 5.23 (1H, s, H1), 5.49 (1H, d, H1, *J* 3.8); δ_C (D_2O, 100 MHz): 62.3 (C5), 62.4 (C6), 62.6 (C6), 62.6 (C5), 75.3, 76.0, 76.4, 77.5 (C2, C4), 80.4, 80.8 (C3), 97.5, 103.0 (C1); LRMS (ESI +ve): 103 (100%, $M+2H^+$), 223 (41%, $M+NH_4^+$), 228 (65%, $M+Na^+$), 269 (90%, $M+MeCN+Na^+$), 428 (52%, $2M+NH_4^+$), 433 (82%, $2M+Na^+$); Lit.[23] $[\alpha]_D^{20}$ -22.2 (*c* 0.9, H_2O).

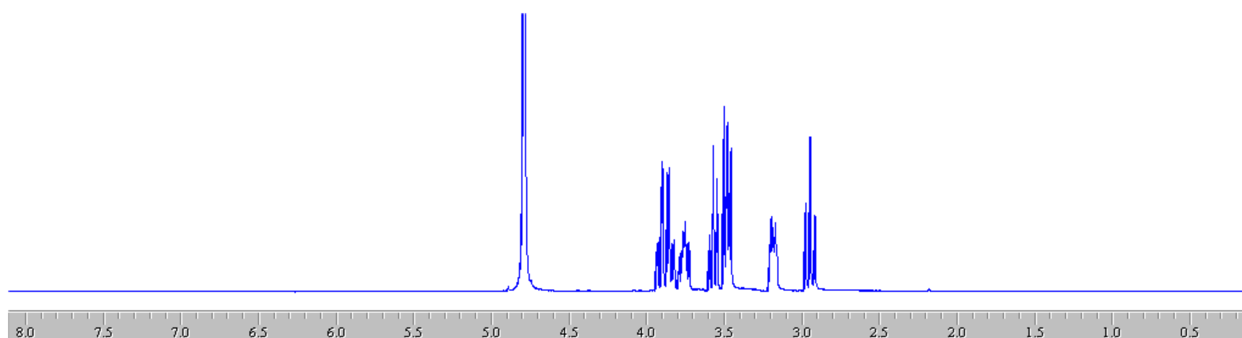
1-Deoxynojirimycin hydrochloride, 2.6.HCl

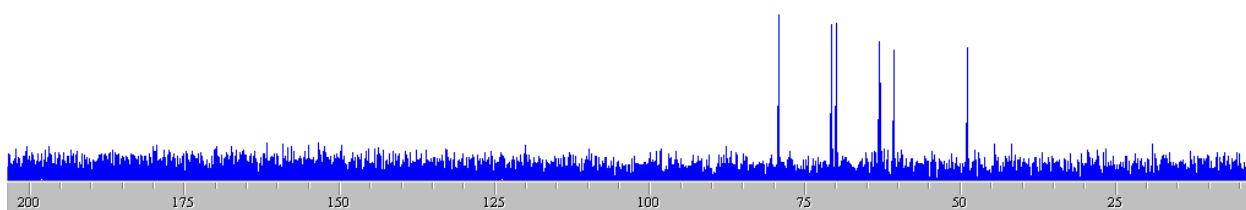


Azido-glucose **2.23** (12.0 g, 49.0 mmol) was dissolved in water (150 mL), Dowex (50W-X2, H^+) (20 g) added, and stirred at 70°C. TLC analysis (EtOAc) after 3 h showed the complete consumption of starting material (R_f 0.75) and the formation of a single product (R_f 0.30). The

reaction mixture was then filtered, and the Dowex rinsed with water (50 mL). The solution was cooled to RT, Pd (10% on C, 5.0 g, 4.7 mmol) added and the mixture shaken under hydrogen. The reaction vessel was evacuated and recharged with hydrogen every 15-30 min until a reduction of pressure in the vessel was observed, after which the vessel was evacuated and recharged a final time. The pressure of hydrogen was then maintained at 40-50 psi for a further 2 h and then left shaking under H_2 overnight, after which time no further reduction in pressure was observed. TLC analysis (14:3:1:1:1 industrial methylated spirits/water/pyridine/AcOH/n-BuOH) showed the complete consumption of starting material (R_f 0.95) and the formation of a major product (R_f 0.55). The reaction mixture was filtered, acidified to pH 2 with 32% aqueous HCl and concentrated to approx. 20 mL under reduced pressure. The remaining solution was then coevaporated with methanol until a white crystalline precipitate was observed. The mixture was refrigerated and the precipitate collected by filtration to give **2.6.HCl** (7.37 g, 74%) as a white crystalline solid.

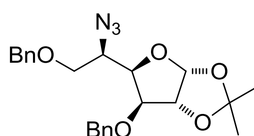
Data for DNJ.HCl: HRMS (ESI +ve): $C_6H_{14}NO_5$ found 164.0922; $(M+H^+)$ requires 163.0917; $[\alpha]_D^{23}$ +36.9 (*c* 1.10, H_2O); mp 200-202°C; δ_H (D_2O, 400 MHz): 2.95 (1H, t, H1a, $J_{gem} = J_{1a,2}$ 12.0), 3.18 (1H, ddd, H5, $J_{5,4}$ 10.4, $J_{5,6a}$ 5.1, $J_{5,6b}$ 3.3), 3.48 (1H, dd, H1b, J_{gem} 11.9, $J_{1b,2}$ 5.1), 3.48 (1H, t, H3, $J_{3,4}$ $J_{3,2}$ 9.3), 3.57 (1H, dd, H4, $J_{4,5}$ 10.4, $J_{4,3}$ 9.3), 3.75 (1H, ddd, H2, $J_{2,1a}$ 11.9, $J_{2,3}$ 9.2, $J_{2,1b}$ 5.1), 3.84 (1H, dd, H6a, J_{gem} 12.9, $J_{6a,5}$ 5.1), 3.91 (1H, dd, H6b, J_{gem} 12.9, $J_{6b,5}$ 3.3); δ_C (D_2O, 100 MHz): 48.9 (C1), 60.7 (C5), 63.0 (C6), 70.0 (C2), 70.8 (C4), 79.2 (C3); LRMS (ESI +ve): 164 (68%, $M+H^+$), 327 (100%, $2M+H^+$); [Lit.[99] m.p. 196-198°C; $[\alpha]_D^{22}$ +38 (*c* 1, H_2O)].





2.6.2. Synthesis of DMDP

5-Azido-3,6-di-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose, 2.26

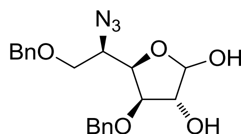


Benzyl bromide (3.6 mL, 30.3 mmol) and NaH (60% in mineral oil, 1.20 g, 30.0 mmol) were added to a stirring solution of diol **2.22** (2.45 g, 10.0 mmol) in DMF (10 mL) over 4 Å molecular sieves at 0°C. The reaction mixture was allowed to reach RT and TLC analysis (1:2 EtOAc/CyH) after 90 min revealed the absence of starting material (R_f 0.15) and the presence of a major product (R_f 0.75). The reaction mixture was quenched with 20% v/v brine/water (100 mL) and EtOAc (100 mL) added. The aqueous phase was discarded and the organic layer washed with 20% v/v brine/water (2 x 100 mL) before being dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography gave the fully protected azido-glucose **2.26** (3.51 g, 82%) as a white crystalline solid.

HRMS (ESI +ve): $C_{23}H_{27}N_3NaO_5$ found 448.1833; $(M+Na^+)$ requires 448.1843; $[\alpha]_D^{25}$ -30.8 (*c* 1.04, $CHCl_3$); m.p. 65-68°C; ν_{max} (thin film): 2098 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.32, 1.48 (2 x 3H, s, Me), 3.64 (1H, dd, H6a, J_{gem} 10.1, $J_{6a,5}$ 7.1), 3.91 (1H, dd, H6b, J_{gem} 10.2, $J_{6b,5}$ 2.3), 4.00-4.08 (3H, m, H3, H4, H5), 4.57-4.62 (4H, m, H2, 3 x C$\underline{H}_2$Ph), 4.69 (1H, d, C$\underline{H}_2$Ph, J_{gem} 11.4), 5.89 (1H, d, H1, $J_{1,2}$ 3.8), 7.27-7.40 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 26.3, 26.8 (Me), 59.0 (C5), 71.1 (C6), 72.2, 73.4 ($\underline{C}H_2$Ph), 78.6 (C4), 81.5 (C3), 81.7 (C2), 105.3 (C1), 112.0 ($\underline{C}Me_2$),

127.6, 127.6, 127.8, 128.0, 128.1, 128.4, 128.5 (Ar<u>C</u>H), 137.1, 137.9 (Ar<u>C</u>); LRMS (ESI +ve): 484 (100%, M+MeCN+NH_4^+); [Lit.[81] m.p. 66-67°C; $[\alpha]_D^{20}$ -35 (*c* 1.16, $CHCl_3$)].

5-Azido-3,6-di-*O*-benzyl-5-deoxy-D-glucofuranose, 2.27

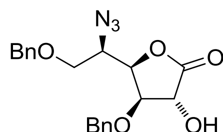


Fully protected **2.26** (6.20 g, 14.6 mmol) was dissolved in 1:7 water/1,4-dioxane (40 mL) and *p*TSA (6.0 g, 31.6 mmol) was added. The reaction mixture heated to 90°C and further 10 mL of water was added in portions over 30 min, maintaining a homogenous mixture. TLC analysis (1:2 EtOAc/CyH) after 2.5 h revealed a trace of starting material (R_f 0.75) and the formation of a major product (R_f 0.10). The reaction mixture was then cooled to RT, quenched with sat. $NaHCO_3$ (150 mL) and the aqueous extracted with EtOAc (3 x 150 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography afforded the title lactol **2.27** (5.16 g, 92%) as a pale yellow oil, which crystallised on standing, in a 3:2 ratio of anomers.

HRMS (ESI +ve): $C_{20}H_{23}N_3NaO_5$ found 408.1520; (M+Na^+) requires 408.1530; $[\alpha]_D^{25}$ -9.4 (*c* 1.17, $CHCl_3$); m.p. 60-62°C; ν_{max} (thin film): 3417 (s, OH), 2100 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 3.64 (1H, dd, H6a^A, J_{gem} 10.1, $J_{6a,5}$ 7.2), 3.71 (1H, dd, H6a^B, J_{gem} 10.0, $J_{6b,5}$ 6.8), 3.85 (1H, dd, H6b^A, J_{gem} 10.2, $J_{6b,5}$ 2.5), 3.91-3.99 (3H, m, H5^A, H5^B, H6b^B), 3.99-4.02 (2H, m, H3^A, H3^B), 4.13 (1H, dd, H2^A, $J_{2,1}$ 3.8, $J_{2,3}$ 1.1), 4.16 (1H, dd, H4^B, *J* 3.2, 9.6), 4.17 (1H, dd, H4^A, *J* 3.7, 9.6), 4.24 (1H, s, H2^B), 4.58 (2H, s, C<u>H</u>$_2$Ph^A), 4.59, 4.62 (2 x 1H, d, C<u>H</u>$_2$Ph^B, J_{gem} 12.0), 4.61, 4.66 (2 x 1H, d, C<u>H</u>$_2$Ph^A, J_{gem} 11.4), 4.64, 4.67 (2 x 1H, d, C<u>H</u>$_2$Ph^B, J_{gem} 11.1), 5.10 (1H, s, H1^B), 5.47 (1H, d, H1^A, $J_{1,2}$ 3.8), 7.27-7.41 (20H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 59.4 (C5^A), 60.1 (C5^B), 70.9 (C6^A), 71.0 (C6^B), 72.3 (<u>C</u>H$_2$Ph^A), 73.2 (<u>C</u>H$_2$Ph^B), 73.5 (<u>C</u>H$_2$Ph^A), 73.6 (<u>C</u>H$_2$Ph^B), 74.1 (C2^A), 76.6 (C2^B), 77.4 (C4^A), 79.7 (C4^B), 82.0 (C3^B), 83.2 (C3^A), 97.2 (C1^A), 103.8 (C1^B), 127.7, 127.7, 128.0, 128.1, 128.4, 128.4, 128.5, 128.8 (Ar<u>C</u>H), 136.4, 137.3, 137.8, 137.8 (Ar<u>C</u>);

LRMS (ESI +ve): 444 (100%, M+MeCN+NH_4^+); Lit.[100] 58-59°C; Lit.[101] $[\alpha]_D$ -2.9 (*c* 0.018, $CHCl_3$).

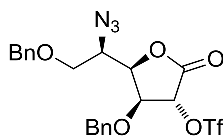
5-Azido-3,6-di-*O*-benzyl-5-deoxy-D-glucono-1,4-lactone, 2.28D



K_2CO_3 (365 mg, 2.64 mmol) and iodine (670 mg, 2.64 mg) were added to a warm solution of lactol **2.27** (507 mg, 1.32 mmol) and the reaction mixture stirred at reflux for 4 h. TLC analysis (1:1 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.50) and formation of a major product (R_f 0.85). Heating was ceased and saturated $Na_2S_2O_3$ added slowly until a biphasic mixture was obtained, followed by addition of EtOAc (25 mL). This mixture was stirred vigorously until the iodine was visibly quenched and the organic layer collected. The remaining aqueous suspension was extracted with EtOAc (2 x 25 mL) and the combined organic fractions dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography afforded the title lactone **2.28D** (463 mg, 92%) as colourless oil which crystallised on standing.

HRMS (ESI +ve): $C_{20}H_{21}N_3NaO_5$ found 406.1367; (M+Na^+) requires 406.1373; $[\alpha]_D^{25}$ +19.2 (*c* 1.09, $CHCl_3$); m.p. 60-62°C; ν_{max} (thin film): 3418 (s, OH), 2105 (s, N_3), 1792 (s, CO); δ_H ($CDCl_3$, 400 MHz): 3.21 (1H, br-s, OH), 3.76 (1H, dd, H6a J_{gem} 10.2, $J_{6a,5}$ 6.8), 3.83 (1H, dd, H6b J_{gem} 10.1, $J_{6b,5}$ 3.3), 4.06 (1H, ddd, H5 $J_{5,4}$ 7.5, $J_{5,6a}$ 6.8, $J_{5,6b}$ 3.3), 4.26 (1H, dd, H3 $J_{3,2}$ 4.2, $J_{3,4}$ 5.8), 4.52 (1H, d, H2 $J_{2,3}$ 4.2), 4.55 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.9), 4.60 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.9), 4.63 (1H, dd, H4 $J_{4,3}$ 5.8, $J_{4,5}$ 7.5), 4.63 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.6), 4.73 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.6), 7.29-7.40 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 59.9 (C5), 69.7 (C6), 71.7 (C2), 72.6, 73.6 ($\underline{C}H_2$Ph), 78.3 (C4), 79.5 (C3), 127.7, 127.9, 128.0, 128.3, 128.5, 128.6 (Ar$\underline{C}$H), 136.6, 137.4 (Ar$\underline{C}$), 174.6 (C1); LRMS (ESI +ve): 401 (89%, M+NH_4^+), 406 (65%, M+Na^+), 442 (78%, M+MeCN+NH_4^+), 789 (100%, 2M+Na^+).

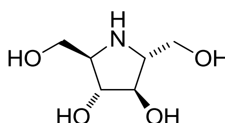
5-Azido-3,6-di-*O*-benzyl-5-deoxy-2-*O*-trifluoromethanesulfonyl-D-glucono-1,4-lactone, 2.29D



Trifluromethanesulfonic anhydride (0.18 mL, 1.07 mmol) was added dropwise to a solution of lactone **2.28D** (340 mg, 0.89 mmol) in DCM (3 mL) in the presence of pyridine (0.18 mL, 2.23 mmol) at -40°C. The reaction mixture was stirred for 1 h between -35°C and -25°C after which time TLC analysis (1:2 EtOAc/CyH) revealed the absence of starting material (R_f 0.40) and the formation of a major product (R_f 0.55). The reaction mixture was then partitioned between 2 M aqueous HCl (10 mL) and DCM (10 mL) and the organic phase washed with 2 M aqueous HCl (2 x 10 mL) before being dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography gave the title triflate **2.29D** (452 mg, 99%) as a colourless oil.

HRMS (ESI +ve); $C_{21}H_{20}F_3N_3NaO_7S$ found 538.0869; (M+Na$^+$) requires 538.0866; $[\alpha]_D^{25}$ +14.8 (*c* 1.42, $CHCl_3$); ν_{max} (thin film): 2111 (s, N_3), 1811 (s, CO); δ_H ($CDCl_3$, 400 MHz): 3.77 (1H, dd, H6a J_{gem} 10.1, $J_{6a,5}$ 6.3), 3.82 (1H, dd, H6b J_{gem} 10.1, $J_{6b,5}$ 3.8), 4.06 (1H, ddd, H5 $J_{5,4}$ 7.3, $J_{5,6a}$ 6.3, $J_{5,6b}$ 3.8), 4.46 (1H, dd, H3 $J_{3,2}$ 4.0, $J_{3,4}$ 5.6), 4.55 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.9), 4.60 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.9), 4.62 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.4), 4.63 (1H, dd, H4 $J_{4,3}$ 5.6, $J_{4,5}$ 7.3), 4.74 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.4), 4.39 (1H, d, H2 $J_{2,3}$ 4.0), 7.30-7.40 (10H, m, Ph); 59.3 (C5), 69.2 (C6), 73.4, 73.7 ($\underline{C}H_2$Ph), 77.0 (C3), 78.0 (C4), 79.8 (C2), 127.6, 128.0, 128.4, 128.5, 128.8, 128.9 (Ar$\underline{C}$H), 135.2, 137.0 (Ar$\underline{C}$), 165.8 (C1); LRMS (ESI +ve): 533 (62%, M+NH_4^+), 538 (61%, M+Na$^+$), 565 (74%, M+MeOH+NH_4^+), 570 (77%, M+MeOH+Na$^+$), 606 (100%, M+MeCN+MeOH+NH_4^+).

2,5-Dideoxy-2,5-imino-D-mannitol [DMDP], 2.12D



Pd (10% on C, 82 mg, 77 μmol) was added to a degassed solution of lactone **2.29D** (390 mg, 0.76 mmol) in ethanol (5 mL). The reaction mixture was flushed with hydrogen and stirred under hydrogen for 1 h at RT. TLC analysis (1:2 EtOAc/CyH) revealed the formation of one major product (R_f 0.20), the corresponding bicyclic lactone **2.30**:

Crude data: ν_{max} (thin film): 1824 (s, CO); δ_H ($CDCl_3$, 400 MHz): 3.84 (1H, dd, H6a J_{gem} 10.1, $J_{6a,5}$ 7.0), 3.96 (1H, dd, H6b J_{gem} 10.1, $J_{6b,5}$ 8.3), 4.17 (1H, a-t, H5 $J_{5,6}$ 7.6), 4.40 (1H, d, H3 or H4 J 2.4), 4.43 (1H, d, C<u>H</u>$_2$Ph J_{gem} 12.0), 4.47 (1H, d, C<u>H</u>$_2$Ph J_{gem} 12.0), 4.59 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.9), 4.80 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.9), 4.80 (1H, dd, H3 or H4 J 2.4, 0.8), 4.84 (1H, a-s, H2), 7.25-7.34 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 60.2 (C2), 62.9 (C5), 64.6 (C6), 73.1, 73.4 (<u>C</u>H$_2$Ph), 78.6, 79.7 (C3 or C4), 128.0, 128.0, 128.1, 128.5, 128.7, 128.8 (Ar<u>C</u>H), 135.2, 135.9 (Ar<u>C</u>), 165.4 (C1).

The catalyst was removed by filtration through a short Celite pad and $NaBH_4$ (140 mg, 3.70 mmol) was added directly to the filtrate in small portions. The reaction mixture was stirred at RT for 16 h, to give 1,4-di-*O*-benzyl-DMDP **2.31**:

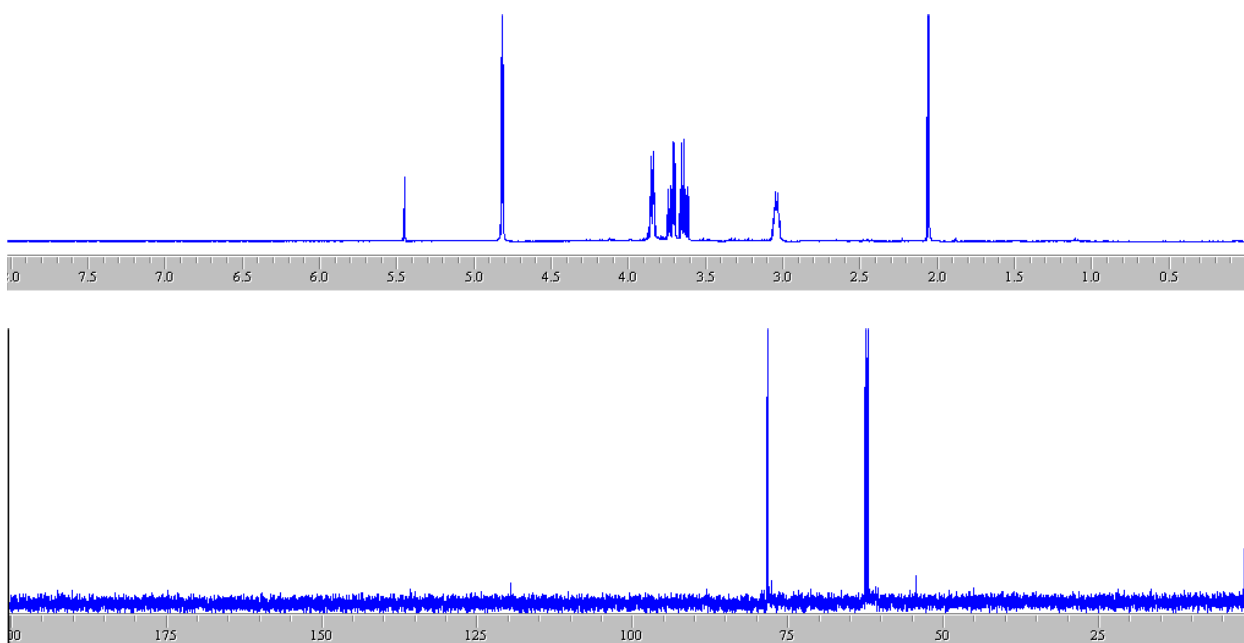
Crude data: δ_C ($CDCl_3$, 100 MHz): 63.5 (C1), 64.1, 64.3 (C2, C5), 71.4 (C6), 72.8, 74.3 (<u>C</u>H$_2$Ph), 79.2 (C4), 88.2 (C3), 128.5, 128.7, 128.8, 128.8, 129.3, 129.4 (Ar<u>C</u>H), 139.5, 139.7 (Ar<u>C</u>).

2 M HCl(aq) (3 mL) and Pd (10% on C, 150 mg, 0.14 mmol) were then added and the reaction mixture was stirred for a further 16 h under hydrogen at RT. The catalyst was removed by filtration and the filtrate concentrated to approx. 5 mL. The concentrate was then loaded onto a column of Dowex (50W-X8, H^+), washed sequentially with EtOH and water, and the amine

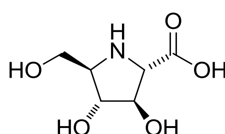
liberated with 2 M NH_3(aq). The ammoniacal fractions were combined and concentrated under reduced pressure to give DMDP **2.12D** (85 mg, 69%) as a pale yellow gum.

HRMS (ESI +ve); $C_6H_{14}NO_4$ found 164.0919; $(M+H^+)$ requires 164.0917; $[\alpha]_D^{21}$ +50.4 (*c* 0.89, H_2O); ν_{max} (thin film, Ge): 3332 (s, NH/OH); δ_H (D_2O, 400 MHz): 3.04-3.09 (2H, m, H2/H5), 3.66 (2H, dd, H1a/H6a J_{gem} 11.6, $J_{1a,2}/J_{6a,5}$ 6.3), 3.74 (2H, dd, H1b/H6b J_{gem} 11.6, $J_{1a,2}/J_{6a,5}$ 4.3), 3.87 (2H, m, H3/H4); δ_C (D_2O, 100 MHz): 62.2 (C2/C5), 62.5 (C1/C6), 78.3 (C3/C4); LRMS (ESI -ve): 162 (39%, $[M-H]^-$), 325 (100%, $[2M-H]^-$); Lit.[102] $[\alpha]_D^{20}$ +54.3 (*c* 1.2, H_2O).

The same sequence in the enantiomeric series gave L-DMDP **2.12L** {$[\alpha]_D^{25}$ -56.6 (*c* 1.22, H_2O)}, (346 mg, 2.12 mmol), in 81% yield from lactone **2.28L** (1.00 g, 2.61 mmol). The NMR data was identical to that of its enantiomer.



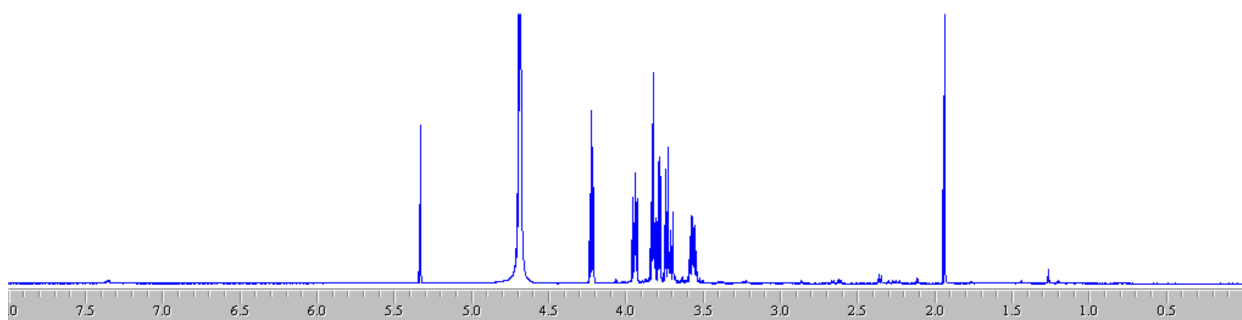
2,5-Dideoxy-2,5-imino-D-mannonic acid, 2.24D

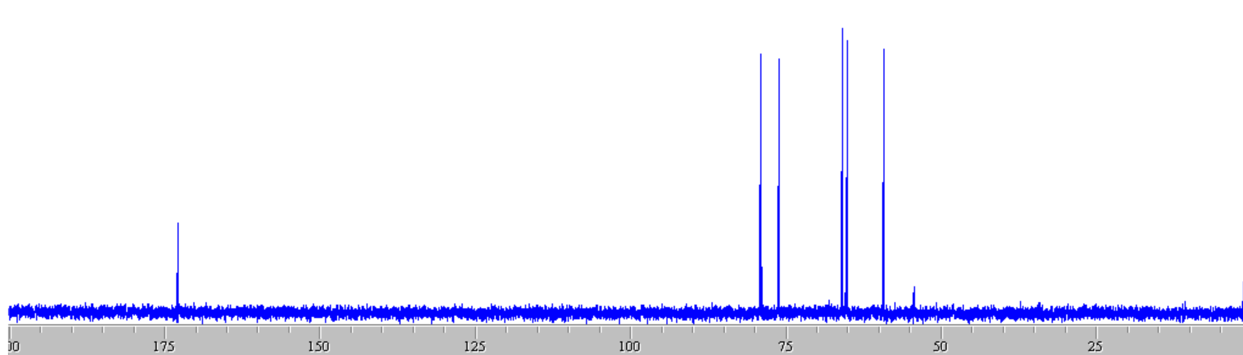


Pd (10% on C, 180 mg, 0.17 mmol) was added to a degassed solution of lactone **2.29D** (450 mg, 0.87 mmol) in 3:7 water/1,4-dioxane (5 mL) and saturated with water (1.5 mL). The reaction mixture was flushed with hydrogen and stirred under hydrogen for 4 h at RT after which IR analysis revealed the absence of azide functionality. 2 M HCl(aq) (1 mL) was injected into the reaction vessel and the mixture stirred for a further 16 h under hydrogen. The catalyst was then removed by filtration through a short pad of Celite and the filtrate concentrated to approx 2 mL under reduced pressure. The concentrate was then loaded onto a column of Dowex (50W-X8, H^+), washed with water, and the amine liberated with 2 M NH_3(aq). The ammoniacal fractions were combined and concentrated under reduced pressure to give the sugar amino acid **2.24D** (119 mg, 77%) as pale yellow gum, which crystallised on standing.

HRMS (ESI +ve); $C_6H_{11}NNaO_5$ found 200.0526; (M+Na^+) requires 200.0529; $[\alpha]_D^{25}$ +14.7 (*c* 1.13, H_2O); m.p. 176°C-decomp; ν_{max} (thin film, Ge): 3356 (s, NH/OH), 1634 (s, CO); δ_H (D_2O, 400 MHz): 3.69 (1H, a-dt, H5 $J_{5,6a}$ 6.8, $J_{5,6b}$ = $J_{5,4}$ 5.1), 3.84 (1H, dd, H6a J_{gem} 12.4, $J_{6a,5}$ 6.8), 3.92 (1H, dd, H6b J_{gem} 12.4, $J_{6b,5}$ 4.5), 3.95 (1H, d, H2 $J_{2,3}$ 4.3), 4.06 (1H, dd, H4 $J_{4,3}$ 4.5, $J_{4,5}$ 5.3), 4.34 (1H, t, H3 $J_{3,2}$, $J_{3,4}$ 4.5); δ_C (D_2O, 100 MHz): 59.3 (C6), 65.3 (C5), 66.0 (C2), 76.2 (C4), 79.0 (C3), 173.0 (C1); LRMS (ESI -ve): 176 (69%, $[M-H]^-$), 353 (100%, $[2M-H]^-$).

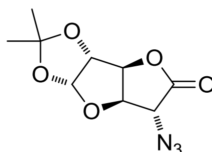
The same sequence in the enantiomeric series gave sugar amino acid **2.24L** {$[\alpha]_D^{25}$ -15.1 (*c* 0.96, H_2O); m.p. 174°C-decomp}, (396 mg, 2.25 mmol) in 86% yield from lactone **2.28L** (1.00 g, 2.61 mmol). The NMR data was identical to that of its enantiomer.





2.6.3. Synthesis of DNJNAc and DGJNAc

5-Azido-5-deoxy-1,2-*O*-isopropylidene-β-L-idurono-3,6-lactone, 2.20

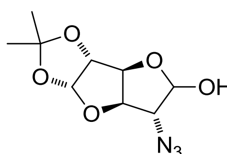


Trifluoromethanesulfonic anhydride (10.0 mL, 59.6 mmol) was added dropwise to a solution of lactone **2.5D** (10.0 g, 46.3 mmol) in DCM (100 mL) and pyridine (10.0 mL, 124 mmol) at -40°C, and the reaction mixture stirred at -35 to -25°C. TLC analysis (1:1 EtOAc/CyH) after 1 h revealed the complete consumption of the starting material (R_f 0.20) and the formation of a major product (R_f 0.70). The mixture was diluted with DCM (150 mL), washed with 2 M HCl (3 x 150 mL) and the organic fraction dried over $MgSO_4$, filtered and concentrated. The crude residue was dissolved in DMF (100 mL), cooled to -30°C and sodium azide (3.70 g, 59.6 mmol) added. TLC analysis (1:2 EtOAc/CyH) after 1 h revealed the complete consumption of the starting material (R_f 0.50) and the formation of a major product (R_f 0.70). The reaction mixture was partitioned between EtOAc (200 mL) and 4:1 water/brine (100 mL) and the aqueous layer discarded. The organic fraction was washed with 4:1 water/brine (2 x 100 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude title compound **2.20** (11.1 g, assumed 99%) as a white crystalline solid, which was used without further purification.

Data for chromatographed sample:

HRMS (ESI +ve): $C_{10}H_{15}N_3NaO_6$ found 296.0856; (M+MeOH+Na^+) requires 296.0853; $[\alpha]_D^{25}$ +261.4 (*c* 1.01, $CHCl_3$); m.p. 112-114°C; ν_{max} (thin film): 2125 (s, N_3), 1793 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.34, 1.50 (2 x 3H, s, Me), 4.23 (1H, s, H5), 4.65 (1H, d, H3 or H4 *J* 3.2), 4.83 (1H, d, H2 $J_{1,2}$ 3.6), 4.94 (1H, d, H3 or H4 *J* 3.3), 5.93 (1H, d, H1 $J_{1,2}$ 3.6); δ_C ($CDCl_3$, 100 MHz): 26.5, 27.0 (Me), 61.2 (C5), 81.1 (C3 or C4), 81.8 (C2), 84.9 (C3 or C4), 106.3 (C1), 113.4 ($\underline{C}Me_2$), 170.7 (C6); LRMS (ESI +ve): 296 (100%, M+MeOH+Na^+), 452 (76%), 531 (63%); [Lit.[75] 114-116°C; $[\alpha]_D^{20}$ +243 (*c* 1.1, $CHCl_3$)].

5-Azido-5-deoxy-1,2-*O*-isopropylidene-β-L-*ido*-hexodialdo-1,4:3,6-difuranose, 2.34

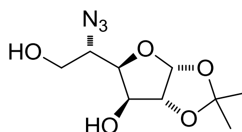


Diisobutylaluminium hydride (1.5 M in PhMe, 34.0 mL, 51.0 mmol) was added dropwise to a solution of crude azido-lactone **2.20** (11.1 g, assumed 46.1 mmol) in DCM (100 mL) at -78°C. The mixture was stirred at this temperature for 1 h, after which TLC analysis (1:2 EtOAc/CyH) revealed the complete consumption of the starting material (R_f 0.70) and the formation of a major product (R_f 0.45). DCM (300 mL) and sat. potassium sodium tartrate (400 mL) were added and the mixture stirred for 18 h. The organic layer was collected and the aqueous extracted with DCM (6 x 100 mL). The combined organic fractions were filtered, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude title compound **2.34** (8.70 g, assumed 78%) as a white crystalline solid in a 2:1 ratio of anomers, which was used without further purification.

HRMS (ESI +ve): $C_{10}H_{17}N_3NaO_6$ found 298.1010; (M+MeOH+Na^+) requires 298.1010; $[\alpha]_D^{25}$ -26.9 (*c* 1.32, $CHCl_3$); m.p. 54-56°C; ν_{max} (thin film): 3442 (br, s, OH), 2112 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.32 (3H, s, Me^B), 1.34 (3H, s, Me^A), 1.50 (6H, s, Me^A, Me^B), 3.25 (1H, d, OH^A $J_{OH,6}$ 7.2), 3.43 (1H, d, OH^B $J_{OH,6}$ 9.9), 4.06-4.08 (2H, m, $H5^A$, $H5^B$), 4.63 (1H, d, $H2^B$ $J_{2,1}$ 3.4), 4.71

(1H, s, $H3^B$ or $H4^B$ J 3.0), 4.74-4.75 (2H, m, $H2^A$, $H3^A$ or $H4^A$), 4.78-4.80 (2H, m, $H3^A$ or $H4^A$, $H3^B$ or $H4^B$), 5.33 (1H, d, $H6^A$ $J_{1,OH}$ 6.7), 5.51 (1H, dd, $H6^B$ $J_{1,2}$ 3.7, $J_{1,OH}$ 9.6), 5.87 (1H, d, $H1^B$ $J_{1,2}$ 3.5), 6.00 (1H, d, $H1^A$ $J_{1,2}$ 3.5); δ_C ($CDCl_3$, 100 MHz): 26.5, 27.2 (Me^B), 26.7, 27.3 (Me^A), 66.5 ($C5^B$), 69.1 ($C5^A$), 83.5 ($C2^B$), 83.6, 85.0 ($C3^B$, $C4^B$), 84.7, 85.3, 87.0 ($C2^A$, $C3^A$, $C4^A$), 98.9 ($C6^B$), 102.5 ($C6^A$), 106.5 ($C1^B$), 106.9 ($C1^A$), 112.7 ($\underline{C}Me_2^B$), 113.1 ($\underline{C}Me_2^A$); LRMS (ESI +ve): 298 (91%, M+MeOH+Na^+), 573 (100%, 2M+2MeOH+Na^+).

5-Azido-5-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose, 2.35



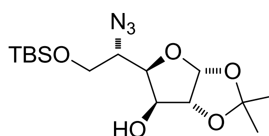
Sodium borohydride (540 mg, 14.3 mmol) was added to a solution of crude lactol **2.34** (8.70 g, assumed 35.8 mmol) in methanol (100 mL) at -30°C, before stirring at an internal temperature between -20 and -10°C. TLC analysis (1:2 EtOAc/CyH) after 1 h revealed the complete consumption of the starting material (R_f 0.45) and the formation of a major product (R_f 0.10). The mixture was neutralized with glacial acetic acid, concentrated under reduced pressure and partitioned between EtOAc (200 mL) and sat. $NaHCO_3$/brine (1:1, 200 mL). The aqueous layer was discarded and the organic washed sequentially with sat. $NaHCO_3$/brine (1:1, 100 mL) and brine (100 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude title compound **2.35** (8.18 g, assumed 93%) as a white crystalline solid, which was used without further purification.

Data for chromatographed sample:

HRMS (ESI +ve): $C_9H_{15}N_3NaO_5$ found 268.0906; (M+Na^+) requires 268.0904; $[\alpha]_D^{25}$ -69.6 (*c* 0.94, $CHCl_3$); m.p. 120-122°C; ν_{max} (thin film): 3334 (br, s, OH), 2108 (s, N_3); δ_H ($(CD_3)_2CO$, 400 MHz): 1.27, 1.41 (2 x 3H, s, Me), 3.66 (1H, dt, H6a J_{gem} 10.8, $J_{6a,5} = J_{6a,OH6}$ 5.5), 3.70-3.75 (1H, m, H5), 3.78 (1H, ddd, H6b J_{gem} 10.6, $J_{6b,5}$ 3.3, $J_{6b,OH6}$ 5.3), 4.16-4.18 (2H, m, H3, H4), 4.27 (1H, t, OH6 $J_{OH6,6}$ 5.6), 4.51 (1H, dd, OH3 $J_{OH3,3}$ 4.8, J 0.8), 4.54 (1H, d, H2, $J_{2,1}$ 3.8), 5.91 (1H,

d, H1 $J_{1,2}$ 3.8); δ_C ($(CD_3)_2CO$, 100 MHz): 26.4, 27.1 (Me), 62.4 (C6), 64.6 (C5), 75.1, 81.5 (C3, C4), 86.6 (C2), 105.5 (C1), 111.9 (CMe$_2$); LRMS (ESI +ve): 268 (86%, M+Na$^+$), 513 (100%, 2M+Na$^+$); [Lit.[23] 119-121°C, $[\alpha]_D^{20}$ -77.0 (*c* 1.04, MeOH)].

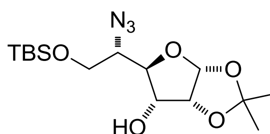
5-Azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose, 2.36



tert-Butyldimethylsilyl chloride (8.55 g, 56.8 mmol) was added to a solution of crude diol **2.35** (8.18 g, assumed 33.4 mmol) in pyridine (100 mL). The mixture was stirred at RT for 7 h, after which TLC analysis (1:2 EtOAc/CyH) revealed the complete consumption of the starting material (R_f 0.10) and the formation of a major product (R_f 0.70). The mixture was quenched with methanol (2 mL), concentrated under reduced pressure and dissolved in EtOAc (250 mL). The organic phase was washed with 2 M HCl (3 x 150 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (1:19 to 3:17 EtOAc/CyH) afforded the title compound **2.36** (11.9 g, assumed 99%, and 72% over 5 steps) as a colourless oil.

HRMS (ESI +ve): $C_{15}H_{29}N_3NaO_5Si$ found 382.1770; (M+Na$^+$) requires 382.1769; $[\alpha]_D^{25}$ -12.7 (*c* 1.07, $CHCl_3$); ν_{max} (thin film): 3463 (br, s, OH), 2101 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.12 (6H, s, SiMe$_2$), 0.91 (9H, s, CMe$_3$), 1.32, 1.50 (2 x 3H, s, CMe$_2$), 3.24 (1H, s, br, OH), 3.63-3.69 (1H, m, H5), 3.76-3.81 (2H, m, H6), 4.12 (1H, dd, H4 $J_{4,3}$ 2.5, $J_{4,5}$ 7.8), 4.20 (1H, a-s, H3), 4.54 (1H, d, H2 $J_{2,1}$ 3.8), 5.96 (1H, d, H1 $J_{1,2}$ 3.8); δ_C ($CDCl_3$, 100 MHz): -5.7 (SiMe$_2$), 18.1 (CMe$_3$), 25.7 (CMe$_3$), 26.2, 26.8 (CMe$_2$), 62.0 (C5), 63.3 (C6), 75.4 (C3), 82.4 (C4), 85.0 (C2), 104.6 (C1), 111.7 (CMe$_2$); LRMS (ESI +ve): 382 (87%, M+Na$^+$), 741 (100%, 2M+Na$^+$); Lit.[103] $[\alpha]_D^{20}$ -11.5 (*c* 0.8, CH_2Cl_2).

5-Azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene-β-L-talofuranose, 2.38L

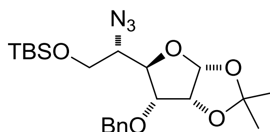


Pyridinium chlorochromate (4.80 g, 22.3 mmol) was added to a solution of alcohol **2.36** (2.00 g, 5.57 mmol) in DCM (30 mL) in the presence of 3 Å molecular sieves and the mixture stirred for 18 h. TLC analysis (1:2 EtOAc/CyH) revealed the complete consumption of the starting material (R_f 0.65) and the formation of a major product (R_f 0.30, streaking to 0.80). The mixture was filtered through Celite, concentrated and passed down a short column of silica gel (1:5 to 1:3 EtOAc/CyH), and fractions containing the product were combined, concentrated and dissolved in ethanol (20 mL). Aqueous sodium borohydride (210 mg, 5.56 mmol in 5 mL) was added to this solution dropwise at 0°C, before allowing to reach RT. TLC analysis (1:2 EtOAc/CyH) after 2 h revealed the complete consumption of the starting material and the formation of a major product (R_f 0.60). The mixture was neutralized with glacial acetic acid, concentrated under reduced pressure and partitioned between EtOAc (50 mL) and $NaHCO_3$(aq) (50 mL). The aqueous layer was discarded and the organic layer washed with $NaHCO_3$(aq) (50 mL) and brine (50 mL) before being dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:49 to 3:17 EtOAc/CyH) afforded the title compound **2.38L** (1.57 g, 79%) as a colourless oil.

HRMS (ESI +ve): $C_{15}H_{29}N_3NaO_5Si$ found 382.1770; (M+Na$^+$) requires 382.1769; $[\alpha]_D^{25}$ +74.9 (*c* 0.94, $CHCl_3$); ν_{max} (thin film): 3475 (br, s, OH), 2100 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.10, 0.10 (2 x 3H, s, Si<u>Me</u>$_2$), 0.91 (9H, s, C<u>Me</u>$_3$), 1.36, 1.55 (2 x 3H, s, C<u>Me</u>$_2$), 2.57 (1H, d, OH $J_{OH,3}$ 10.0), 3.63 (1H, ddd, H5 $J_{5,4}$ 3.5, $J_{5,6a}$ 5.3, $J_{5,6b}$ 7.7), 3.80 (1H, dd, H4 $J_{4,3}$ 8.6, $J_{4,5}$ 3.5), 3.86 (1H, dd, H6a J_{gem} 10.5, $J_{6a,5}$ 5.4), 3.91 (1H, dd, H6b J_{gem} 10.5, $J_{6b,5}$ 7.7), 4.04 (1H, ddd, H3 $J_{3,2}$ 5.2, $J_{3,4}$ 8.7, $J_{3,OH}$ 9.9), 4.58 (1H, dd, H2 $J_{2,1}$ 3.9, $J_{2,3}$ 5.2), 5.81 (1H, d, H1 $J_{1,2}$ 3.9); δ_C ($CDCl_3$, 100 MHz): -5.6, -5.5 (Si<u>Me</u>$_2$), 18.2 (<u>C</u>Me$_3$), 25.7 (C<u>Me</u>$_3$), 26.5, 26.5 (C<u>Me</u>$_2$), 62.5 (C5), 63.9 (C6),

72.2 (C3), 78.4 (C2), 79.3 (C4), 104.1 (C1), 112.9 (CMe$_2$); LRMS (ESI +ve): 382 (89%, M+Na$^+$), 741 (100%, 2M+Na$^+$).

5-Azido-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene-β-L-talofuranose, 2.39L

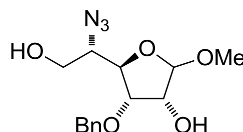


Sodium hydride (60% in mineral oil, 400 mg, 10.0 mmol) was added to a solution of alcohol **2.38L** (2.38 g, 6.63 mmol) and benzyl bromide (1.20 mL, 10.1 mmol) in DMF (15 mL) at 0°C, before stirring at RT for 1 h. TLC analysis (1:3 EtOAc/CyH) revealed the complete consumption of the starting material (R_f 0.30) and the formation of a major product (R_f 0.65). The reaction mixture was partitioned between EtOAc (50 mL) and 1:1 water/brine (50 mL) and the aqueous layer discarded. The organic fraction was washed with 1:1 water/brine (2 x 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:49 to 1:19 EtOAc/CyH) afforded the title compound **2.39L** (2.88 g, 97%) as a colourless oil.

HRMS (ESI +ve): $C_{22}H_{35}N_3NaO_5Si$ found 472.2240; (M+Na$^+$) requires 472.2238; $[\alpha]_D^{25}$ +101.5 (*c* 0.56, $CHCl_3$); ν_{max} (thin film): 2098 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.08, 0.09 (2 x 3H, s, SiMe$_2$), 0.90 (9H, s, CMe$_3$), 1.35, 1.57 (2 x 3H, s, CMe$_2$), 3.56 (1H, ddd, H5 $J_{5,4}$ 2.0, $J_{5,6a}$ 4.5, $J_{5,6b}$ 8.8), 3.84 (1H, dd, H6a J_{gem} 10.5, $J_{6a,5}$ 4.5), 3.88 (1H, dd, H3 $J_{3,2}$ 4.3, $J_{3,4}$ 8.8), 3.90 (1H, dd, H6b J_{gem} 10.5, $J_{6b,5}$ 8.8), 4.04 (1H, dd, H4 $J_{4,3}$ 8.8, $J_{4,5}$ 2.0), 4.56 (1H, dd, H2 $J_{2,1}$ 3.8, $J_{2,3}$ 4.2), 4.57, 4.80 (2 x 1H, d, CH$_2$Ph J_{gem} 12.0), 5.72 (1H, d, H1 $J_{1,2}$ 3.7), 7.31-7.39 (5H, m, Ph); δ_C ($CDCl_3$, 100 MHz): -5.6, -5.5 (SiMe$_2$), 18.2 (CMe$_3$), 25.8 (CMe$_3$), 26.4, 26.8 (CMe$_2$), 62.2 (C5), 64.5 (C6), 72.3 (CH$_2$Ph), 76.9 (C2), 76.9 (C4), 77.8 (C3), 104.3 (C1), 113.2 (CMe$_2$), 128.1, 128.3, 128.6 (ArCH), 137.3 (ArC); LRMS (ESI +ve): 472 (78%, M+Na$^+$), 921 (100%, 2M+Na$^+$).

Similar treatment of alcohol **2.38D** (3.66 g, 10.2 mmol) in a suitably scaled procedure gave fully protected **2.39D** (4.42 g, 97%); $[\alpha]_D^{25}$ -95.2 (*c* 0.59, $CHCl_3$).

Methyl 5-azido-3-*O*-benzyl-5-deoxy-L-talofuranoside, 2.40L

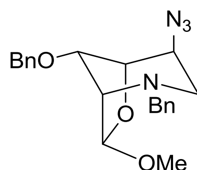


Fully protected **2.39L** (2.55 g, 5.68 mmol) was dissolved in a mixture of methanol (20 mL) and acetyl chloride (0.2 mL). The reaction mixture was stirred at 50°C for 3 h after which TLC analysis (1:2 EtOAc/CyH) revealed the complete consumption of the starting material (R_f 0.70) and the formation of a major product (R_f 0.10). The reaction was quenched by the addition of $NaHCO_3$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:9 to 3:2 EtOAc/CyH) afforded the title compound **2.40L** (1.71 g, 97%) as a partially crystalline 5:1 mixture of anomers.

HRMS (ESI +ve): $C_{14}H_{19}N_3NaO_5$ found 332.1225; ($M+Na^+$) requires 332.1217; ν_{max} (thin film): 3425 (br, s, OH), 2118 (s, N_3); δ_H ($CDCl_3$, 400 MHz, major anomer only): 3.38 (3H, s, Me), 3.42 (1H, dt, H5 $J_{5,4} = J_{5,6a}$ 6.1, $J_{5,6b}$ 4.4), 3.71 (1H, dd, H6a J_{gem} 11.4, $J_{6a,5}$ 6.3), 3.76 (1H, dd, H6b J_{gem} 11.4, $J_{6b,5}$ 4.5), 4.03 (1H, d, H2 $J_{2,3}$ 4.3), 4.11 (1H, dd, H4 $J_{4,3}$ 7.6, $J_{4,5}$ 6.1), 4.19 (1H, dd, H3 $J_{3,2}$ 4.3, $J_{3,4}$ 7.6), 4.54, 4.61 (2 x 1H, d, C<u>H</u>$_2$Ph, J_{gem} 11.6), 4.88 (1H, s, H1), 7.34-7.42 (5H, m, Ph); δ_C ($CDCl_3$, 100 MHz, major anomer only): 55.6 (Me), 62.5 (C6), 65.4 (C5), 72.5 (C2), 73.1 (<u>C</u>H$_2$Ph), 79.6 (C3), 81.0 (C4), 108.6 (C1), 128.2, 128.7, 128.8 (<u>ArCH</u>), 136.4 (Ar<u>C</u>); LRMS (ESI +ve): 332 (76%, $M+Na^+$), 641 (100%, $2M+Na^+$).

Similar treatment of fully protected **2.39D** (3.08 g, 6.86 mmol) in a suitably scaled procedure gave glycoside **2.40D** (2.00 g, 94%) as a 5:1 mixture of anomers.

Methyl 5-azido-2-*N*-benzyl-3-*O*-benzyl-2,6-imino-2,5,6-trideoxy-α-L-galactofuranoside, 2.42Lα



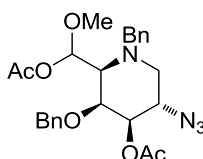
Trifluoromethanesulfonic anhydride (2.50 mL, 14.9 mmol) was added dropwise to a solution of talofuranoside **2.40L** (1.69 g, 5.47 mmol) in DCM (15 mL) and pyridine (2.50 mL, 30.9 mmol) at -40°C, and the reaction mixture stirred at -35 to -25°C. TLC analysis (1:2 EtOAc/CyH) after 2 h revealed the complete consumption of the starting material (R_f 0.15) and the formation of a major product (R_f 0.70). The mixture was diluted with DCM (85 mL), washed with 2 M HCl (3 x 100 mL) and the organic fraction dried over $MgSO_4$, filtered and concentrated. The crude residue was dissolved in THF (30 mL), cooled to -30°C and benzylamine (1.80 mL, 16.8 mmol) added dropwise. The mixture was allowed to warm to RT and TLC anaysis (1:20:79 TEA/EtOAc/CyH) after 2 h revealed the presence of two major components (R_f 0.30, 0.40), thought to be the two anomeric mono-substituted triflates. The reaction was stirred at 60°C for a further 16 h after which TLC analysis (1:20:79 TEA/EtOAc/CyH) revealed one new major product (R_f 0.75) and the persistence of the minor mono-substituted triflate (R_f 0.35). The reaction mixture was concentrated under reduced pressure and purification by flash column chromatography (1:0:99 to 1:4:95 TEA/EtOAc/CyH) afforded the title compound **2.42Lα** (1.28 g, 61%) as a pale yellow oil, and a single anomer.

HRMS (ESI +ve): $C_{21}H_{25}N_4O_3$ found 381.1919; ($M+H^+$) requires 381.1921; $[\alpha]_D^{25}$ +22.8 (*c* 1.11, $CHCl_3$); ν_{max} (thin film): 2109 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 2.74 (1H, d, H6a J_{gem} 12.9), 3.21 (1H, a-s, H2), 3.51 (1H, dd, H6b J_{gem} 12.9, $J_{6b,5}$ 4.3), 3.54 (3H, s, Me), 3.86 (1H, a-t, H5 $J_{5,4}$ 4.5, $J_{5,6b}$ 4.5), 3.89, 4.00 (2 x 1H, d, NC$\underline{H}_2$Ph J_{gem} 13.6), 4.11 (1H, s, H3), 4.29 (1H, d, H4 $J_{4,5}$ 5.2), 4.50, 4.57 (2 x 1H, d, OC$\underline{H}_2$Ph J_{gem} 11.7), 5.31 (1H, d, H1 $J_{1,2}$ 2.8), 7.25-7.36 (10H, m, Ph);

δ_C ($CDCl_3$, 100 MHz): 48.6 (C6), 57.6 (Me), 59.7 (C5), 59.9 (N<u>C</u>H$_2$Ph), 61.4 (C2), 71.0 (O<u>C</u>H$_2$Ph), 78.7 (C4), 81.5 (C3), 109.6 (C1), 127.0, 127.7, 127.8, 128.3, 128.4, 128.4 (Ar<u>C</u>H), 137.6, 139.1 (Ar<u>C</u>); LRMS (ESI +ve): 381 (93%, M+H$^+$), 403 (100%, M+Na$^+$), 492 (65%), 783 (55%, 2M+Na$^+$).

Similar treatment of methyl talofuranoside **2.40D** (685 mg, 2.22 mmol) in a suitably scaled procedure gave methyl galactofuranoside **2.42Dα** (530 mg, 63%); $[\alpha]_D^{25}$ -21.4 (*c* 1.20, $CHCl_3$).

4-*O*-Acetyl-5-azido-2-*N*-benzyl-3-*O*-benzyl-2,6-imino-2,5,6-trideoxy-L-galactose acetyl methyl acetal, 2.43L



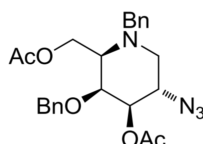
Boron trifluoride diethyl etherate (0.60 mL, 4.9 mmol) was added dropwise to a solution of methyl galactofuranoside **2.42Lα** (1.22 g, 3.21 mmol) in acetic anhydride (10 mL) at -30°C before warming to RT. TLC analysis (1:30:69 TEA/EtOAc/CyH) after 3 h revealed the formation of a major product (R_f 0.50) and the persistence of starting material (R_f 0.75). A further portion of boron trifluride diethyl etherate (0.40 mL, 3.2 mmol) was added and the mixture stirred for a further 3 h, after which time the reaction was complete. The mixture was concentrated under reduced pressure, dissolved in EtOAc (100 mL) and washed with sat. $NaHCO_3$(aq) (3 x 100 mL). The organic fraction was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:2:97 to 1:20:79 TEA/EtOAc/CyH) gave the title mixed acetal **2.43L** (1.44 g, 93%) as a pale yellow oil, in a 4:1 ratio of diastereoisomers.

HRMS (ESI +ve): $C_{25}H_{31}N_4O_6$ found 483.2232; (M+H$^+$) requires 483.2238; ν_{max} (thin film): 2106 (s, N_3), 1744 (s, CO); δ_H ($CDCl_3$, 400 MHz): 2.04, 2.11 (2 x 3H, s, $MeCO_2^B$), 2.10, 2.13 (2

x 3H, s, MeCO$_2^{A}$), 2.35 (1H, dd, H6a^{A} J_{gem} 13.8, $J_{6a,5}$ 10.6), 2.40 (1H, dd, H6a^{B} J_{gem} 13.9, $J_{6a,5}$ 10.6), 3.02-3.07 (3H, m, H6b^{B}, H2$^{A\&B}$), 3.09 (1H, dd, H6b^{A} J_{gem} 13.9, $J_{6b,5}$ 4.5), 3.29 (3H, s, OMe^{B}), 3.47 (3H, s, OMe^{A}), 3.88 (2H, s, NC$\underline{H}_2$Ph^{A}), 3.93 (2H, s, NC$\underline{H}_2$Ph^{B}), 4.04 (1H, dd, H3^{A} J 1.8, 2.8), 4.08-4.13 (1H, m, H5^{B}), 4.10 (1H, dt, H5^{A} $J_{5,4} = J_{5,6a}$ 10.1, $J_{5,6b}$ 4.4), 4.28 (1H, dd, H3^{B} J 1.5, 2.8), 4.49, 4.66 (2 x 1H, d, OC$\underline{H}_2$Ph^{A} J_{gem} 11.0), 4.58, 4.78 (2 x 1H, d, OC$\underline{H}_2$Ph^{B} J_{gem} 11.6), 4.83-4.86 (1H, m, H4^{B}), 4.86 (1H, dd, H4^{A} $J_{4,3}$ 2.8, $J_{4,5}$ 9.6), 6.14 (1H, d, H1^{B} $J_{1,2}$ 7.8), 6.30 (1H, d, H1^{A} $J_{1,2}$ 7.6), 7.21-7.42 (20H, m, Ph); (CDCl$_3$, 100 MHz): 20.9, 21.0 ($\underline{Me}$CO$_2^{B}$), 20.0, 21.1 ($\underline{Me}$CO$_2^{A}$), 51.5 (C6^{A}), 51.8 (C6^{B}), 53.8 (N$\underline{C}$H$_2$Ph^{B}), 53.8 (C5^{B}), 54.3 (C5^{A}), 54.6 (N$\underline{C}$H$_2$Ph^{A}), 56.3 (OMe^{A}), 56.9 (OMe^{B}), 63.1 (C2^{A}), 64.1 (C2^{B}), 74.9 (O$\underline{C}$H$_2$Ph^{A}), 75.1 (O$\underline{C}$H$_2$Ph^{B}), 76.0 (C3^{A}), 76.8 (C3^{B}), 77.2 (C4^{B}), 77.6 (C4^{A}), 95.0 (C1$^{A\&B}$), 127.0, 127.1, 127.4, 127.5, 127.7, 127.8, 128.3, 128.4, 128.5, 128.6 (Ar$\underline{C}$H), 138.0, 138.2, 139.3, 139.5 (Ar$\underline{C}$), 170.1, 170.2, 170.6, 171.0 (Me$\underline{C}$O$_2$); LRMS (ESI +ve): 482 (100%, M+H$^+$), 505 (99%, M+Na$^+$), 987 (2M+Na$^+$).

Similar treatment of methyl galactofuranoside **2.42Dα** (499 mg, 1.31 mmol) in a suitably scaled procedure gave mixed acetal **2.43D** (529 mg, 84%) as a 3:2 mixture of diastereoisomers.

3,6-Di-*O*-acetyl-2-azido-1-*N*-benzyl-4-*O*-benzyl-1,5-imino-1,2,5-trideoxy-D-galactitol, 2.46D



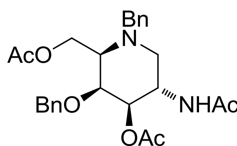
DIBAL (1.5 M in PhMe, 4.8 mL, 7.2 mmol) was added dropwise to a solution of 4-*O*-acetyl-5-azido-2-*N*,3-*O*-dibenzyl-2,6-imino-2,5,6-trideoxy-L-galactose acetyl methyl acetal **2.43L** (693 mg, 1.44 mmol) in DCM (5 mL) at -78°C. The mixture was stirred at this temperature for 45 min after which TLC analysis (1:40:59 TEA/EtOAc/CyH) revealed the absence of starting material (R_f 0.65) and the formation of several products (R_f 0.75, 0.60, 0.30). The reaction was quenched

with EtOAc (15 mL) and sat. potassium sodium tartrate solution (20 mL) added before stirring vigorously for 2 h. TLC analysis of the organic layer at this point revealed the presence of a major component (R_f 0.60) and a minor component (R_f 0.30). The organic layer was collected and the aqueous extracted with EtOAc (3 x 20 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was dissolved in MeOH (10 mL) and stirred with sodium borohydride (55 mg, 1.46 mmol) at RT for 1 h. TLC analysis (1:40:59 TEA/EtOAc/CyH) revealed one major component (R_f 0.30). The reaction mixture was concentrated under reduced pressure, co-evaporated with methanol three times, dissolved in a mixture of pyridine (5 mL) and acetic anhydride (5 mL) and stirred for 16 h at RT. TLC analysis (1:40:59 TEA/CyH) revealed the formation of a major product (R_f 0.65). The reaction mixture was concentrated under reduced pressure and purified by flash column chromatography (1:2:97 to 1:10:89 TEA/EtOAc/CyH) to afford the title compound **2.46D** (541 mg, 83%) as a colourless oil.

HRMS (ESI +ve): $C_{24}H_{28}N_4NaO_5$ found 475.1958; ($M+Na^+$) requires 475.1952; $[\alpha]_D^{25}$ +79.8 (*c* 0.43, $CHCl_3$); ν_{max} (thin film): 2107 (s, N_3), 1742 (s, ester); δ_H ($CDCl_3$, 400 MHz): 2.03. 2.12 (2 x 3H, s, Me), 2.26 (1H, dd, H1a J_{gem} 12.6, $J_{1a,2}$ 8.8), 2.95-2.99 (1H, m, H5), 3.01 (1H, dd, H1b J_{gem} 12.6, $J_{1b,2}$ 4.0), 3.63, 3.87 (2 x 1H, d, N$\underline{H_2}$Ph J_{gem} 14.1), 3.95 (1H, dt, H2 $J_{2,1a}$ 8.4, $J_{2,1b}$ 4.0, $J_{2,3}$ 8.4), 4.03 (1H, t, H4 $J_{4,3} = J_{4,5}$ 2.5), 4.20 (1H, dd, H6a J_{gem} 11.6, $J_{6a,5}$ 6.1), 4.53 (1H, dd, H6b J_{gem} 11.4, $J_{6b,5}$ 6.3), 4.55, 4.74 (2 x 1H, d, OC$\underline{H_2}$Ph J_{gem} 11.6), 4.87 (1H, dd, H3 $J_{3,2}$ 8.6, $J_{3,4}$ 2.5), 7.26-7.39 (10H, m, Ph); δ_C ($CDCl_3$, 126 MHz): 21.0, 21.0 (Me), 51.4 (C1), 56.4 (C2), 56.9 (N$\underline{C}H_2$Ph), 60.7 (C5), 61.9 (C6), 74.3 (O$\underline{C}H_2$Ph), 74.7 (C4), 75.1 (C3), 127.3, 127.8, 127.9, 128.4, 128.5, 128.7 (Ar$\underline{C}$H), 137.8, 138.0 (Ar$\underline{C}$), 170.1, 170.6 ($\underline{C}$OMe); LRMS (ESI +ve): 453 (74%, $M+H^+$), 475 (76%, $M+Na^+$), 927 (100%, $2M+Na^+$).

Similar treatment of mixed acetal **2.43D** (515 mg, 1.07 mmol) in a suitably scaled procedure gave piperidine **2.46L** (351 mg, 73%); $[\alpha]_D^{25}$ -75.6 (*c* 0.87, $CHCl_3$).

2-Acetamido-3,6-di-*O*-acetyl-1-*N*-benzyl-4-*O*-benzyl-1,5-imino-1,2,5-trideoxy-D-galactitol, 2.48D



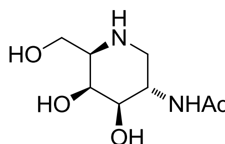
Powdered zinc (1.5 g, 23 mmol) was added to a solution of 3,6-di-*O*-acetyl-2-azido-1-*N*-benzyl-4-*O*-benzyl-1,5-imino-1,2,5-trideoxy-D-galactitol **2.46D** (520 mg, 1.15 mmol) in 3:2:1 THF/AcOH/Ac_2O (12 mL) and stirred vigorously before the dropwise addition of sat. $CuSO_4$(aq) (3 mL) to initiate the reaction. TLC analysis (1:50:49 TEA/EtOAc/CyH) after 20 min revealed the formation of a major product (R_f 0.15) and the persistence of starting material (R_f 0.80). A further portion of zinc (300 mg, 4.6 mmol) was added and the reaction stirred for a further 10 min, after which TLC analysis (1:50:49 TEA/EtOAc/CyH) revealed the reaction was complete. The reaction mixture was filtered through Celite, concentrated under reduced pressure and purified by flash column chromatography (1:5:94 to 1:70:29 TEA/EtOAc/CyH) to afford the title compound **2.48D** (426 mg, 79%) as a white crystalline solid.

HRMS (ESI +ve): $C_{26}H_{22}N_2NaO_6$ found 491.2145; ($M+Na^+$) requires 491.2153; $[\alpha]_D^{25}$ +26.2 (*c* 1.07, Me_2CO); m.p. 112-114°C; ν_{max} (thin film): 3300 (m, NH), 1738 (s, ester), 1653 (s, CO amide I), 1539 (m, NH amide II); δ_H (($(CD_3)_2CO$ 100 MHz):1.81, 2.01, 2.07 (3 x 3H, s, Me), 2.27 (1H, dd, H1a J_{gem} 12.6, $J_{1a,2}$ 6.3), 3.00 (1H, dd, H1b J_{gem} 12.6, $J_{1b,2}$ 3.5), 3.23-3.28 (1H, m, H5), 3.74, 3.85 (2 x 1H, d, NC$\underline{H}_2$Ph J_{gem} 14.1), 4.12 (1H, dd, H4 J 3.0, 3.8), 4.24-4.30 (1H, m, H2), 4.34 (1H, dd, H6a J_{gem} 11.9, $J_{6a,5}$ 4.3), 4.58, 4.75 (2 x 1H, d, OC$\underline{H}_2$Ph J_{gem} 11.6), 4.60 (1H, d, H6b J_{gem} 11.9), 5.07-5.10 (1H, m, H3), 6.90 (1H, d, NH $J_{NH,2}$ 8.1), 7.21-7.40 (10H, m, Ph); δ_C ($(CD_3)_2CO$ 126 MHz): 21.0, 21.0, 22.9 (Me), 46.6 (C2), 50.1 (C1), 58.2 (N$\underline{C}H_2$Ph), 61.5 (C5), 61.6 (C6), 72.5 (C3), 73.4 (O$\underline{C}H_2$Bn), 75.5 (C4), 127.6, 128.1, 128.2, 128.9, 128.9, 129.3

(ArC̲H), 139.4, 140.1 (ArC̲), 169.6, 170.2, 170.7 (C̲OMe); LRMS (ESI -ve): 467 (52%, $M-H^+$), 503 (100%, $M+^{35}Cl^-$), 505 (71%, $M+^{37}Cl^-$).

Similar treatment of azide **2.46L** (330 mg, 0.730 mmol) in a suitably scaled procedure gave piperidine **2.48L** (267 mg, 78%); $[\alpha]_D^{25}$ -28.6 (*c* 1.06, Me_2CO); m.p. 113-115°C.

2-Acetamido-1,5-imino-1,2,5-trideoxy-D-galactitol [DGJNAc], 2.16D



Sodium methoxide (10-15 mg, cat.) was added to a stirring solution of piperidine **2.48D** (406 mg, 0.868 mmol) in methanol (10 mL) at RT. TLC analysis (1:99 TEA/EtOAc) after 16 h revealed the absence of starting material (R_f 0.65) and the formation of a single product (R_f 0.25). The reaction mixture was concentrated under reduced pressure and dissolved in 10:2:1 water/2 M HCl/1,4-dioxane (13 mL). Palladium (10% on C, 100 mg, 94.0 μmol) was added and the vessel degassed before charging with hydrogen. The mixture was stirred at RT for 24 h after which no benzylated derivatives were observed by LRMS. The reaction mixture was filtered (GF/A glass microfibre), concentrated under reduced pressure, and the absence of benzylated intermediates confirmed by ^1H NMR.

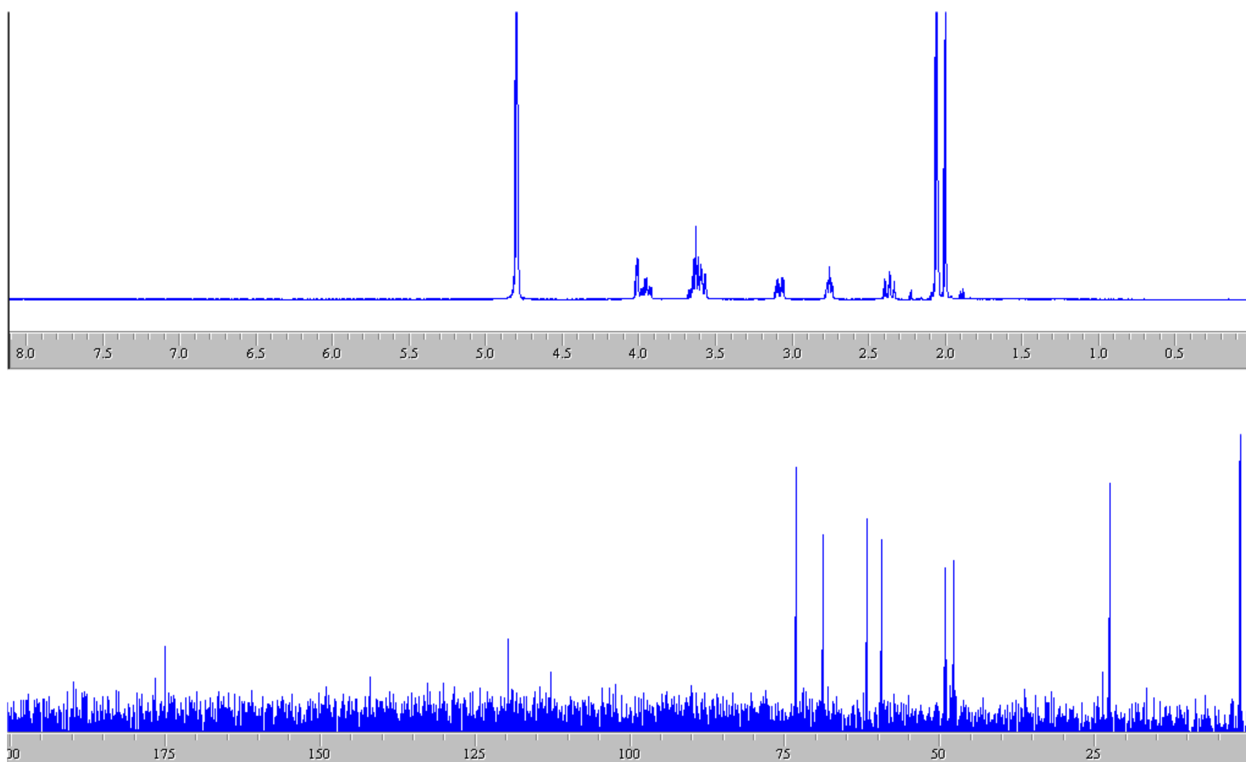
DGJNAc.HCl: δ_H (D_2O, 200 MHz): 2.03 (3H, s, Me), 2.89 (1H, t, H1a J_{gem}=$J_{1a,2}$ 12.4), 3.42 (1H, ddd, H5 $J_{5,4}$ 1.2, $J_{5,6a}$ 8.0, $J_{5,6b}$ 5.6), 3.49 (1H, dd, H1b J_{gem} 12.6, $J_{1,b,2}$ 5.1), 3.79 (1H, dd, H3 $J_{3,2}$ 10.6, $J_{3,4}$ 2.8), 3.82 (1H, dd, H6a J_{gem} 12.5, $J_{6a,5}$ 8.0), 3.90 (1H, dd, H6b J_{gem} 12.3, $J_{6b,5}$ 5.6), 4.19 (1H, dd, H4 $J_{4,3}$ 2.7, $J_{4,5}$ 1.2), 4.34 (1H, ddd, H2 $J_{2,1a}$ 12.1, $J_{2,1b}$ 5.1, $J_{2,3}$ 10.7).

The crude was loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water until neutral fractions were obtained. The product was liberated with 2M NH_3 and the

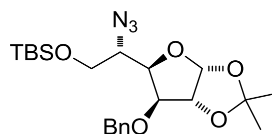
ammonical fractions concentrated under reduced pressure, assisted by co-evaporation with ethanol, to give DGJNAc **2.16D** (174 mg, 98%) as a white crystalline solid.

HRMS (ESI +ve): $C_8H_{16}N_2NaO_4$ found 227.1001; ($M+Na^+$) requires 227.1002; $[\alpha]_D^{25}$ +41.9 (*c* 0.67, H_2O); m.p. 150-154°C; ν_{max} (thin film, Ge): 3287 (br, s, OH/NH), 1637 (s, CO amide I), 1561 (s, NH amide II); δ_H (D_2O, 400 MHz): 2.00 (3H, s, Me), 2.37 (1H, dd, H1a J_{gem} 12.9, $J_{1a,2}$ 11.6), 2.76 (1H, dt, H5 $J_{5,4}$ 1.3, $J_{5,6a} = J_{5,6b}$ 6.6), 3.08 (1H, dd, H1b J_{gem} 12.9, $J_{1b,2}$ 5.1), 3.58 (1H, dd, H3 $J_{3,2}$ 10.6, $J_{3,4}$ 3.0), 3.61 (1H, dd, H6a J_{gem} 11.1, $J_{6a,5}$ 6.3), 3.65 (1H, dd, H6b J_{gem} 11.1, $J_{6b,5}$ 6.6), 3.96 (1H, dt, H2 $J_{2,1a}$ 11.1, $J_{2,1b}$ 5.1, $J_{2,3}$ 11.1), 4.01 (1H, dd, H4 $J_{4,3}$ 3.0, $J_{4,5}$ 1.4); δ_C (D_2O, 100 MHz): 22.7 (Me), 47.7 (C1), 49.1 (C2), 59.4 (C5), 61.9 (C6), 68.9 (C4), 73.2 (C3), 175.2 (<u>C</u>OMe); LRMS (ESI +ve): 205 (77%, $M+H^+$), 431 (100%, $2M+Na^+$); Lit.[56] $[\alpha]_D^{20}$ +37 (*c* 1, MeOH).

Similar treatment of piperidine **2.48L** (231 mg, 0.49 mmol) gave L-DGJNAc **2.16L** (99 mg, 98%); $[\alpha]_D^{25}$ -46.6 (*c* 0.73, H_2O); m.p. 152-156°C.



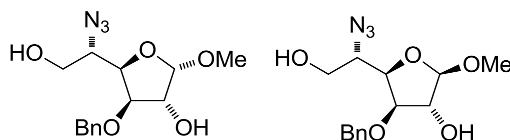
5-Azido-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose, 2.52



Sodium hydride (60% in mineral oil, 1.00 g, 25.0 mmol) was added to a solution of alcohol **2.36** (5.90 g, 16.4 mmol) and benzyl bromide (3.0 mL, 25 mmol) in DMF (50 mL) at 0°C and the mixture allowed to warm to RT. TLC analysis (1:3 EtOAc/CyH) after 1 h revealed the complete consumption of starting material (R_f 0.30) and the formation of a single product (R_f 0.60). The reaction mixture was partitioned between EtOAc (150 mL) and 1:1 brine/water (150 mL), the aqueous phase discarded and the organic washed with 1:1 brine/water (2 x 150 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:49 to 1:9 EtOAc/CyH) afforded the title compound **2.52** (7.36 g, 100%) as a colourless oil.

HRMS (ESI +ve): $C_{22}H_{35}N_3NaO_5Si$ found 472.2225; (M+Na^+) requires 472.2238; $[\alpha]_D^{25}$ -39.4 (*c* 1.78, $CHCl_3$); ν_{max} (thin film): 2100 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.01, 0.03 (2 x 3H, s, Si$\underline{Me}_2$), 0.87 (9H, s, C$\underline{Me}_3$), 1.33, 1.49 (2 x 3H, s, C$\underline{Me}_2$), 3.53 (1H, dd, H6a J_{gem} 11.4, $J_{6a,5}$ 6.3), 3.68-3.72 (2H, m, H5, H6b), 3.89 (1H, d, H3 $J_{3,4}$ 3.0), 4.28 (1H, dd, H4 $J_{4,3}$ 3.0, $J_{4,5}$ 8.3), 4.44 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.9), 4.67 (1H, d, H2 $J_{2,1}$ 3.8), 4.71 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.9), 5.97 (1H, d, H1 $J_{1,2}$ 3.8), 7.30-7.39 (5H, m, Ph); δ_C ($CDCl_3$, 100 MHz): -5.7, -5.6 (Si$\underline{Me}_2$), 18.1 ($\underline{C}Me_3$), 25.7 (C$\underline{Me}_3$), 26.4, 26.7 (C$\underline{Me}_2$), 61.9 (C5), 63.1 (C6), 71.5 ($\underline{C}H_2$Ph), 78.8 (C4), 81.3 (C3), 81.8 (C2), 104.7 (C1), 111.9 ($\underline{C}Me_2$), 128.0, 128.2, 128.6 (Ar$\underline{C}$H), 136.8 (Ar$\underline{C}$); LRMS (ESI +ve): 472 (67%, M+Na^+), 551 (56%), 916 (93%, 2M+NH_4^+), 921 (100%, 2M+Na^+).

Methyl 5-azido-3-*O*-benzyl-5-deoxy-L-idofuranosides, 2.53



Fully protected idose derivative **2.52** (7.36 g, 16.4 mmol) was dissolved in a mixture of methanol (50 mL) and acetyl chloride (2.5 mL). The reaction mixture was stirred at 50°C for 1 h after which TLC analysis revealed the complete consumption of the starting material (1:3 EtOAc/CyH: R_f 0.60) and the formation of two products (1:3 EtOAc/CyH: R_f 0.00; 1:4 acetone/PhMe: R_f 0.25, 0.35). The reaction was quenched by the addition of $NaHCO_3$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:49 to 1:3 acetone/PhMe) afforded the title compounds: **2.52β** (R_f 0.35, 2.42 g, 48%) as a white crystalline solid and **2.52α** (R_f 0.25, 2.19 g, 43%) as a colourless oil.

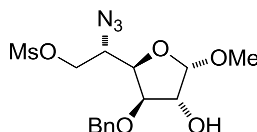
Data for 2.52β

HRMS (ESI +ve): $C_{14}H_{19}N_3NaO_5$ found 332.1215; ($M+Na^+$) requires 332.1217; m.p. 46-50°C; $[\alpha]_D^{25}$ +79.1 (*c* 1.39, $CHCl_3$); ν_{max} (thin film): 3424 (s, br, OH), 2105 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 2.04 (1H, t, OH6 $J_{OH,6}$ 6.3), 2.85 (1H, d, OH2 $J_{OH,2}$ 6.8), 3.51 (3H, s, Me), 3.57 (1H, dt, H6a J_{gem} 11.6, $J_{6a,5} = J_{6a,OH}$ 6.1), 3.66 (1H, ddd, H6b J_{gem} 11.6, $J_{6b,5}$ 4.0, $J_{6b,OH}$ 6.6), 3.82 (1H, dt, H5 $J_{5,4} = J_{5,6a}$ 6.4, $J_{5,6b}$ 4.0), 3.99 (1H, dd, H3 $J_{3,2}$ 3.8, $J_{3,4}$ 5.6), 4.28 (1H, t, H4 $J_{4,3} = J_{4,5}$ 6.0), 4.37 (1H, ddd, H2 $J_{2,1}$ 4.5, $J_{2,3}$ 3.8, $J_{2,OH}$ 7.1), 4.55, 4.81 (2 x 1H, d, OC$\underline{H}_2$Ph J_{gem} 11.9), 5.05 (1H, d, H1 $J_{1,2}$ 4.8), 7.30-7.39 (5H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 55.8 (Me), 62.4 (C6), 62.9 (C5), 71.7 (O$\underline{C}H_2$Ph), 76.4 (C2), 77.9 (C4), 83.1 (C3), 101.6 (C1), 127.9, 128.0, 129.5 (Ar$\underline{C}$H), 137.2 (Ar$\underline{C}$); LRMS (ESI +ve): 332 (91%, $M+Na^+$), 348 (78%, $M+K^+$), 641 (100%, $2M+Na^+$).

Data for 2.52α

HRMS (ESI +ve): $C_{14}H_{19}N_3NaO_5$ found 332.1213; ($M+Na^+$) requires 332.1217; $[\alpha]_D^{25}$ -41.4 (*c* 0.92, $CHCl_3$); ν_{max} (thin film): 3417 (s, br, OH), 2106 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 2.20 (1H, t, OH6 $J_{OH,6}$ 6.5), 2.51 (1H, d, OH2 $J_{OH,2}$ 4.3), 3.47 (3H, s, Me), 3.50 (1H, dt, H6a J_{gem} 11.6, $J_{6a,5}$ = $J_{6a,OH}$ 6.1), 3.65 (1H, ddd, H6b J_{gem} 11.6, $J_{6b,5}$ 3.5, $J_{6b,OH}$ 6.1), 3.91 (1H, ddd, H5 $J_{5,4}$ 8.1, $J_{5,6a}$ 5.8, $J_{5,6b}$ 3.8), 3.94 (1H, dd, H3 $J_{3,2}$ 2.3, $J_{3,4}$ 5.6), 4.33 (1H, dt, H2 $J_{2,1}$ = $J_{2,3}$ 2.0, $J_{2,OH}$ 4.0), 4.35 (1H, dd, H4 $J_{4,3}$ 5.6, $J_{4,5}$ 8.3), 4.49, 4.73 (2 x 1H, d, $OC\underline{H}_2Ph$ J_{gem} 11.9), 4.86 (1H, d, H1 $J_{1,2}$ 1.5), 7.30-7.39 (5H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 56.2 (Me), 62.2 (C6), 64.2 (C5), 72.1 ($O\underline{C}H_2Ph$), 78.3 (C2), 80.7 (C4), 82.3 (C3), 109.6 (C1), 128.1, 128.1, 129.6 (Ar$\underline{C}$H), 137.0 (Ar$\underline{C}$); LRMS (ESI +ve): 332 (74%, $M+Na^+$), 348 (57%, $M+K^+$), 641 (100%, $2M+Na^+$).

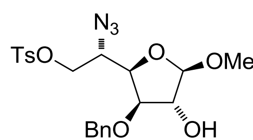
Methyl 5-azido-3-*O*-benzyl-5-deoxy-6-*O*-methanesulfonyl-β-L-idofuranoside, 2.57



Methanesulfonyl chloride (0.16 mL, 2.1 mmol) was added dropwise to a solution of idoside **2.52β** (542 mg, 1.75 mmol) and 2,4,6-collidine (0.55 mL, 4.2 mmol) in DCM (5 mL) at 0°C. TLC analysis (1:4 acetone/PhMe) after 3 h revealed the complete consumption of starting material (R_f 0.35), the formation of a major product (R_f 0.45) and a minor product (R_f 0.50). The mixture was diluted with DCM (15 mL), washed with 2 M HCl(aq) (3 x 20 mL) and the organic phase dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:99 to 1:19 acetone/PhMe) afforded the title mesylate **2.57** (582 mg, 86%) as a colourless oil.

HRMS (ESI +ve): $C_{15}H_{21}N_3NaO_7S$ found 410.0991; ($M+Na^+$) requires 410.0992; $[\alpha]_D^{25}$ +72.6 (*c* 0.97, $CHCl_3$); ν_{max} (thin film): 3518 (s, br, OH), 2110 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 2.74 (1H, d, OH $J_{OH,2}$ 7.6), 3.02 (3H, s, $SO_2\underline{Me}$), 3.49 (3H, s, O$\underline{Me}$), 3.95 (1H, dt, H5 $J_{5,4}$=$J_{5,6a}$ 6.3, $J_{5,6b}$ 4.3), 4.05 (1H, dd, H3 $J_{3,2}$ 4.3, $J_{3,4}$ 6.1), 4.20 (1H, dd, H6a J_{gem} 10.6, $J_{6a,5}$ 6.7), 4.25 (1H, dd, H6b J_{gem} 10.6, $J_{6b,5}$ 4.3), 4.26 (1H, t, H4 $J_{4,3}$=$J_{4,5}$ 6.0), 4.37 (1H, dt, H2 $J_{2,1}$ = $J_{2,3}$ 4.5, $J_{2,OH}$ 7.6), 4.57, 4.83 (2 x 1H, d, C$\underline{H}_2$Ph J_{gem} 11.6), 5.01 (1H, d, H1 $J_{1,2}$ 4.8), 7.30-7.40 (5H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 37.5 ($SO_2\underline{Me}$), 55.9 (O$\underline{Me}$), 60.0 (C5), 68.5 (C6), 71.9 ($\underline{C}H_2$Ph), 76.6 (C2), 76.7 (C4), 82.8 (C3), 101.7 (C1), 128.1, 128.6 (Ar$\underline{C}$H), 137.1 (Ar$\underline{C}$); LRMS (ESI +ve): 410 (100%, $M+Na^+$).

Methyl 5-azido-3-*O*-benzyl-5-deoxy-6-*O*-*p*-toluenesulfonyl-α-L-idofuranoside, 2.60

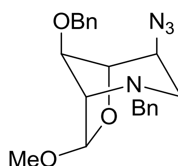


p-Toluenesulfonyl chloride (450 mg, 2.36 mmol) was added to a solution of idoside **2.52α** (358 mg, 1.16 mmol) and 2,4,6-collidine (0.60 mL, 4.5 mmol) in DCM (5 mL) RT. TLC analysis (1:4 acetone/PhMe) after 24 h revealed a trace of starting material (R_f 0.25), the formation of a major product (R_f 0.50) and a minor product (R_f 0.75). The mixture was diluted with DCM (10 mL), washed with 2 M HCl(aq) (3 x 10 mL) and the organic phase dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:99 to 1:19 acetone/PhMe) afforded the title tosylate **2.60** (397 mg, 74%) as a colourless oil.

HRMS (ESI +ve): $C_{21}H_{25}N_3NaO_7S$ found 486.1303; ($M+Na^+$) requires 486.1305; $[\alpha]_D^{25}$ -7.0 (*c* 1.32, $CHCl_3$); ν_{max} (thin film): 3443 (s, br, OH), 2105 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 2.07 (1H, br, s, OH), 2.44 (3H, s, $SO_2C_6H_4\underline{Me}$), 3.40 (3H, s, O$\underline{Me}$), 3.90-3.95 (2H, m, H3, H5), 4.05 (1H, dd, H6a J_{gem} 10.4, $J_{6a,5}$ 5.8), 4.09 (1H, dd, H6b J_{gem} 10.4, $J_{6b,5}$ 3.8), 4.28-4.29 (1H, m, H2), 4.30

(1H, dd, H4 J 6.1, 7.6), 4.46, 4.67 (2 x 1H, d, C$\underline{H}_2$Ph J_{gem} 11.6), 4.81 (1H, d, H1 $J_{1,2}$ 1.5), 7.30-7.39 (7H, m, Ph), 7.74-7.77 (2H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 21.7 ($SO_2C_6H_4\underline{Me}$), 56.1 (O$\underline{Me}$), 60.8 (C5), 68.9 (C6), 72.3 ($\underline{C}H_2$Ph), 78.3 (C2), 79.7 (C4), 82.5 (C3), 109.6 (C1), 128.0, 128.1, 128.2, 128.6, 129.9 (Ar$\underline{C}$H), 132.5, 137.0, 145.1 (Ar$\underline{C}$); LRMS (ESI +ve): 486 (100%, $M+Na^+$).

Methyl 5-azido-2-*N*-benzyl-3-*O*-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside, 2.55β

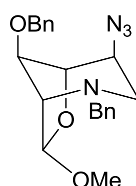


Trifluromethanesulfonic anhydride (0.30 mL, 1.8 mmol) was added dropwise to a solution of mesylate **2.57** (560 mg, 1.45 mmol) and pyridine (0.30 mL, 3.7 mmol) in DCM (6 mL) at -40°C and stirred at -30°C. TLC analysis (1:4 acetone/PhMe) after 1 h revealed the complete consumption of starting material (R_f 0.45) and the formation of a single product (R_f 0.65). The mixture was diluted with DCM (15 mL), washed with 2 M HCl(aq) (3 x 20 mL) and the organic phase dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude triflate was dissolved in benzylamine (2.5 mL) and stirred at 100°C for 18 h after which TLC analysis (1:33:66 TEA/EtOAc/CyH) revealed the complete consumption of the triflate (R_f 0.30) and the formation of a major product (R_f 0.70). The reaction mixture was preadsorbed onto silica gel and purification by flash column chromatography (1:0:99 to 1:5:94 TEA/EtOAc/CyH) afforded the title bicyclic imino-guloside **2.55β** (386 mg, 70%) as a pale yellow crystalline solid.

HRMS (ESI +ve): $C_{21}H_{24}N_4NaO_3$ found 403.1729; ($M+Na^+$) requires 403.1741; m.p. 50-54°C; $[\alpha]_D^{25}$ +120.3 (c 0.46, $CHCl_3$); ν_{max} (thin film): 2114 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 3.02 (1H, dd, H6a J_{gem} 13.1, $J_{6a,5}$ 4.3), 3.06 (1H, d, H6b J_{gem} 13.1), 3.18 (1H, d, H2 $J_{2,3}$ 4.3), 3.35 (3H, s, Me), 3.49-3.52 (1H, m, H5), 3.73 (2H, s, NC$\underline{H}_2$Ph), 4.16 (1H, t, H3 $J_{3,2} = J_{3,4}$ 4.6), 4.26 (1H, t, H4 $J_{4,3}$

= $J_{4,5}$ 4.6), 4.51, 4.63 (2 x 1H, d, OC<u>H</u>$_2$Ph J_{gem} 11.9), 4.94 (1H, s, H1), 7.28-7.47 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 49.4 (C6), 55.4 (Me), 56.3 (C5), 60.1 (N<u>C</u>H_2Ph), 61.5 (C2), 70.7 (O<u>C</u>H_2Ph), 72.2 (C4), 73.1 (C3), 101.4 (C1), 127.5, 127.6, 127.7, 128.3, 128.4, 129.1 (Ar<u>C</u>H), 137.5, 137.9 (Ar<u>C</u>); LRMS (ESI +ve): 381 (75%, $M+H^+$), 403 (77%, $M+Na^+$), 783 (100%, $M+Na^+$).

Methyl 5-azido-2-*N*-benzyl-3-*O*-benzyl-2,6-imino-2,5,6-trideoxy-α-L-gulofuranoside, 2.55α

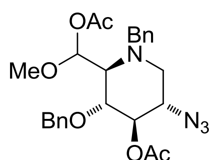


Trifluromethanesulfonic anhydride (0.20 mL, 1.2 mmol) was added dropwise to a solution of tosylate **2.60** (483 mg, 1.04 mmol) and pyridine (0.20 mL, 2.5 mmol) in DCM (5 mL) at -40°C and stirred at -30°C. TLC analysis (1:2 EtOAc/CyH) after 1 h revealed the complete consumption of starting material (R_f 0.15) and the formation of a single product (R_f 0.55). The mixture was diluted with DCM (15 mL), washed with 2 M HCl(aq) (3 x 20 mL) and the organic phase dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude triflate was dissolved in benzylamine (2.5 mL) and stirred at 100°C for 18 h after which TLC analysis (1:19:80 TEA/EtOAc/CyH) revealed the complete consumption of the triflate (R_f 0.35) and the formation of a major product (R_f 0.60). The reaction mixture was preadsorbed onto silica gel and purification by flash column chromatography (1:0:99 to 1:5:94 TEA/EtOAc/CyH) afforded the title imino-guloside **2.55α** (267 mg, 68%) as a pale yellow oil.

HRMS (ESI +ve): $C_{21}H_{25}N_4O_3$ found 381.1921; ($M+H^+$) requires 381.1921; $[\alpha]_D^{25}$ +39.2 (*c* 1.05, $CHCl_3$); ν_{max} (thin film): 2114 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 3.00 (1H, d, H6a J_{gem} 12.9), 3.27 (1H, t, H2 $J_{2,1}$=$J_{2,3}$ 3.7), 3.50 (3H, s, Me), 3.56 (1H, t, H5 $J_{5,4}$=$J_{5,6b}$ 4.7), 3.77 (1H, dd, H6b

J_{gem}12.9, $J_{6b,5}$ 5.6), 3.83 (1H, t, H3 $J_{3,2}$=$J_{3,4}$ 4.5), 3.92, 4.06 (2 x 1H, d, NC<u>H</u>$_2$Ph J_{gem} 13.4), 4.24 (1H, t, H4 $J_{4,3}$=$J_{4,5}$ 4.5), 4.47, 4.64 (2 x 1H, d, OC<u>H</u>$_2$Ph J_{gem} 12.1), 5.02 (1H, d, H1 $J_{1,2}$ 2.8), 7.25-7.37 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 49.0 (C6), 56.4 (C5), 56.8 (Me), 57.6 (C2), 60.1 (N<u>C</u>H$_2$Ph) , 71.5 (O<u>C</u>H$_2$Ph), 74.9 (C4), 75.1 (C3), 107.3 (C1), 127.1, 127.6, 127.7, 128.2, 128.3, 129.1 (Ar<u>C</u>H), 137.7, 138.4 (Ar<u>C</u>); LRMS (ESI +ve): 381 (97%, M+H$^+$), 403 (100%, M+Na$^+$).

4-*O*-Acetyl-5-azido-2-*N*-benzyl-3-*O*-benzyl-2,6-imino-2,5,6-trideoxy-L-gulose acetyl methyl acetal, 2.59

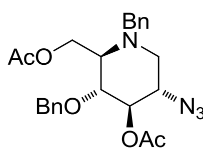


Boron trifluoride diethyl etherate (0.40 mL, 3.2 mmol) was added dropwise to a solution of imino-guloside **2.55β** (384 mg, 1.01 mmol) in acetic anhydride (4 mL) at 0°C before warming to RT. TLC analysis (1:19:80 TEA/EtOAc/CyH) after 36 h revealed the formation of a major product (R_f 0.50) and the complete consumption of starting material (R_f 0.60). The mixture was concentrated under reduced pressure, dissolved in EtOAc (20 mL) and washed with sat. $NaHCO_3$(aq) (3 x 20 mL). The organic fraction was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:0:99 to 1:9:90 TEA/EtOAc/CyH) gave the title mixed acetal **2.59** (373 mg, 75%) as a pale yellow oil, in a 2:1 ratio of diastereoisomers.

Similar treatment of imino-guloside **2.55α** (96 mg, 0.25 mmol, R_f 0.55 in 1:19:80 TEA/EtOAc/CyH) with boron trifluoride diethyl etherate for 3 h gave the title mixed acetal **2.59** (86 mg, 71%) as a pale yellow oil, in a 3:1 ratio of diastereoisomers.

HRMS (ESI +ve): $C_{25}H_{31}N_4O_6$ found 483.2242; ($M+H^+$) requires 483.2238; ν_{max} (thin film): 2104 (s, N_3), 1746 (s, CO); δ_H ($CDCl_3$, 400 MHz): 2.06 (3H, s, CO<u>Me</u>^{B}), 2.07, 2.09 (2 x 3H, s, CO<u>Me</u>^{A}), 2.14 (3H, s, CO<u>Me</u>^{B}), 2.16-2.23 (2H, m, H6a$^{A\&B}$), 2.82-2.85 (2H, m, H2$^{A\&B}$), 2.96 (1H, dd, H6b^A J_{gem} 12.4, $J_{6b,5}$ 4.8), 2.96 (1H, dd, H6b^B J_{gem} 11.9, $J_{6b,5}$ 4.5), 3.35 (3H, s, O<u>Me</u>^{B}), 3.39 (3H, s, O<u>Me</u>^{A}), 3.42 (1H, d, NC<u>H</u>$_2$Ph^B J_{gem} 14.1), 3.45-3.52 (2H, m, H5$^{A\&B}$), 3.57 (1H, d, NC<u>H</u>$_2$Ph^A J_{gem} 13.4), 3.64 (1H, t, H3^B $J_{3,2}$=$J_{3,4}$ 8.1), 3.70 (1H, t, H3^A $J_{3,2}$=$J_{3,4}$ 8.8), 4.29 (1H, d, NC<u>H</u>$_2$Ph^A J_{gem} 13.4), 4.38 (1H, d, NC<u>H</u>$_2$Ph^B J_{gem} 14.1), 4.63, 4.68 (2 x 1H, d, OC<u>H</u>$_2$Ph^A J_{gem} 11.1), 4.64, 4.73 (2 x 1H, d, OC<u>H</u>$_2$Ph^B J_{gem} 10.9), 5.08-5.13 (2H, m, H4$^{A\&B}$), 6.14 (1H, d, H1^B $J_{1,2}$ 1.8), 6.23 (1H, d, H1^A $J_{1,2}$ 2.3), 7.24-7.39 (20H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 21.0, 21.0 (CO<u>Me</u>^{A}), 21.1, 21.1 (CO<u>Me</u>^{B}), 52.0 (C6^A), 52.4 (C6^B), 57.0 (N<u>C</u>$_2$Ph^A), 57.2 (O<u>Me</u>^{B}), 57.6 (O<u>Me</u>^{A}), 58.3 (N<u>C</u>H$_2$Ph^B), 58.7 (C5^A), 58.9 (C5^B), 66.2 (C2^A), 66.8 (C2^B), 74.2 (O<u>C</u>H$_2$Ph^A), 74.3 (O<u>C</u>H$_2$Ph^B), 76.6, 76.8, 76.9, 76.9 (C3$^{A\&B}$, C4$^{A\&B}$), 96.9 (C1^A), 97.2 (C1^B), 127.1, 127.2, 127.5, 127.7, 127.7, 127.7, 128.0, 128.1, 128.4, 128.4, 128.6, 128.9 (Ar<u>C</u>H), 137.9, 138.7, 138.7, 139.0 (Ar<u>C</u>), 170.0, 170.2, 170.2, 171.0 (<u>C</u>OMe); LRMS (ESI +ve): 483 (97%, $M+H^+$), 505 (100%, $M+Na^+$), 987 (92%, $2M+Na^+$).

3,6-Di-*O*-acetyl-2-azido-1-*N*-benzyl-4-*O*-benzyl-1,5-imino-1,2,5-trideoxy-D-glucitol, 2.62

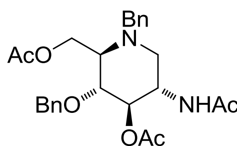


DIBAL (1.5 M in PhMe, 2.5 mL, 3.8 mmol) was added dropwise to a solution of mixed acetal **2.59** (361 mg, 0.749 mmol) in DCM (3 mL) at -78°C. The mixture was stirred at this temperature for 40 min after which TLC analysis (20:79:1 EtOAc/CyH/TEA) revealed the absence of starting material (R_f 0.40) and the formation of a major product (R_f 0.25). The reaction was quenched with EtOAc (20 mL) and sat. potassium sodium tartrate solution (20 mL) added before stirring vigorously for 2 h. The organic layer was collected and the aqueous extracted with EtOAc (3 x

10 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude intermediate was dissolved in MeOH (4 mL), cooled to -10°C and stirred with sodium borohydride (15 mg, 0.397 mmol) for 2 h. TLC analysis (40:59:1 EtOAc/CyH/TEA) revealed the complete consumption of starting intermediate (R_f 0.55) and the formation of a major component (R_f 0.25). The reaction mixture was quenched with glacial acetic acid, concentrated under reduced pressure, dissolved in a mixture of pyridine (2 mL) and acetic anhydride (2 mL) and stirred for 16 h at RT. TLC analysis (20:59:1 EtOAc/CyH/TEA) revealed the formation of a major product (R_f 0.40). The reaction mixture was concentrated under reduced pressure and purified by flash column chromatography (3:96:1 to 15:84:1 EtOAc/CyH/TEA) to afford the azido-piperidine **2.62** (264 mg, 78%) as a white crystalline solid.

HRMS (ESI +ve): $C_{24}H_{28}N_4NaO_5$ found 475.1957; ($M+H^+$) requires 475.1952; m.p. 89-91°C; $[\alpha]_D^{22}$ +3.3 (*c* 1.20, $CHCl_3$); ν_{max} (thin film): 2104 (s, N_3); 1743 (s, CO); δ_H ($CDCl_3$, 400 MHz): 2.06 (1H, t, H1a J_{gem}=$J_{1a,2}$ 11.4), 2.07, 2.08 (2 x 3H, s, Me), 2.55 (1H, dt, H5 $J_{5,4}$ 9.6, $J_{5,6}$ 2.7), 2.98 (1H, dd, H1b J_{gem} 11.6, $J_{1b,2}$ 4.8), 3.27 (1H, d, NC$\underline{H}_2$Ph J_{gem} 13.3), 3.47 (1H, dt, H2 $J_{2,1a}$=$J_{2,3}$ 10.4, $J_{2,1b}$ 4.8), 3.66 (1H, t, H4 $J_{4,3}$=$J_{4,5}$ 9.4), 4.09 (1H, d, NC$\underline{H}_2$Ph J_{gem} 13.3), 4.31 (1H, dd, H6a J_{gem} 12.6, $J_{6a,5}$ 2.7), 4.59 (1H, d, OC$\underline{H}_2$Ph J_{gem} 10.9), 4.64 (1H, dd, H6b J_{gem} 12.8, $J_{6b,5}$ 2.4), 4.66 (1H, d, OC$\underline{H}_2$Ph J_{gem} 10.9), 5.08 (1H, t, H3 $J_{3,2}$=$J_{3,4}$ 9.6), 7.26-7.38 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 20.9, 20.9 (Me), 53.6 (C1), 56.5 (N$\underline{C}H_2$Ph), 59.3 (C2), 59.9 (C6), 63.9 (C5), 75.0 (O$\underline{C}H_2$Ph), 76.9 (C4), 77.4 (C3), 127.4, 128.0, 128.0, 128.5, 128.5, 128.7 (Ar*C*H), 137.3, 137.6 (Ar$\underline{C}$), 170.0, 170.6 ($\underline{C}$O); LRMS (ESI +ve): 453 (94%, $M+H^+$), 475 (100%, $M+Na^+$), 927 (99%, $2M+Na^+$).

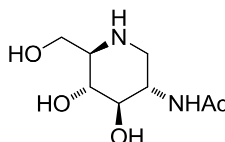
2-Acetamido-3,6-di-*O*-acetyl-1-*N*-benzyl-4-*O*-benzyl-1,5-imino-1,2,5-trideoxy-D-glucitol, 2.63



Powdered zinc (450 mg, 6.88 mmol) was added to a solution of azido-piperidine **2.62** (113 mg, 0.250 mmol) in 3:2:1 THF/AcOH/Ac_2O (3 mL) and stirred vigorously before the dropwise addition of sat. $CuSO_4$(aq) (0.8 mL) to initiate the reaction. TLC analysis (20:79:1 EtOAc/CyH/TEA) after 20 min revealed the complete consumption of starting material (R_f 0.50) and the formation of a major product (R_f 0.10). The reaction mixture was filtered, concentrated under reduced pressure and purified by flash column chromatography (10:89:1 to 70:29:1 EtOAc/CyH/TEA) to afford the title acetamido-piperidine **2.63** (92 mg, 79%) as a white crystalline solid.

HRMS (ESI +ve): $C_{26}H_{33}N_2O_6$ found 469.2334; (M+H$^+$) requires 469.2333; m.p. 149-151°C; $[\alpha]_D^{25}$ +0.9 (*c* 0.92, Me_2CO); ν_{max} (thin film): 1739 (s, CO ester), 1660 (s, CO amide I), 1546 (m, NH amide II); δ_H (($(CD_3)_2CO$), 400 MHz): 1.74 (3H, s, <u>Me</u>CONH), 1.98, 2.06 (2 x 3H, s, <u>Me</u>CO_2), 2.14 (1H, dd, H1a J_{gem} 11.6, $J_{1a,2}$ 10.4), 2.63 (1H, dt, H5 $J_{5,4}$ 8.8, $J_{5,6}$ 3.0), 2.85 (1H, dd, H1b J_{gem} 11.6. $J_{1b,2}$ 4.5), 3.34 (1H, d, NC<u>H</u>$_2$Ph J_{gem} 13.6), 3.72 (1H, t, H4 $J_{4,3}$=$J_{4,5}$ 8.8), 4.05 (1H, dq, H2 $J_{2,1a}$ 9.9, $J_{2,1b}$ 4.5, $J_{2,3}$=$J_{2,NH}$ 9.9), 4.13 (1H, d, NC<u>H</u>$_2$Ph J_{gem} 13.6), 4.35 (1H, dd, H6a J_{gem} 12.6, $J_{6a,5}$ 3.3), 4.64 (1H, dd, H6b J_{gem} 12.6, $J_{6b,5}$ 2.8), 4.65, 4.72 (2 x 1H, d, OC<u>H</u>$_2$Ph J_{gem} 11.1), 4.90 (1H, dd, H3 $J_{3,2}$ 9.6, $J_{3,4}$ 8.6), 6.77 (1H, d, NH $J_{NH,2}$ 9.3), 7.21-7.37 (10H, m, Ph); δ_C (($(CD_3)_2CO$), 100 MHz): 20.8, 21.0 (<u>Me</u>CO_2), 22.9 (<u>Me</u>CONH), 49.0 (C2), 54.6 (C1), 57.3 (N<u>C</u>H_2Ph), 60.6 (C6), 64.7 (C5), 75.2 (O<u>C</u>H_2Ph), 77.7 (C3), 77.9 (C4), 127.8, 128.4, 128.7, 128.1, 128.1, 128.5 (Ar<u>C</u>H), 139.3, 140.0 (Ar<u>C</u>), 169.5, 170.9, 170.9 (CO); LRMS (ESI +ve): 469 (100%, M+H$^+$), 491 (64%, M+Na$^+$).

2-Acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol [DNJNAc], 2.17D



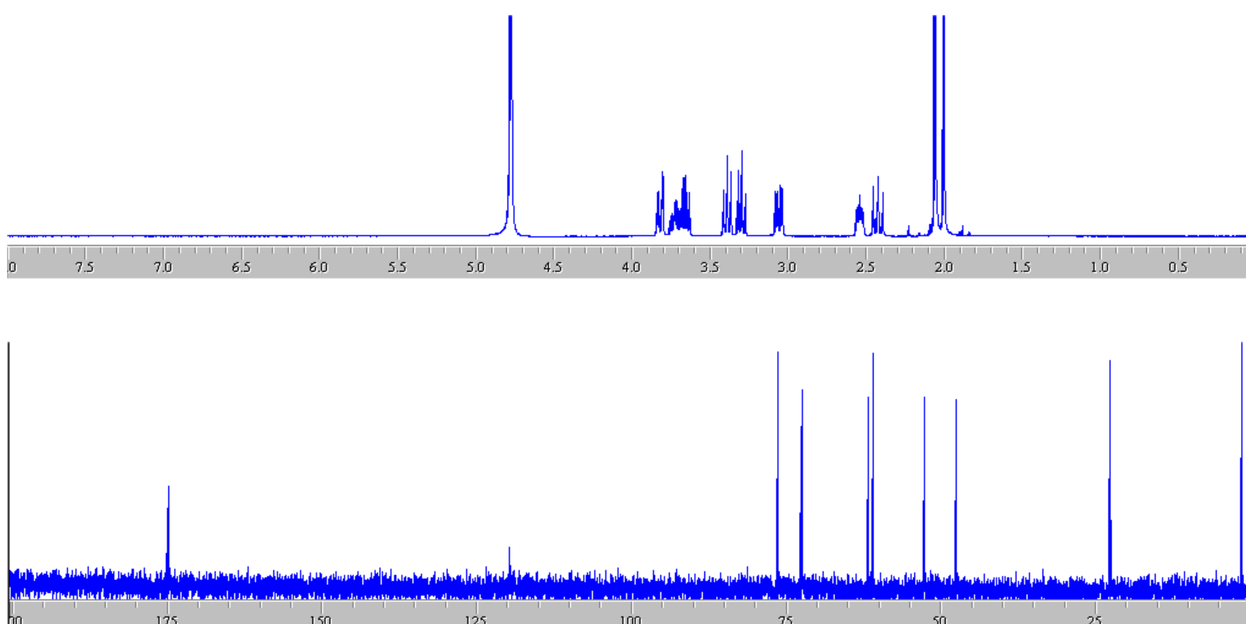
Sodium methoxide (6 mg, cat.) was added to a stirring solution of protected piperidine **2.63** (163 mg, 0.348 mmol) in methanol (5 mL) at RT. TLC analysis (99:1 EtOAc/TEA) after 16 h revealed the absence of starting material (R_f 0.70) and the formation of a single product (R_f 0.25). The reaction mixture was concentrated under reduced pressure and dissolved in 13:3:4 water/2 M HCl/1,4-dioxane (5 mL). Palladium (10% on C, 47 mg, 44 μmol) was added and the vessel degassed before charging with hydrogen. The mixture was stirred at RT for 24 h after which no benzylated derivatives were observed by LRMS. The reaction mixture was filtered (GF/A glass microfibre), concentrated under reduced pressure, and the absence of benzylated intermediates confirmed by ^{1}H NMR.

DNJNAc.HCl δ_H (D_2O, 400 MHz): 2.03 (3H, s, Me), 2.99 (1H, t, H1a J_{gem}=$J_{1a,2}$ 12.5), 3.23 (1H, ddd, H5 $J_{5,4}$ 10.1, $J_{5,6a}$ 5.0, $J_{5,6b}$ 3.2), 3.50 (1H, dd, H1b J_{gem} 12.6, $J_{1b,2}$ 5.0), 3.63 (1H, t, H3 $J_{3,2}$=$J_{3,4}$ 9.6), 3.67 (1H, dd, H4 $J_{4,3}$ 9.1, $J_{4,5}$ 10.2), 3.90 (1H, dd, H6a J_{gem} 12.8, $J_{6a,5}$ 5.1), 3.96 (1H, dd, H6b J_{gem} 12.8, $J_{6b,5}$ 3.2), 3.72 (1H, ddd, H2 $J_{2,1a}$ 12.3, $J_{2,1b}$ 5.0, $J_{2,3}$ 10.1).

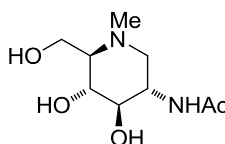
The crude was loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water until neutral fractions were obtained. The product was liberated with 2 M NH_3 (aq) and the ammonical fractions concentrated under reduced pressure, to give DNJNAc **2.17D** (67 mg, 94%) as a white crystalline solid.

HRMS (ESI +ve): $C_8H_{17}N_2O_4$ found 205.1183; (M+H^+) requires 205.1183; m.p. 224-226 ^{o}C; $[\alpha]_D^{25}$ +14.6 (*c* 0.86, H_2O); ν_{max} (thin film, Ge): 3286 (s, br, OH/NH), 1638 (s, CO amide I), 1561 (s, NH amide II); δ_H (D_2O, 400 MHz): 2.00 (3H, s, Me), 2.43 (1H, dd, H1a J_{gem} 12.6, $J_{1a,2}$

11.5), 2.54 (1H, ddd, H5 $J_{5,4}$ 9.7, $J_{5,6a}$ 6.1, $J_{5,6b}$ 3.0), 3.06 (1H, dd, H1b J_{gem} 12.6, $J_{1b,2}$ 4.9), 3.30 (1H, t, H3 $J_{3,2}$=$J_{3,4}$ 9.7), 3.39 (1H, dd, H4 $J_{4,3}$ 9.1, $J_{4,5}$ 10.0), 3.66 (1H, dd, H6a J_{gem} 11.6, $J_{6a,5}$ 6.1), 3.72 (1H, ddd, H2 $J_{2,1a}$ 11.4, $J_{2,1b}$ 4.9, $J_{2,3}$ 10.2), 3.82 (1H, dd, H6b J_{gem} 11.6, $J_{6b,5}$ 2.9); δ_C (D_2O, 100 MHz): 22.6 (Me), 47.5 (C1), 52.8 (C2), 61.0 (C5), 61.8 (C6), 72.5 (C3), 76.4 (C4), 174.9 (<u>C</u>O); LRMS (ESI +ve): 205 (100%, M+H$^+$), 227 (90%, M+Na$^+$), 431 (91%, 2M+Na$^+$); [Lit.[64] m.p. 226-227°C; $[\alpha]_D^{20}$ +16.4 (*c* 1.0, H_2O)].



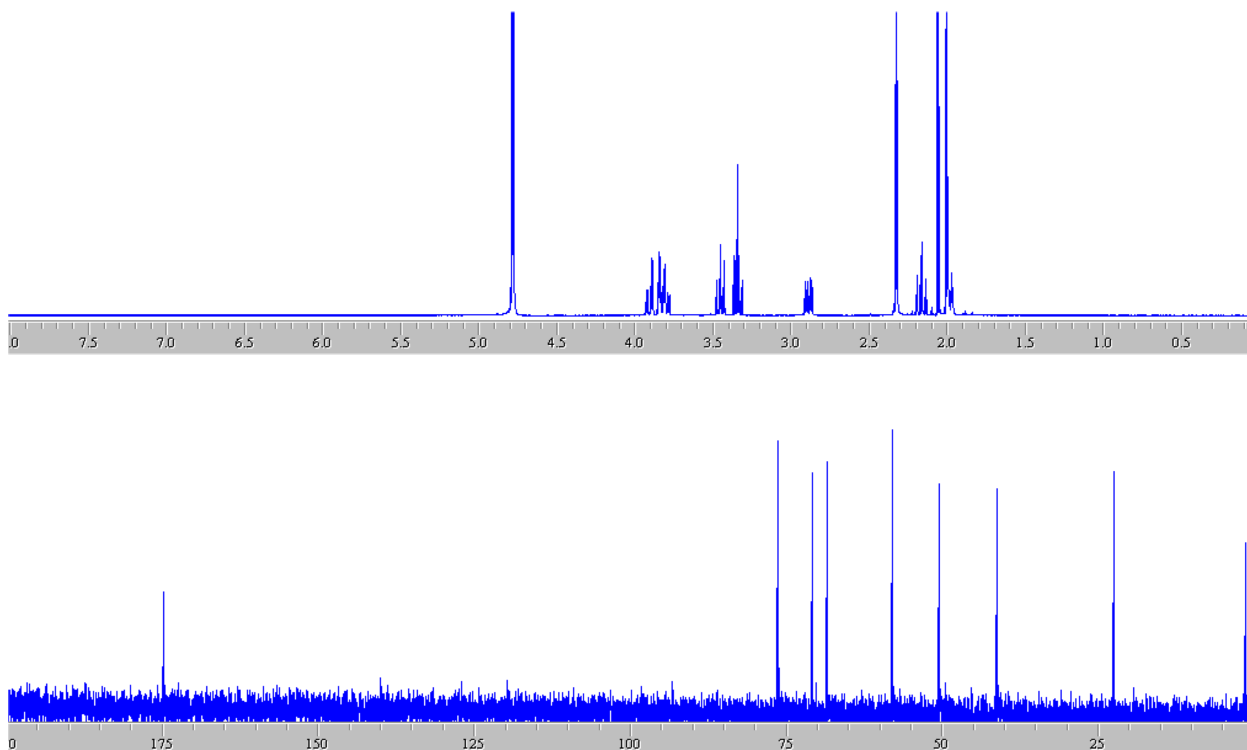
N-Methyl-DNJNAc, 2.64



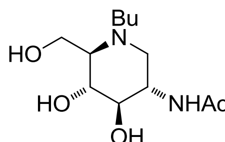
Formaldehyde (39.5% aqueous, 0.10 mL, 1.5 mmol) and palladium (10% on C, 5 mg, 5 μmol) were added to a solution of DNJNAc **2.17D** (11.5 mg, 56.9 μmol) in water (1 mL). The vessel was degassed, charged with hydrogen and the mixture stirred under hydrogen for 24 h. The

catalyst was removed by filtration, the filtrate concentrated under reduced pressure and loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water until neutral fractions were obtained. The product was liberated with 2 M NH_3 (aq) and the ammonical fractions concentrated under reduced pressure, to give *N*-methyl-DNJNAc **2.64** (11.6 mg, 94%) as a white crystalline solid.

HRMS (ESI +ve): $C_9H_{19}N_2O_4$ found 219.1340 ; ($M+H^+$) requires 219.1339; m.p. 248°C-decomp; $[\alpha]_D^{25}$ +20.3 (*c* 0.53, H_2O); ν_{max} (thin film, Ge): 3300 (s, br, OH), 1635 (s, CO amide I), 1565 (s, NH amide II); δ_H (D_2O, 400 MHz): 1.99 (1H, dt, H5 $J_{5,4}$ 9.9, $J_{5,6}$ 2.8), 2.01 (3H, s, COMe), 2.17 (1H, t, H1a J_{gem}=$J_{1a,2}$ 11.6), 2.33 (3H, s, NMe), 2.89 (1H, dd, H1b J_{gem} 11.6, $J_{1b,2}$ 4.8), 3.34 (1H, dd, H3 $J_{3,2}$ 10.1, $J_{3,4}$ 9.3), 3.46 (1H, t, H4 $J_{4,3}$=$J_{4,5}$ 9.6), 3.78-3.85 (2H, m, H2, H6a), 3.82 (1H, dd, H6b J_{gem} 12.9, $J_{6b,5}$ 2.5); δ_C (D_2O, 100 MHz): 22.6 (COMe), 41.3 (NMe), 50.5 (C2), 58.1 (C1), 58.1 (C6), 68.5 (C5), 70.9 (C4), 76.3 (C3), 174.9 (CO); LRMS (ESI +ve): 219 (70%, $M+H^+$), 459 (100%, $2M+Na^+$).

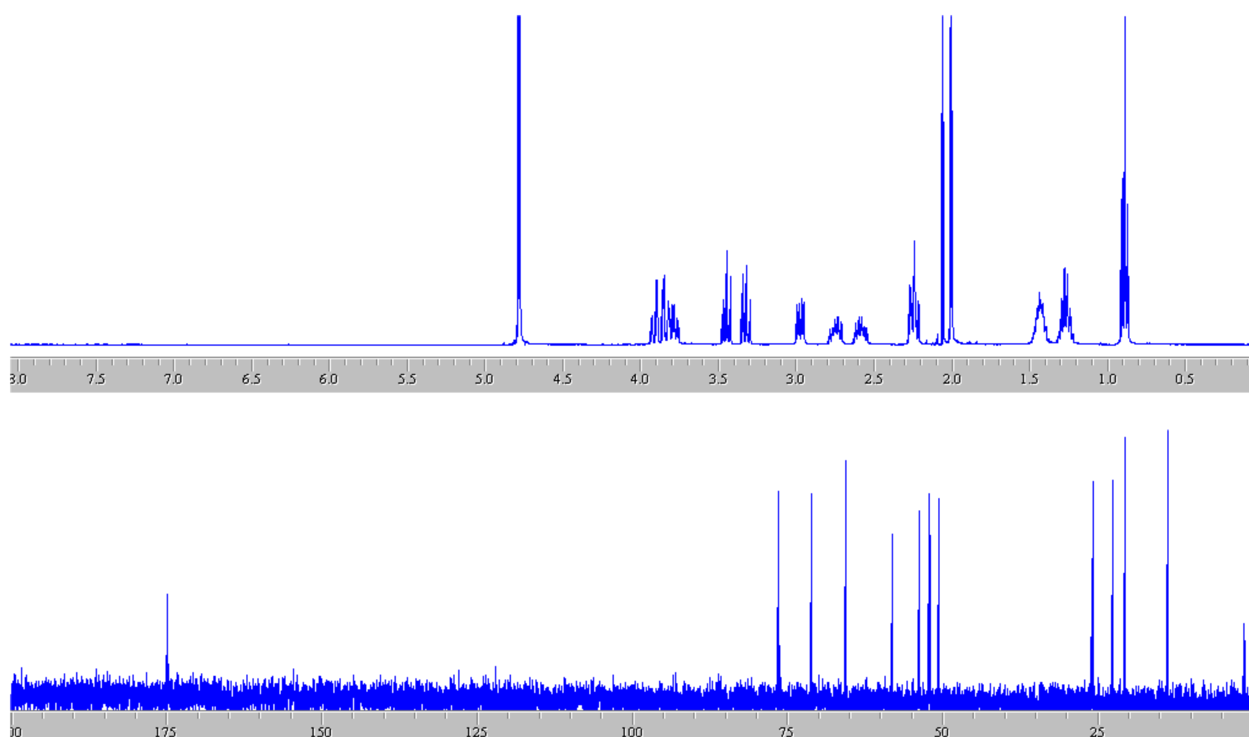


N-Butyl-DNJNAc, 2.65



Butraldehyde (0.10 mL, 1.1 mmol) and palladium (10% on C, 5 mg, 5 μmol) were added to a solution of DNJNAc **2.17D** (11.0 mg, 53.9 μmol) in 1:1 water/1,4-dioxane (2 mL). The vessel was degassed, charged with hydrogen and the mixture stirred under hydrogen for 24 h. The catalyst was removed by filtration, the filtrate concentrated under reduced pressure and loaded onto a short a short column of Dowex (50W-X8, H^+) and the resin washed with water until neutral fractions were obtained. The product was liberated with 3:17 [25% NH_3(aq)]/MeOH and the ammonical fractions concentrated under reduced pressure to give N-butyl-DNJNAc **2.65** (13.4 mg, 96%) as a white crystalline solid.

HRMS (ESI +ve): $C_{12}H_{25}N_2O_4$ found 261.1816; (M+H^+) requires 261.1809; m.p. 192-196°C; $[\alpha]_D^{25}$ +13.3 (c 0.56, H_2O); ν_{max} (thin film, Ge): 3296 (s, br, OH), 1640 (s, CO amide I), 1562 (s, NH amide II); δ_H (D_2O, 400 MHz): 0.89 (3H, t, $(CH_2)_3\underline{Me}$ J 7.3), 1.27 (2H, sext, $(CH_2)_2C\underline{H_2}Me$ J 7.3), 1.40-1.48 (2H, m, $CH_2C\underline{H_2}Et$), 2.01 (3H, s, CO$\underline{Me}$), 2.25 (1H, t, H1a J_{gem}=$J_{1a,2}$ 11.6), 2.25 (1H, dt, H5 $J_{5,4}$ 9.6, $J_{5,6}$ 2.5), 2.55-2.62 (1H, m, $C\underline{H_2}Pr$), 2.71-2.79 (1H, m, $C\underline{H_2}Pr$), 2.98 (1H, dd, H1b J_{gem} 11.9, $J_{1b,2}$ 4.5), 3.32 (1H, dd, H3 $J_{3,2}$ 10.1, $J_{3,4}$ 9.3), 3.45 (1H, t, H4 $J_{4,3}$=$J_{4,5}$ 9.3), 3.79 (1H, dt, H2 $J_{2,1a}$ 10.9, $J_{2,1b}$ 4.5, $J_{2,3}$ 10.9), 3.84 (1H, dd, H6a J_{gem} 12.6, $J_{6a,5}$ 2.8), 3.92 (1H, dd, H6b J_{gem} 12.6, $J_{6b,5}$ 2.3); δ_C (D_2O, 100 MHz): 13.8 ($(CH_2)_3\underline{Me}$), 20.8 ($(CH_2)_2\underline{C}H_2Me$), 22.6 (CO$\underline{Me}$), 25.9 ($CH_2\underline{C}H_2Et$), 50.6 (C2), 52.2 ($\underline{C}H_2Pr$), 53.9 (C1), 58.2 (C6), 65.7 (C5), 71.2 (C4), 76.4 (C3), 174.9 ($\underline{C}O$); LRMS (ESI +ve): 261 (94%, M+H^+), 331 (74%, M+MeOH+K^+), 543 (100%, 2M+Na^+).



2.7. References

1. Kitahara, T.; Ogawa, T.; Naganuma, T.; Matsui, M. *Agr Biol Chem Tokyo* **1974,** *38*, 2189-2190.
2. Weymouth-Wilson, A. C.; Clarkson, R. A.; Jones, N. A.; Best, D.; Wilson, F. X.; Pino-Gonzalez, M. S.; Fleet, G. W. J. *Tetrahedron Letters* **2009,** *50*, 6307-6310.
3. Bichard, C. J. F.; Bruce, I.; Hughes, D. J.; Girdhar, A.; Fleet, G. W. J.; Watkin, D. J. *Tetrahedron: Asymmetry* **1993,** *4*, 1579-1589.
4. Sowa, W. *Can J Chem* **1969,** *47*, 3931-3934.
5. Yagi, M.; Kouno, T.; Aoyagi, Y.; Murai, H. *J Agr Chem Soc Jpn* **1976,** *50*, 571-572.
6. Fellows, L. E.; Bell, E. A.; Lynn, D. G.; Pilkiewicz, F.; Miura, I.; Nakanishi, K. *J Chem Soc Chem Comm* **1979**, 977-978.
7. Asano, N.; Yasuda, K.; Kizu, H.; Kato, A.; Fan, J.-Q.; Nash, R. J.; Fleet, G. W. J.; Molyneux, R. J. *Eur. J. Biochem.* **2001,** *268*, 35-41.
8. Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J Med Chem* **2005,** *48*, 2036-2044.
9. Takahata, H.; Banba, Y.; Sasatani, M.; Nemoto, H.; Kato, A.; Adachi, I. *Tetrahedron* **2004,** *60*, 8199-8205.
10. Takahata, H.; Banba, Y.; Ouchi, H.; Nemoto, H. *Org Lett* **2003,** *5*, 2527-2529.
11. Hashimoto, H.; Hayakawa, M. *Chem Lett* **1989**, 1881-1884.
12. El Sayed H. El Ashry, A. E. N. *Synthesis of Naturally Occurring Nitrogen Heterocycles from Carbohydrates*; Blackwells, 2005.
13. Paulsen, H. *Angew Chem Int Ed* **1966,** *5*, 495-510.
14. Paulsen, H.; Sangster, I.; Heyns, K. *Chem Ber* **1967,** *100*, 802-815.
15. Behling, J.; Farid, P.; Medich, J. R.; Scaros, M. G.; Prunier, M.; Weier, R. M.; Khanna, I. *Synth Commun* **1991,** *21*, 1383-1386.

16. Wennekes, T.; Lang, B.; Leeman, M.; van der Marel, G. A.; Smits, E.; Weber, M.; van Wiltenburg, J.; Wolberg, M.; Aerts, J. M. F. G.; Overkleeft, H. S. *Org Process Res Dev* **2008,** *12*, 414-423.
17. Wennekes, T.; Meijer, A. J.; Groen, A. K.; Boot, R. G.; Groener, J. E.; van Eijk, M.; Ottenhoff, R.; Bijl, N.; Ghauharali, K.; Song, H.; O'Shea, T. J.; Liu, H. L.; Yew, N.; Copeland, D.; van den Berg, R. J.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. M. *J Med Chem* **2010,** *53*, 689-698.
18. Wennekes, T.; van den Berg, R. J. B. H. N.; Donker, W.; van der Marel, G. A.; Donker, W.; van der Marel, G. A.; Strijland, A.; Aerts, J. M. F. G.; Overkleeft, H. S. *J Org Chem* **2007,** *72*, 1088-1097.
19. Danieli, E.; Lalot, J.; Murphy, P. V. *Tetrahedron* **2007,** *63*, 6827-6834.
20. Fügedi, P.; Csíki, Z. *Synthesis* **2010,** *2010*, 2626-2630.
21. Jadhav, V. H.; Bande, O. P.; Puranik, V. G.; Dhavale, D. D. *Tetrahedron: Asymmetry* **2010,** *21*, 163-170.
22. Song, X. Z.; Hollingsworth, R. I. *Tetrahedron Letters* **2007,** *48*, 3115-3118.
23. Dax, K.; Gaigg, B.; Grassberger, V.; Kölblinger, B.; Stütz, A. E. *J Carbohydr Chem* **1990,** *9*, 479-499.
24. Anzeveno, P. B.; Creemer, L. J. *Tetrahedron Letters* **1990,** *31*, 2085-2088.
25. Kinast, G.; Schedel, M. *Angew Chem Int Ed* **1981,** *20*, 805-806.
26. Concia, A. L.; Lozano, C.; Castillo, J. A.; Parella, T.; Joglar, J.; Clapés, P. *Chem Eur J* **2009,** *15*, 3808-3816.
27. D'Alonzo, D.; Guaragna, A.; Palumbo, G. *Curr Med Chem* **2009,** *16*, 473-505.
28. Minami, Y.; Kurlyarna, C.; Ikeda, K.; Kato, A.; Takebayashi, K.; Adachi, I.; Fleet, G. W. J.; Kettawan, A.; Karnoto, T.; Asano, N. *Bioorg Med Chem* **2008,** *16*, 2734-2740.
29. Dondoni, A.; Giovannini, P. P.; Perrone, D. *J Org Chem* **2002,** *67*, 7203-7214.
30. Hong, Z.; Liu, L.; Sugiyama, M.; Fu, Y.; Wong, C.-H. *J Am Chem Soc* **2009,** *131*, 8352-8353.
31. Fleet, G. W. J.; Smith, P. W. *Tetrahedron Letters* **1985,** *26*, 1469-1472.
32. Lee, R. E.; Smith, M. D.; Nash, R. J.; Griffiths, R. C.; McNeil, M.; Grewal, R. K.; Yan, W. X.; Besra, G. S.; Brennan, P. J.; Fleet, G. W. J. *Tetrahedron Letters* **1997,** *38*, 6733-6736.
33. Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *Journal of Medicinal Chemistry* **1994,** *37*, 3701-3706.
34. Asano, N.; Yamauchi, T.; Kagamifuchi, K.; Shimizu, N.; Takahashi, S.; Takatsuka, H.; Ikeda, K.; Kizu, H.; Chuakul, W.; Kettawan, A.; Okamoto, T. *J Nat Prod* **2005,** *68*, 1238-1242.
35. Kato, A.; Yamashita, Y.; Nakagawa, S.; Koike, Y.; Adachi, I.; Hollinshead, J.; Nash, R. J.; Ikeda, K.; Asano, N. *Bioorg Med Chem* **2010,** *18*, 3790-3794.
36. Best, D.; Wang, C.; Weymouth-Wilson, A. C.; Clarkson, R. A.; Wilson, F. X.; Nash, R. J.; Miyauchi, S.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2010,** *21*, 311-319.
37. Tsou, E.-L.; Yeh, Y.-T.; Liang, P.-H.; Cheng, W.-C. *Tetrahedron* **2009,** *65*, 93-100.
38. Wang, Y. F.; Takaoka, Y.; Wong, C. H. *Angew Chem Int Ed* **1994,** *33*, 1242-1244.
39. Card, P. J.; Hitz, W. D. *J Org Chem* **1985,** *50*, 891-893.
40. Legler, G.; Korth, A.; Berger, A.; Ekhart, C.; Gradnig, G.; Stutz, A. E. *Carbohydr Res* **1993,** *250*, 67-77.
41. Hung, R. R.; Straub, J. A.; Whitesides, G. M. *J Org Chem* **1991,** *56*, 3849-3855.
42. Garcia-Moreno, M. I.; Rodriguez-Lucena, D.; Mellet, C. O.; Fernandez, J. M. G. *J Org Chem* **2004,** *69*, 3578-3581.
43. Garcia-Moreno, M. I.; Aguilar, M.; Mellet, C. O.; Fernandez, J. M. G. *Org Lett* **2006,** *8*, 297-299.
44. Baxter, E. W.; Reitz, A. B. *J Org Chem* **1994,** *59*, 3175-3185.

45. Chorghade, M. S.; Cseke, C. T.; Liu, P. S. *Tetrahedron: Asymmetry* **1994,** *5*, 2251-2254.
46. Behr, J.-B.; Guillerm, G. *Tetrahedron Letters* **2007,** *48*, 2369-2372.
47. Fleet, G. W. J.; Smith, P. W. *Tetrahedron* **1987,** *43*, 971-978.
48. Park, K. H.; Yoon, Y. J.; Lee, S. G. *Tetrahedron Letters* **1994,** *35*, 9737-9740.
49. Poitout, L.; LeMerrer, Y.; Depezay, J. C. *Tetrahedron Letters* **1996,** *37*, 1609-1612.
50. LeMerrer, Y.; Poitout, L.; Depezay, J. C.; Dosbaa, I.; Geoffroy, S.; Foglietti, M. J. *Bioorg Med Chem* **1997,** *5*, 519-533.
51. Trost, B. M.; Horne, D. B.; Woltering, M. J. *Chem Eur J* **2006,** *12*, 6607-6620.
52. Colobert, F.; Tito, A.; Khiar, N.; Denni, D.; Medina, M. A.; Martin-Lomas, M.; Ruano, J. L. G.; Solladie, G. *J Org Chem* **1998,** *63*, 8918-8921.
53. Yu, C.-Y.; Asano, N.; Ikeda, K.; Wang, M.-X.; Butters, T. D.; Wormald, M. R.; Dwek, R. A.; Winters, A. L.; Nash, R. J.; Fleet, G. W. J. *Chem Commun* **2004**, 1936.
54. Sugiyama, M.; Hong, Z.; Whalen, L. J.; Greenberg, W. A.; Wong, C.-H. *Adv Synth Catal* **2006,** *348*, 2555-2559.
55. Kang, S. H.; Ryu, D. H. *Tetrahedron Letters* **1997,** *38*, 607-610.
56. Schueller, A. M.; Heiker, F. R. *Carbohydr Res* **1990,** *203*, 308-313.
57. Kiso, M.; Kitagawa, M.; Ishida, H.; Hasegawa, A. *J Carbohydr Chem* **1991,** *10*, 25-45.
58. Nishimura, Y.; Satoh, T.; Kudo, T.; Kondo, S.; Takeuchi, T. *Bioorg Med Chem* **1996,** *4*, 91-96.
59. Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *J Antibiot* **1999,** *52*, 348-350.
60. Saotome, C.; Wong, C. H.; Kanie, O. *Chem Biol* **2001,** *8*, 1061-1070.
61. Best, D.; Chairatana, P.; Glawar, A. F. G.; Crabtree, E.; Butters, T. D.; Wilson, F. X.; Yu, C. Y.; Wang, W. B.; Jia, Y. M.; Adachi, I.; Kato, A.; Fleet, G. W. J. *Tetrahedron Letters* **2010,** *51*, 2222-2224.
62. Fleet, G. W. J.; Smith, P. W.; Nash, R. J.; Fellows, L. E.; Parekh, R. B.; Rademacher, T. W. *Chem Lett* **1986**, 1051-1054.
63. Fleet, G. W. J.; Fellows, L. E.; Smith, P. W. *Tetrahedron* **1987,** *43*, 979-990.
64. Boshagen, H.; Heiker, F. R.; Schuller, A. M. *Carbohydr Res* **1987,** *164*, 141-148.
65. Kappes, E.; Legler, G. *J Carbohydr Chem* **1989,** *8*, 371-388.
66. Ho, C. W.; Popat, S. D.; Liu, T. W.; Tsai, K. C.; Ho, M. J.; Chen, W. H.; Yang, A. S.; Lin, C. H. *ACS Chem Biol* **2010,** *5*, 489-497.
67. Gradnig, G.; Legler, G.; Stutz, A. E. *Carbohydr Res* **1996,** *287*, 49-57.
68. Kajimoto, T.; Liu, K. K. C.; Pederson, R. L.; Zhong, Z. Y.; Ichikawa, Y.; Porco, J. A.; Wong, C. H. *J Am Chem Soc* **1991,** *113*, 6187-6196.
69. Furneaux, R. H.; Gainsford, G. J.; Lynch, G. P.; Yorke, S. C. *Tetrahedron* **1993,** *49*, 9605-9612.
70. Granier, T.; Vasella, A. *Helv Chim Acta* **1998,** *81*, 865-880.
71. Al-Rawi, S.; Hinderlich, S.; Reutter, W.; Giannis, A. *Angew Chem Int Ed* **2004,** *43*, 4366-4370.
72. Csuk, R.; Honig, H.; Nimpf, J.; Weidmann, H. *Tetrahedron Letters* **1980,** *21*, 2135-2136.
73. Bashyal, B. P.; Chow, H. F.; Fellows, L. E.; Fleet, G. W. J. *Tetrahedron* **1987,** *43*, 415-422.
74. Krulle, T. M.; Davis, B.; Ardron, H.; Long, D. D.; Hindle, N. A.; Smith, C.; Brown, D.; Lane, A. L.; Watkin, D. J.; Marquess, D. G.; Fleet, G. W. J. *Chem Commun* **1996**, 1271-1272.
75. Bashyal, B. P.; Chow, H. F.; Fleet, G. W. J. *Tetrahedron* **1987,** *43*, 423-430.
76. Bashyal, B. P.; Chow, H. F.; Fleet, G. W. J. *Tetrahedron Letters* **1986,** *27*, 3205-3208.
77. Weymouth-Wilson, A. C.; Fleet, G. W. J. World Patent WO 10/004269. **2010** Jul 07.
78. Ho, P. T. *Can J Chem* **1979,** *57*, 381-383.

79. Booth, K. V.; Jenkinson, S. F.; Best, D.; Nieto, F. F.; Estevez, R. J.; Wormald, M. R.; Weymouth-Wilson, A. C.; Fleet, G. W. J. *Tetrahedron Letters* **2009,** *50*, 5088-5093.
80. Best, D.; Jenkinson, S. F.; Watkin, D. J.; Booth, K. V.; Fleet, G. W. J. *Acta Crystallogr E* **2007,** *63*, o1056-o1057.
81. Fleet, G. W. J.; Carpenter, N. M.; Petursson, S.; Ramsden, N. G. *Tetrahedron Letters* **1990,** *31*, 409-412.
82. Deno, N. C.; Potter, N. H. *J Am Chem Soc* **1967,** *89*, 3550-3554.
83. Mori, N.; Togo, H. *Tetrahedron* **2005,** *61*, 5915-5925.
84. Sajiki, H.; Hirota, K. *Tetrahedron* **1998,** *54*, 13981-13996.
85. Czech, B. P.; Bartsch, R. A. *J Org Chem* **1984,** *49*, 4076-4078.
86. Wuts, P. G. M. G., T. W. *Greene's Protective Groups in Organic Synthesis*; John Wiley & Sons: New York, 2006.
87. Best, D.; Jenkinson, S. F.; Thompson, A. L.; Watkin, D. J.; Wilson, F. X.; Nash, R. J.; Fleet, G. W. J. *Acta Crystallogr E* **2009,** *65*, o2418-o2419.
88. Jenkinson, S. F.; Kato, A. *Unpublished results*.
89. Shing, T. K. M. *J Chem Soc Chem Comm* **1987**, 262-263.
90. Shing, T. K. M. *Tetrahedron* **1988,** *44*, 7261-7264.
91. Shing, T. K. M.; Aloui, M. *Can J Chem* **1990,** *68*, 1035-1037.
92. Shing, T. K. M.; Aloui, M. *J Chem Soc Chem Comm* **1988**, 1525-1526.
93. Campo, V. L.; Carvalho, I.; Allman, S.; Davis, B. G.; Field, R. A. *Org Biomol Chem* **2007,** *5*, 2645-2657.
94. Winans, K. A.; King, D. S.; Rao, V. R.; Bertozzi, C. R. *Biochemistry* **1999,** *38*, 11700-11710.
95. Glawar, A. F. G. *Unpublished results*.
96. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J Org Chem* **1997,** *62*, 7512-7515.
97. Horton, D.; Tsai, J. H. *Carbohydr Res* **1977,** *58*, 89-108.
98. Nayak, U. G.; Whistler, R. L. *J Org Chem* **1968,** *33*, 3582-3585.
99. Murao, S.; Miyata, S. *Agr Biol Chem Tokyo* **1980,** *44*, 219-221.
100. Broxterman, H. J. G.; Vandermarel, G. A.; Neefjes, J. J.; Ploegh, H. L.; Vanboom, J. H. *Recl Trav Chim Pays-Bas* **1987,** *106*, 571-576.
101. Bird, P.; Dolphin, D. H.; Withers, S. G. *Can J Chem* **1990,** *68*, 317-322.
102. Evans, S. V.; Fellows, L. E.; Shing, T. K. M.; Fleet, G. W. J. *Phytochemistry* **1985,** *24*, 1953-1955.
103. Wrodnigg, T. M.; Diness, F.; Gruber, C.; Hausler, H.; Lundt, I.; Rupitz, K.; Steiner, A. J.; Stutz, A. E.; Tarling, C. A.; Withers, S. G.; Wolfler, H. *Bioorg Med Chem* **2004,** *12*, 3485-3495.

3. Branched Pyrrolidines: The Synthesis of isoDMDP

3.1 Introduction

Branched chain iminosugars, that is those derived from carbon branched sugar backbones, are a subclass of iminosugars that has not received a great deal of attention. The archetypes are the isofagomines and the branched immucillins (e.g. DADMe-ImmH **3.4**), with most branched chain iminosugars in the literature being based on these structures (**Figure 3.1**). Isofagomine [IFG] **3.1** does not occur naturally and was designed as a β-glucosidase inhibitor.[1,2] This synthetic piperidine is a very potent competitive inhibitor of β-glucosidase (IC_{50} 0.1 μM from almond)[3] and β-glucocerebrosidase,[4] as well as exhibiting a sub-micromolar IC_{50} against glycogen phosphorylase,[5] as the *xylo* configuration mimics the C3/C4/C5 configuration of D-glucose. Its enantiomer L-IFG is a potent non-competitive inhibitor of β-glucosidase (IC_{50} 70 μM from almond)[6] with a low micromolar IC_{50} against glycogen phosphorylase.[5] Siastatin B **3.2**, which can be viewed as a *ribo* epimer of IFG with an oxidised branch and an anomeric amide, is a natural product isolated from *Streptomyces.*[7] It is a potent *N*-acetyl-neuraminidase inhibitor, mimicking the structure of the pyranose ring of neuraminic acid,[8] and analogues have been designed and sythesised that show high inhibitory activity against α-glucosidase,[9,10] β-glucuronidase,[10,11] and β-galactosidase,[11] as well as α-*N*-acetyl-hexosaminidases.[12,13] The deoxy *ribo*-configured analogue **3.3** is a very potent α-fucosidase inhibitor (IC_{50} 9.0 nM from bovine kidney),[14] mimicking the L-*galacto* configuration of fucose. The indolizidine 6-methyl-L-swainsonine **3.5** provides another example of a powerful branched glycosidase inhibitor, showing more potent inhibition of α-rhamnosidase (IC_{50} 0.034 μM from *Penicillium decumbens*) than its unbranched parent L-swainsonine.[15] Branched derivatives of D-swainsonine have shown markedly reduced inhibition of β-mannosidases in comparison to the parent iminosugar.[16]

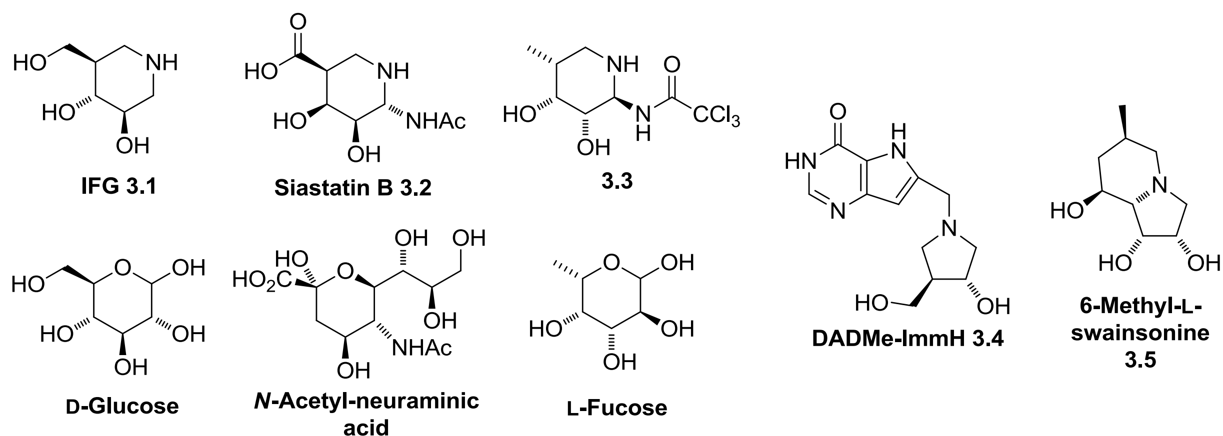


Figure 3.1: Some branched chain iminosugars and natural enzyme substrates that they mimic

The branched immucillins, as well as their un-branched counterparts, are purine nucleoside phosphorylase (PNP) inhibitors and often display extremely potent inhibition.[17,18] DADMe-ImmH **3.4**, for example, is an inhibitor of human PNP with K_i 16 pM.[18] Biological screening of the parent iminosugars of the immucillins for glycosidase inhibition is rarely reported, presumably as glycosidase inhibition is not the design for these molecules, and may also be a consequence of the observation that branching often removes glycosidase inhibition.[19,20] Branched pyrrolidine iminosugars in general are very rarely reported as potent glycosidase inhibitors. One of the very few examples is the branched pyrrolidine amino acid **3.7**, which is a potent inhibitor of influenza neuraminidase,[21] and branched analogues and isomers of DAB **3.9D** and LAB **3.9L** have recently provided very interesting results in the area of biologically active branched iminosugars (**Figure 3.2**). 4-*C*-Methyl-DAB **3.10** and -LAB are both potent α-glucosidase inhibitors. With a sub-micromolar IC_{50}, 4-*C*-methyl-DAB **3.10** is a more potent and more specific inhibitor of α-glucosidases than the parent compound DAB **3.9D**, and similar introduction of a methyl branch to LAB **3.9L** gives complete specificity for α-glucosidases with similar potency.[22] isoDAB **3.12D** is a constitutional isomer of DAB **3.9D** related by a translocation of the terminal hydroxymethyl portion (C5) of the arabinitol backbone by one position, from the C4 position to C3 in the case of DAB **3.9D**, which will be henceforth referred to as the "iso modification" (**Figure 3.2**).

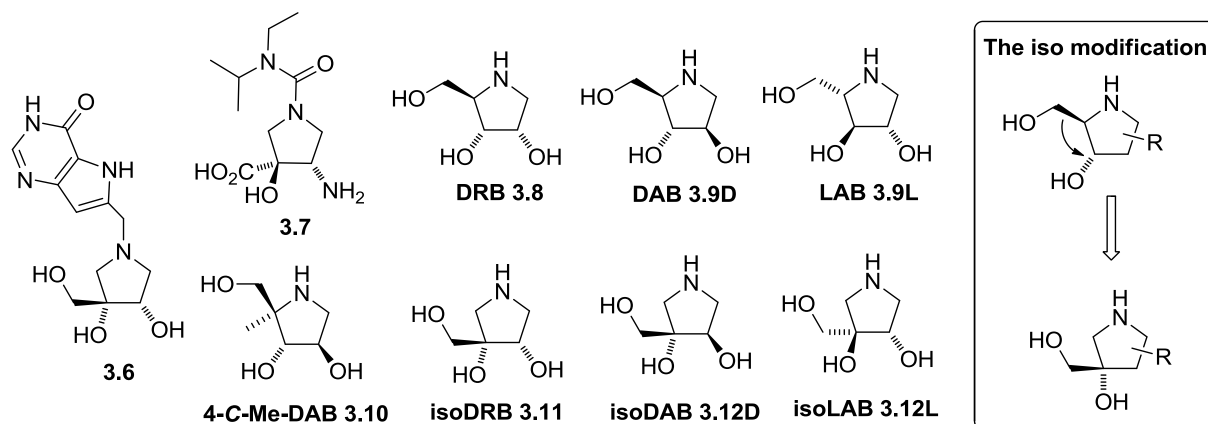


Figure 3.2: Branched pyrrolidines and the iso modification

isoDRB **3.11**, a similarly related constitutional isomer of biologically active natural product 1,4-dideoxy-1,4-imino-D-ribitol [DRB] **3.8**,[23] was found to have very weak inhibition in comparison to the natural product,[19,24] but its corresponding immucillin **3.6** is a nanomolar inhibitor of human PNP.[18] Conversely, isoDAB **3.12D** was found to be a completely specific α-glucosidase inhibitor (IC_{50} 15 μM rat intestinal sucrase) with similar potency to the natural product DAB **3.9D** (IC_{50} 16 μM rat intestinal sucrase).[25] On the other hand, isoLAB **3.12L**, the enantiomer of isoDAB **3.12D** and related to the potent glucosidase inhibitor LAB **3.9L**, displayed no inhibition of any glycosidases. In a surprising turn of events it was discovered that isoLAB can act as a corrector and rescue the function of a cystic fibrosis transmembrane conductance regulator [CFTR] mutant, delF508-CFTR, a mutant protein that is inefficiently trafficked to the plasma membrane, in turn leading to the build up of hyperviscous mucus. *N*B-DNJ has a similar corrector function that has been attributed to its inhibition of endoplasmic reticulum [ER] α-glucosidases, but the pharmacological origin of the effect is not fully understood (also see: **Chapter 1**). The discovery of isoLAB **3.12L** as a CFTR corrector shows that glycosidase inhibition is not required for CFTR rescue and that there are non-inhibitory pathways for the treatment of this disease. isoDAB **3.12D** had no corrector effect at all; whilst it does inhibit α-glucosidases from rice and rats it does not inhibit human ER resident α-glucosidases and would not be expected to function in the same pathway as proposed for *N*B-DNJ. Further investigation

is required before sensible speculation on the nature of these alternative pathways can be made, but therapies targeting such pathways could eventually develop into treatments that avoid side effects attributable to glycosidase inhibition.

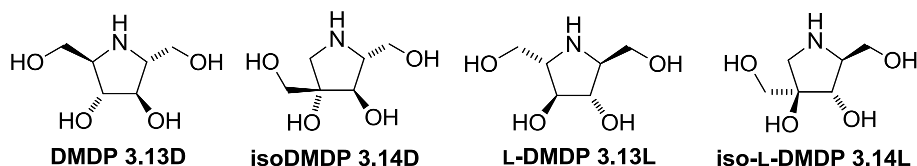


Figure 3.3: isoDMDP targets for this project

Like DAB **3.9D** and LAB **3.9L** (**Figure 3.3**), both enantiomers of DMDP **3.13** are glycosidase inhibitors and investigation of the iso modification of DMDP **3.13**, giving **3.14,** was undertaken, involving branch introduction by a Ho aldol addition and the development of a particularly useful yet woefully underused alcohol protecting group: the benzhydryl ether.

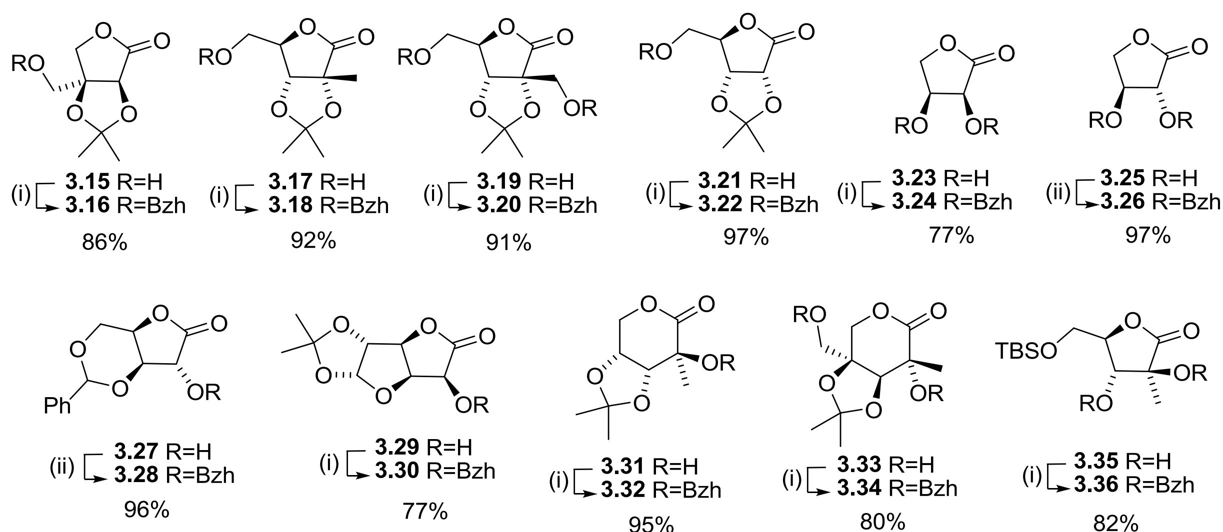
3.2. The Benzhydryl Protecting Group

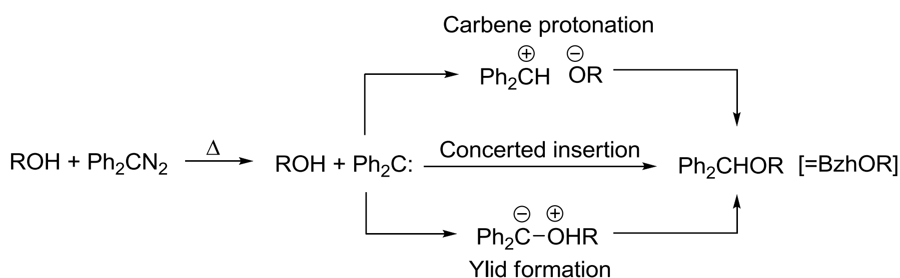
The protection of individual carbohydrate hydroxyl groups is typically achieved by the formation of benzyl and silyl ethers or similar variants on this theme. The introduction of these groups often requires the use of acid or base catalysis;[26] conditions that can give rise to serious complications for many substrates, thus limiting the flexibility of synthetic strategies. Basic conditions introduce the potential for epimerisation adjacent to carbonyls and in the case of silyl groups may result in undesired migration.[27-32] Acidic conditions are incompatible with a host of protecting groups that may already be present in the system, including the almost omnipresent acetals and ketals. There is clearly a need for a reliable protecting group that can be introduced efficiently under neutral conditions.

While not a novel protecting group, benzhydryl (or diphenylmethyl) ethers are relatively scarcely employed as an alcohol protection strategy and their occurrence in carbohydrate chemistry is even rarer. Formation of benzhydryl ethers is conventionally achieved by acid catalysed condensation of the substrate alcohol with benzhydrol.[33] Their introduction *via* the thermal decomposition of diphenyldiazomethane is relatively unstudied, but the potential of this reaction

has been reported.[34] Diphenyldiazomethane itself can be prepared by the oxidation of benzophenone hydrazone with mercuric oxide[35] or Magtrieve™ (CrO_2).[36]

A range of carbohydrate lactone systems with various acid/base sensitivities were subjected to heating with diphenyldiazomethane in toluene or acetonitrile in the absence of any other reagent (**Scheme 3.1**).[37] The resulting fully protected systems were obtained in excellent yields, even in the case of highly hindered neopentyl (**3.15**, **3.19**, **3.33**) and tertiary (**3.31**, **3.33**, **3.35**) hydroxyl groups. No epimerisation was observed in base sensitive lactones **3.23**, **3.25**, **3.27** or **3.29**.





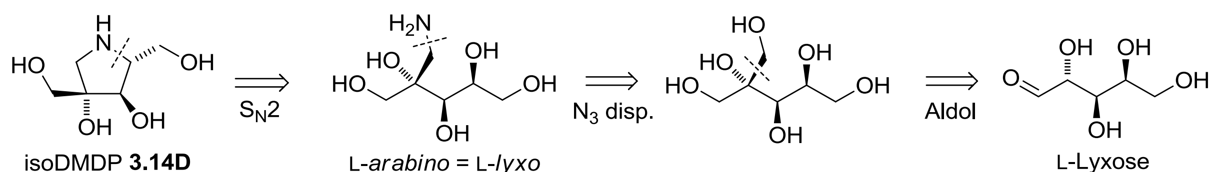
Reagents and conditions: (i) Ph_2CN_2, toluene, reflux, 1 h (ii) Ph_2CN_2, MeCN, reflux, 18 h.
Scheme 3.1: Benzhydryl protection of a range of carbohydrate lactones.

The mechanism of this reaction has not been firmly established. Whilst there appears to be universal agreement in the generation of the diphenylmethylene carbene, the mechanism by which O-H insertion occurs is widely contested. Three mainstream possibilities are concerted insertion, ylid formation and carbene protonation (**Scheme 3.1**). Bethell has presented arguments based on the effect of methanol as an additive on kinetic isotope effects in support of the ylid

pathway, dismissing the possibility of concerted insertion and also arguing against carbene protonation on the basis of product distribution.[38,39] Further evidence for ylid formation has also been presented in more specific systems.[40,41] However, diarylcarbenium ions have been detected by photolysis of diaryldiazomethanes in the presence of alcohols,[42-45] implying that carbene protonation is an operating mechanism, although it has been suggested that this is a minor pathway.[42] A mechanism for concerted O-H insertion involving two alcohol molecules has also been proposed that would be consistent with Bethell's observations.[46] While there is direct spectroscopic evidence for carbene protonation, which is not true for ylid formation, it does not appear to fully explain the kinetic observations[39] and has lead to the conclusion that more than one pathway for O-H insertion is operative.[42,47] The reaction is nevertheless a useful one and this protection strategy has since been employed in viable syntheses of various iminosugars[48-52] and was also utilised in the synthesis of isoDMDP **3.14D**.

3.3. isoDMDP by Intramolecular S_N2

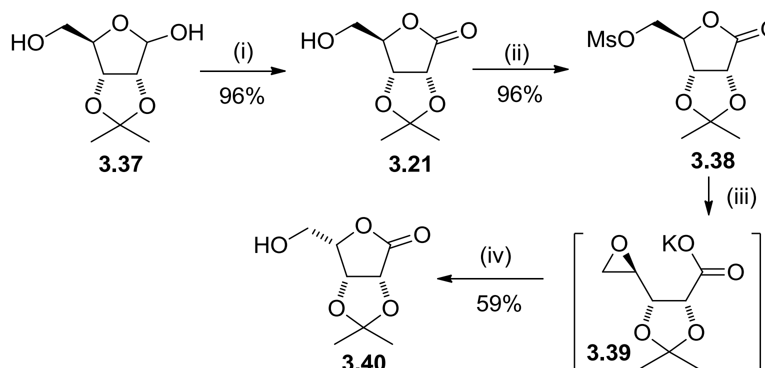
Considering isoDMDP **3.14D** in a retrosynthetic sense, disconnection of the N-C4 bond provides a 4-*C*-azidomethyl substituted L-arabinitol (**Scheme 3.2**). A C4-branched L-arabinitol is equivalent to a C2-branched L-lyxitol, and is available by Ho aldol addition of formaldehyde to L-lyxose.



Scheme 3.2: Retrosynthetic analysis of isoDMDP

The use of a *lyxo* chiron is particularly advantageous as both enantiomers of lyxonolactone are readily available. D-Lyxonolactone is produced by Humphlett oxidation of D-galactose,[53] and L-lyxonolactone is readily available from D-ribose.

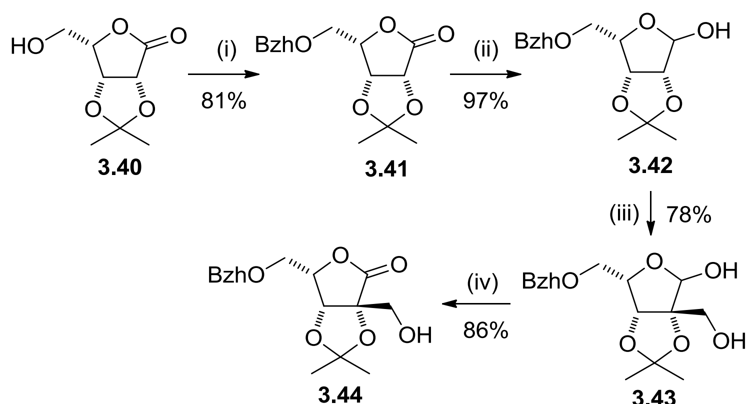
The L-lyxonolactone acetonide **3.40** was prepared from the D-ribose acetonide **3.37** as described in the literature in similar yields (**Scheme 3.3**).[54]



Reagents and conditions: (i) Br_2, $BaCO_3$, H_2O, 0°C to RT, 3.5 h; (ii) MsCl, TEA, DCM, 0-10°C, 2.5 h; (iii) KOH, H_2O, 1,4-dioxane, RT, 1 h; (iv) AcOH, H_2O, 1,4-dioxane, RT, 3 d

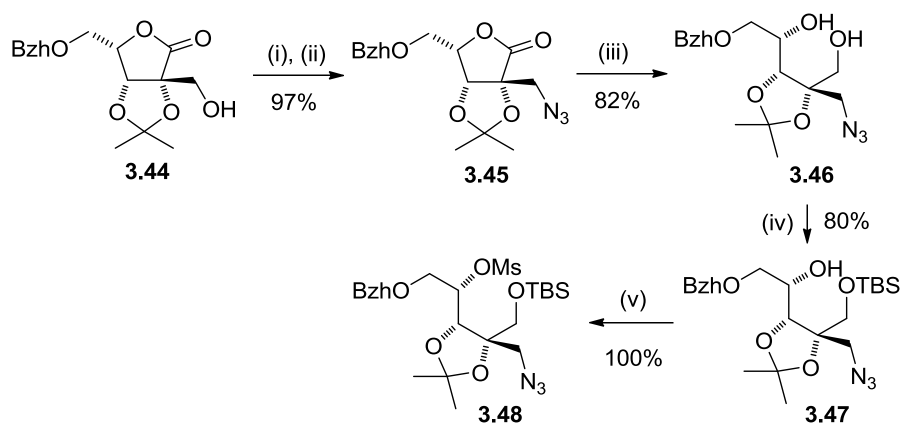
Scheme 3.3: Synthesis of lyxonolactone

Following the extremely efficient bromine oxidation of D-ribose acetonide **3.37** to the corresponding ribonolactone **3.21** in 96% yield, epimerisation of the *ribo* scaffold at C4 was achieved by acid catalysed intramolecular ring opening of intermediate epoxide **3.39**, itself generated by treatment of mesylate **3.38** with potassium hydroxide. With lyxonolactone **3.40** in hand it was necessary to protect OH5 prior to introduction of a hydroxymethyl branch; the branch hydroxyl would otherwise be virtually chemically indistinguishable from OH5 (**Scheme 3.4**). Benzhydryl was identified as a particularly suitable protecting group owing to its ease of introduction and base stability required for the impending Ho aldol addition reaction; silyl ethers are typically labile under these conditions. As lactone **3.40** bears a relatively acidic α-hydrogen, the neutral introduction of benzhydryl was deemed preferable to the typically strongly basic conditions associated with the formation of benzyl ethers. Indeed it may be telling that an efficient procedure for such a benzylation of lyxonolactone **3.40** has never been reported, whereas benzhydryl protection was found to proceed in a satisfying yield of 81%. Reduction of the fully protected lactone **3.41** to the corresponding lactol in 97% yield was achieved with diisobutylaluminium hydride, giving lyxose derivative **3.42** at the appropriate oxidation level for aldol addition.



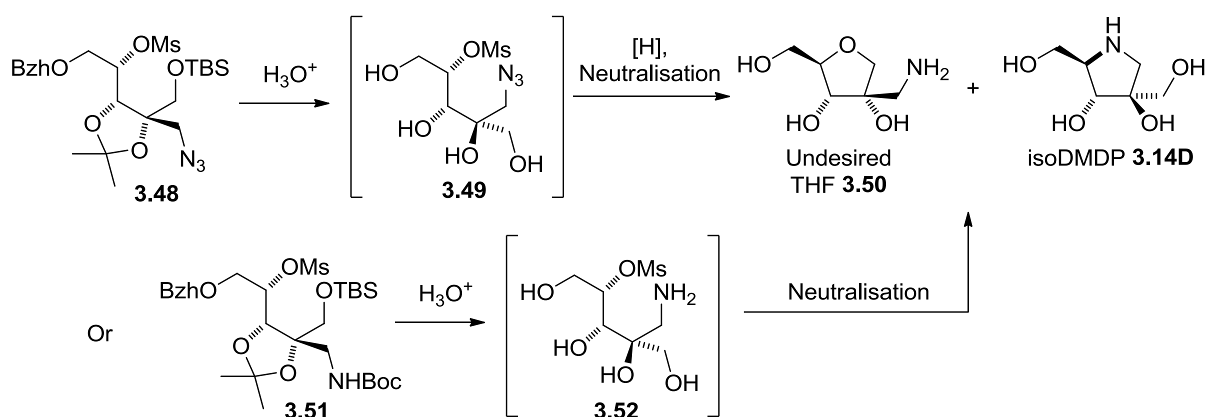
Reagens and conditions: (i) Ph_2CN_2, PhMe, reflux, 1 h; (ii) DIBAL, DCM, -78°C, 90 min; (iii) HCHO, K_2CO_3, MeOH, H_2O, reflux, 4 h; (iv) I_2, K_2CO_3, ^{t}BuOH, reflux, 4 h
Scheme 3.4: Introduction of a hydroxymethyl branch

Treatment of lyxose **3.42** with formaldehyde in basic refluxing aqueous methanol gave the branched lyxose **3.43** in 78% yield, the stereochemical outcome of this Ho aldol[55] reaction being a thermodynamic effect imparted by the isopropylidene group; the *xylo* configured C2 epimer is disfavoured as it cannot exist in a stable furanose form, and is not observed. The anomeric position was then protected as the corresponding lactone, employing iodine oxidation[56] (see also: **Section 2.3**) to cleanly generate branched lactone **3.44** in 86% yield, leaving only the hydroxymethyl branch unprotected. Nitrogen was introduced at the branch by activation of the neopentyl hydroxyl of lactone **3.44** as a triflate ester followed by displacement with sodium azide in DMF. This reaction was remarkably rapid, proceeding in 97% yield within one hour at room temperature to give azido-lactone **3.45** (**Scheme 3.5**).



Reagents and conditions: (i) Tf_2O, Py, DCM, -30°C, 1 h; (ii) NaN_3, DMF, RT, 1 h; (iii) $NaBH_4$, EtOH, RT, 1 h; (iv) TBSCl, imidazole, DMF, RT, 16 h; (v) MsCl, TEA, DCM, 0°C to RT, 2 h
Scheme 3.5: Synthesis of key azido-mesylate **3.48**

Reduction of the lactone **3.45** with sodium borohydride in ethanol gave the required C2-branched lyxitol (= C4-branched arabinitol) **3.46** in 82% yield. In this case workup with ammonium chloride proved critical, the crude reaction mixture being otherwise largely composed of intractable baseline (TLC) material presumably attributable to boron complexation of the diol. Activation of the secondary hydroxyl group was achieved by first protecting the primary hydroxyl function selectively as a silyl ether with *tert*-butyldimethylsilyl chloride and imidazole in DMF at 0°C in 80% yield, giving alcohol **3.47**, followed by mesylation at the remaining free hydroxyl to furnish the azido-mesylate **3.48** in quantitative yield. Unfortunately efforts towards the efficient cyclisation of this 1,4-azido-mesylate **3.48** proved futile (**Scheme 3.6**).

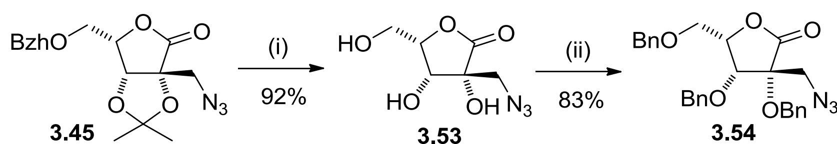


Scheme 3.6: Unsuccessful attempts at clean pyrrolidine formation

The inherent problem in this system is the acetonide-tethered vicinal diol. Ring closure to the desired pyrrolidine isoDMDP **3.14D** ultimately results in a *trans*-configured vicinal diol; a stereochemical relationship incompatible with the acetonide tether as it would constitute a highly strained *trans*-[3.3.0] bicyclic system. Hydrolysis of the offending acetonide was accompanied by global deprotection, with the observed order of acid lability of the protecting groups being silyl>>benzhydryl>acetonide as determined by periodic LRMS analysis, giving the intermediate tetraol **3.49**. Upon azide reduction and neutralisation, mixtures of isoDMDP **3.14D** and a contaminant THF **3.50**, arising from intramolecular displacement of mesylate by the alcohol

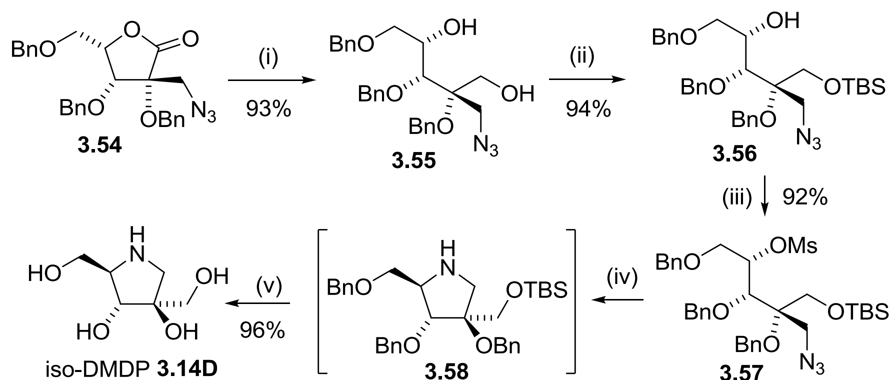
functionality, were obtained in varying ratios (1:2 to 2:1). Similar treatment of the analogous carbamate **3.51** gave similar results and the strategy was redesigned.

The presence of an acetonide is absolutely required to impart stereoselectivity in the Ho aldol addition reaction, but after this transformation its purpose is purely protective and a strategy was developed for its replacement (**Scheme 3.7**).



Reagents and conditions: (i) AcCl, MeOH, 80°C, 18 h; (ii) BnBr, NaH, DMF, 0°C, 4 h
Scheme 3.7: Transprotection of acetonide **3.45**

Branched lactone **3.45** was globally deprotected by methanolysis to give lactone **3.53** in 92% yield after triturative removal of benzhydryl methyl ether, and perbenzylated by treatment with sodium hydride in the presence of benzyl bromide to give **3.54** in 83% yield. As a non-enolisable lactone, **3.54** is stable under these conditions provided that water is rigorously excluded; the product **3.54** is surprisingly vulnerable to hydrolysis, which is induced even on organic-aqueous extractions with sodium bicarbonate and can render the yield of the lactone **3.54** as low as 15%. Quenching the excess sodium hydride with glacial acetic acid and slightly acidifying the reaction mixture prior to aqueous extraction of the salts eliminates this problem.



Reagents and conditions: (i) $NaBH_4$, EtOH, RT, 3 h; (ii) TBSCl, imidazole, DMF, 0°C, 5 h; (iii) MsCl, Py, DCM, RT, 7 h; (iv) H_2, Pd (10% on C), NaOAc, H_2O, 1,4-dioxane, RT, 14 h; (v) H_2, Pd (10% on C), HCl, H_2O, 1,4-dioxane, RT, 24 h
Scheme 3.8: The synthesis of isoDMDP

Lactone **3.54** was then converted into the desired azido-mesylate **3.57** by a sequence of transformations analogous to those employed previously (**Scheme 3.8**); reduction, silylation and mesylation. The azido-mesylate **3.57** cyclised cleanly and efficiently to give isoDMDP **3.14D**. Palladium catalysed reduction of the azide with hydrogen in the presence of sodium acetate gave the intermediate benzylated isoDMDP **3.58**. The reaction mixture was then simply acidified to facilitate hydrogenolysis of the benzyl ethers to give isoDMDP **3.14D** in 96% yield. The overall yield was 30% over twelve steps from the easily obtainable lyxonolactone acetonide **3.40**.

Biological screening for glycosidase inhibition (Atsushi Kato, Univ. Toyama) revealed that isoDMDP **3.14D** is not an inhibitor of any of the enzymes tested, but its enantiomer iso-L-DMDP **3.14L**, which was obtained from D-lyxonolactone by implementation of the same methodology,[57] is a potent and specific inhibitor of α-glucosidases (**Table 3.1**).

	IC_{50} (μM)					
Enzyme	DAB **3.9D**	isoDAB **3.12D**	isoDMDP **3.14D**	LAB **3.9L**	isoLAB **3.12L**	iso-L-DMDP **3.14L**
α-Glucosidase						
Rat intestinal maltase	55	24	NI[a]	0.93	NI	0.19
Rat intestinal isomaltase	5.8	20	NI	0.36	NI	8.8
Rat intestinal sucrase	16	15	NI	1.0	NI	0.38
β-Glucosidase						
Rat intestinal cellobiase	756	NI	NI	NI	NI	NI
β-Galactosidase						
Rat intestinal lactase	323	NI	NI	415	NI	NI
Trehalase						
Rat intestinal trehalase	61	NI	NI	75	NI	NI
Glycogen phosphorylase						
Rabbit muscle	0.33	NI	ND[b]	NI	NI	ND

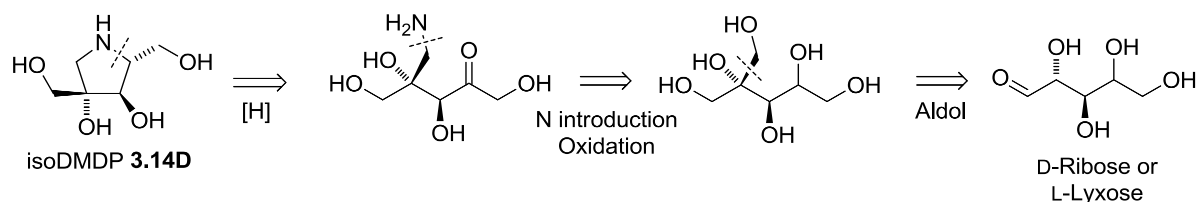
[a]NI: No inhibition (less than 50% inhibition at 1000 μM)
[b]ND: Not determined

Table 3.1: Concentration of iminosugars giving 50% inhibition of various glycosidases and glycogen phosphorylase

This result is in stark contrast to the trend observed in the case of isoDAB **3.12D** and isoLAB **3.12L**. DAB **3.9D** and LAB **3.9L** are potent α-glucosidase inhibitors and the iso modification of DAB **3.9D** to isoDAB **3.12D** increases specificity whilst retaining potency whereas the LAB **3.9L** to isoLAB **3.12L** modification removes inhibition.[25] On the other hand, the activity of DMDP **3.13D** (**Section 2.3**) is completely removed by a similar modification to isoDMDP **3.14D** whereas the inhibitory activity of L-DMDP **3.13L** is retained and slightly increased by the modification to iso-L-DMDP **3.14L**. More curious still are the CFTR-rescuing abilities of these compounds; while isoLAB **3.12L** inhibits no glycosidases screened in these assays, it partially rescues CFTR function to a greater extent than the positive control *N*B-DNJ (a potent α-glucosidase inhibitor), whereas isoDAB **3.14D** is a potent α-glucosidase inhibitor but shows no activity in the CF assays. In the case of isoDMDP **3.14D**, it neither inhibits glycosidases nor rescues CFTR function, but iso-L-DMDP **3.14L** both inhibits α-glucosidases and preliminary communications suggest it may also rescue CFTR function (Frederic Becq, Caroline Norez, Univ. Poitiers). Whether this activity of iso-L-DMDP **3.14L** is associated with ER resident α-glucosidase inhibition, as has been suggested for *N*B-DNJ, is yet to be determined.

3.4. isoDMDP by Intramolecular Reductive Amination

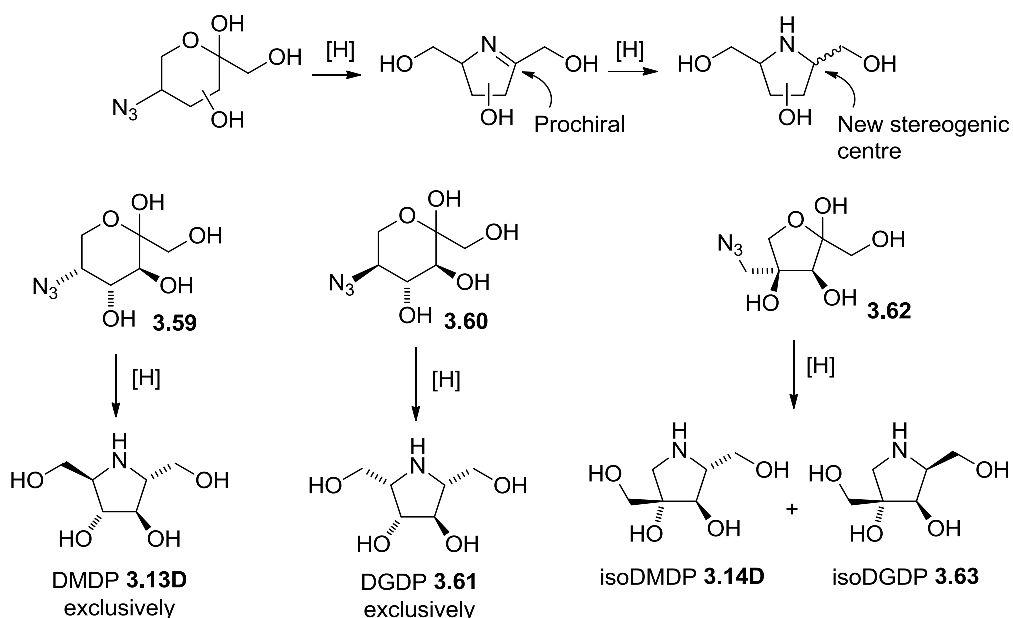
During the development of the efficient route to isoDMDP **3.14L** discussed in **Section 3.3**, an alternative method of cyclisation was considered. The competing side reaction of cyclisation *via* oxygen, giving a THF (**Scheme 3.6**), during attempted pyrrolidine formation by S_N2 was ultimately solved by protection of the problematic hydroxyl. An alternative solution was available in the form of intramolecular reductive amination; a cyclisation reaction which may only proceed *via* an imine. The strategy is essentially identical to that presented in **Scheme 3.2**, but proceeds by oxidation and intramolecular reductive amination rather than S_N2 ring closure (**Scheme 3.9**).



Scheme 3.9: Retrosynthetic analysis of isoDMDP

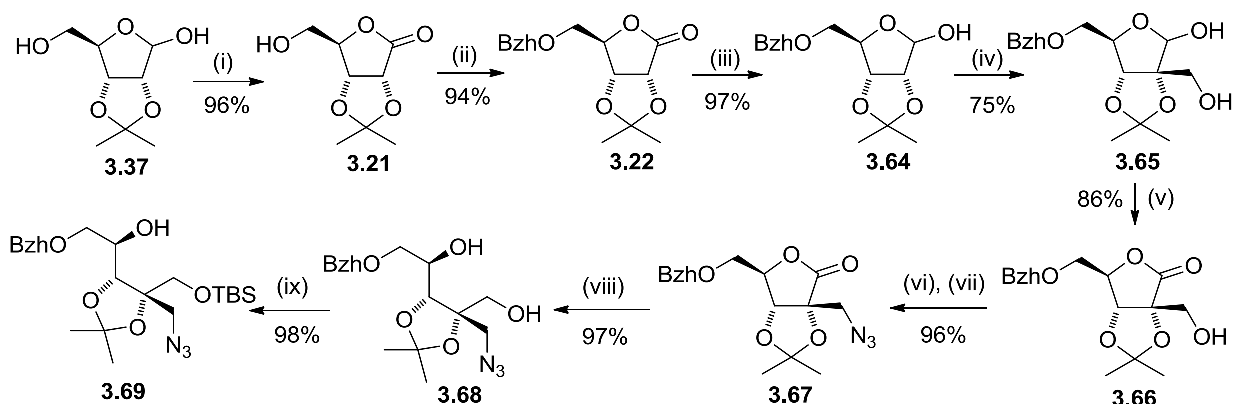
A consequence of employing such an oxidation is the destruction of an asymmetric centre. This in turn has two implications: a) the reductive amination forms a new stereogenic centre and is likely to provide a mixture of diastereoisomers; b) the starting material does not require any particular stereochemical configuration at that centre. The prospect of a diastereomeric mixture can be viewed in either a positive or negative light. On one hand two novel branched pyrrolidines would be obtained for biological screening, and on the other hand such a mixture would require separation, often difficult at such high polarity, and therefore may not be the most efficient of methods. A reductive amination of this type would proceed *via* a prochiral imine, with addition of hydrogen to the *Si* diastereotpic face giving isoDMDP **3.14D** and/or addition to the *Re* face giving 1,4-dideoxy-2-*C*-hydroxymethyl-1,4-imino-L-xylitol [isoDGDP] **3.63**, which is the iso analogue of the natural product and α-glucosidase inhibitor DGDP **3.61** (**Scheme 3.10**). Very high diastereoselectivity has been previously observed for reductive amination in related pyrrolidine systems; hydrogenation of azido-fructose **3.59** is known to generate DMDP **3.13D** with complete selectivity,[58] and similarly azido-sorbose **3.60** gives DGDP **3.61** exclusively.[59,60] A reductive amination strategy was therefore not dismissed, although such degrees of selectivity were not assumed for the branched systems under investigation.

The destruction of the stereogenic centre by oxidation allows use of either epimer D-ribonolactone or L-lyxonolactone as a starting material; as the latter is usually obtained from the former by an epimerisation reaction, D-ribose was clearly the economical choice of starting material.



Scheme 3.10: Stereochemical considerations in reductive amination

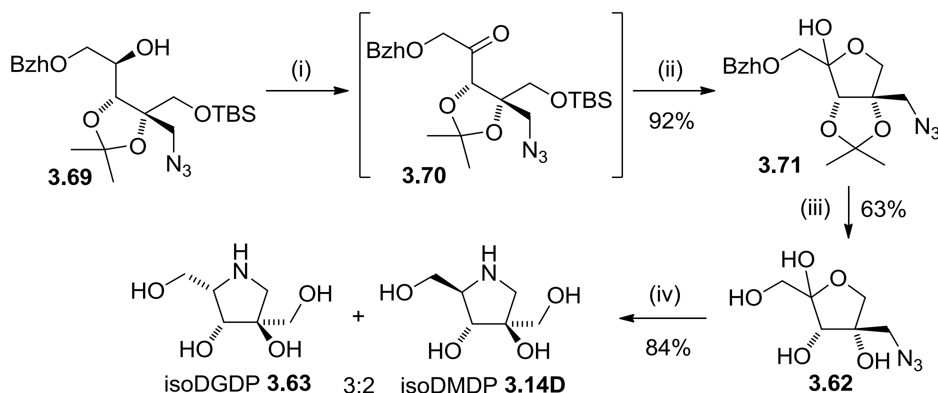
A sequence analogous to that presented in **Section 3.3** was employed to generate the target azido-alcohol intermediate **3.69** from D-ribose acetonide **3.37** (**Scheme 3.11**). This chemistry has been discussed in **Section 3.3** and there is little to add here; the sequence is extremely efficient.



Reagents and conditions: (i) Br_2, $BaCO_3$, H_2O, 0°C to RT, 3.5 h; (ii) Ph_2CN_2, PhMe, reflux, 1 h; (iii) DIBAL, DCM, -78°C, 1 h; (iv) HCHO, K_2CO_3, MeOH, H_2O, reflux, 2 h; (v) I_2, K_2CO_3, ^{t}BuOH, reflux, 90 min; (vi) Tf_2O, Py, DCM, -30°C, 1 h; (vii) NaN_3, DMF, RT, 90 min; (viii) $NaBH_4$, EtOH, RT, 90 min; (ix) TBSCl, imidazole, DMF, 0°C, 5 h

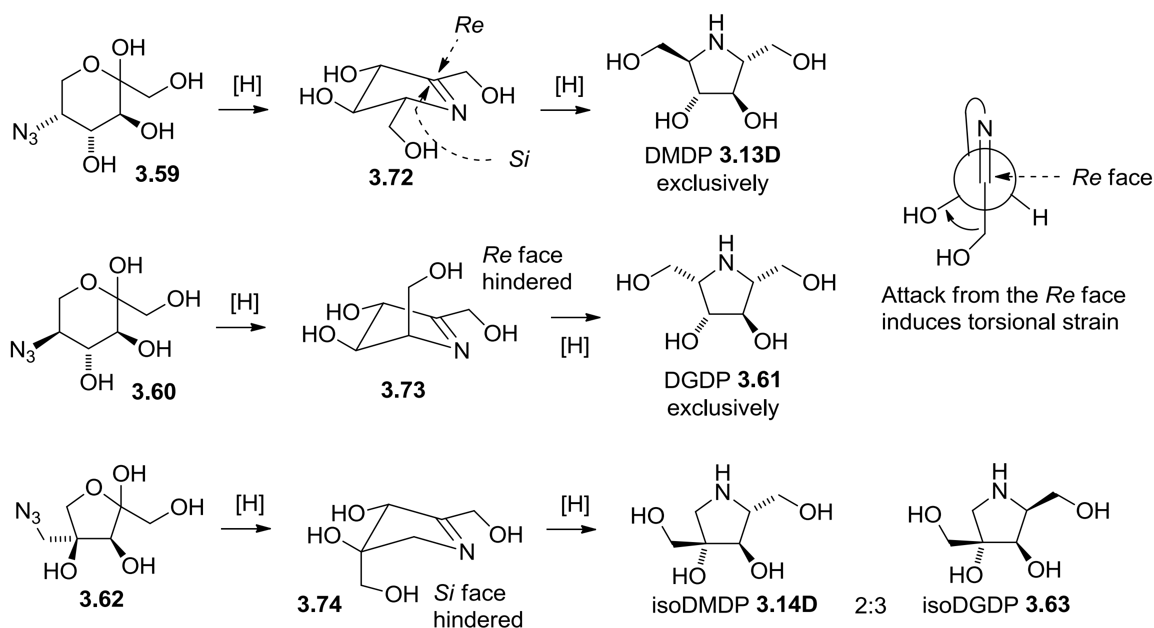
Scheme 3.11: Synthesis of ribitol **3.68**

Oxidation of azido-alcohol **3.69** was achieved with Dess-Martin periodinane and treatment of the crude resultant ketone **3.70** with tetrabutylammonium fluoride cleaved the silyl ether to give the stable furanose **3.71** in 92% yield over two steps (**Scheme 3.12**).



Reagents and conditions: (i) Dess-Martin periodinane, DCM, RT, 2 h; (ii) TBAF, THF, 0°C to RT, 2 h; (iii) *p*TSA, H_2O, 1,4-dioxane, 85°C, 18 h; (iv) H_2, Pd (10% on C), H_2O
Scheme 3.12: isoDMDP and isoDGDP by reductive amination

Global deprotection of ribulose **3.71** proved to be the most challenging transformation of the sequence. Acetonides of tertiary alcohols are often highly resistant to acid catalysed hydrolysis, and are exceptionally stable as part of [3.3.0] fused bicyclic systems.[61] Another limiting factor in this case is the low water solubility of ribulose **3.71** which necessitates a medium of relatively low dielectric constant, often resulting in lethargic hydrolysis.[62] This problem is particularly apparent for the fully protected ketone **3.70** and is one of the reasons for fluoride induced desilylation prior to hydrolysis. Hydrolysis of ribulose **3.71** was achieved in aqueous dioxane at 85°C with catalytic tosic acid, giving the desired free azido-ketose **3.62** in a moderate yield of 63% following chromatographic purification. The transformation appears to proceed cleanly (TLC), and it is likely that the purification procedure is responsible for the disappointing yield. Hydrogenation of the free ketose **3.62** did result in a mixture of diastereomeric pyrrolidines, giving isoDMDP **3.14D** and isoDGDP **3.63** in a combined crude yield of 84%. The diastereomeric ratio was approximately 2:3 isoDMDP/isoDGDP as judged by relative peak integrals in the ^{1}H NMR spectrum. The lack of selectivity in the reductive amination reaction compared to the very high selectivity observed for related systems can be rationalised (**Scheme 3.13**).



Scheme 3.13: Rationalisation of stereoselectivity in reductive amination

In the case of imine **3.72** neither face of the CN bond is clearly more sterically hindered than the other as all substituents would probably lie in pseudoequatorial positions. However, imine **3.72** has been shown to accept hydrogen exclusively from the *Si* face to give DMDP **3.13D**, which is presumably due to the accumulation of torsional strain associated with addition to the *Re* face. In the case of imine **3.73**, the *Si* face does appear less sterically congested than the *Re* face, which, in combination with the torsional strain argument, supports the observation of exclusive addition to the *Si* face to give DGDP **3.61**. In the case of the branched imine **3.74** these steric and torsional factors oppose one another. The hydroxymethyl branch may be expected to sterically hinder the *Si* face and leave the *Re* face more reactive, but the torsional argument predicts that addition to the *Re* face is disfavoured. Hence the net effect is poor selectivity, with the steric bulk of the hydroxymethyl branch marginally dominating over torsional strain to give a slight excess of isoDGDP **3.63** arising from addition of hydrogen to the *Re* face.

Separation of the isomers was achieved with 38% mass recovery to give isoDMDP **3.13D** and isoDGDP **3.63** in a 5:6 ratio (Atsushi Kato, Univ. Toyama). Biological evaluation of isoDGDP is currently underway.

3.5. Conclusions and Future Work

An efficient synthesis of isoDMDP **3.13D** has been developed from L-lyxonolactone, and while isoDMDP **3.13D** itself did not prove to be a biologically interesting branched iminosugar in any of the screens conducted, this may be useful information in terms of structure-activity relationships of iminosugars. Application of the methodology to the readily available D-lyxonolactone allowed synthesis of iso-L-DMDP **3.13L**, which is a potent and selective glycosidase inhibitor and may, according to preliminary reports, have potential applications in the study of cystic fibrosis.

Synthesis of isoDMDP **3.13D** by an unselective reductive amination led to the isolation of isoDGDP **3.63**, which could prove to have interesting biological properties. A logical extension of this project would be a specific synthesis of isoDGDP **3.63**, which should be readily achieved by application of the methodology in **Section 4.2** without epimerisation of D-ribonolactone. The enantiomer, iso-L-DGDP, could be similarly prepared from L-ribonolactone. In this way the enantiomers of isoDGDP **3.63** could be prepared in quantities suitable for more extensive biological evaluation, and may provide more clues for the deconvolution of the mechanisms of CFTR rescue.

3.6. Experimental

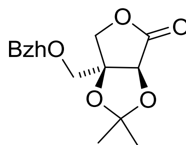
For a general experimental refer to **Section 2.6**.

3.6.1. General method for benzhydryl protection

Diphenyldiazomethane [R] (1.5 eq per hydroxyl substituent) was added to a 0.05 M solution of starting material [SM] in hot PhMe or MeCN. The reaction mixture was stirred at reflux until the absence of purple colour, indicating the complete decomposition of R, was observed (approx. 1 h in PhMe or 12 h in MeCN). The reaction mixture was then analysed by TLC (1:3 EtOAc/CyH unless stated otherwise), and an additional portion of R added if necessary (compounds **20**, **22**).

The reaction mixture was then concentrated under reduced pressure and the crude residue purified by flash column chromatography to afford the product [P] with physical appearance [A].

3'-*O*-Benzhydryl-2,3-*O*-isopropylidene-L-apiono-1,4-lactone, 3.16



SM: **3.15**, 188 mg, 1.0 mmol (R_f 0.0)

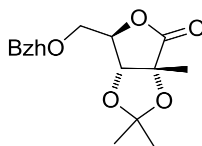
R: 291 mg, 1.5 mmol

P: 305 mg, 86% (R_f 0.30)

A: White crystalline solid

HRMS (ESI +ve): $C_{21}H_{22}NaO_5$ found 377.1355; (M+Na$^+$) requires 377.1359; $[\alpha]_D^{23}$ +26.9 (*c* 1.20, $CHCl_3$); m.p. 87-89°C; ν_{max} (thin film): 1793 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.33, 1.35 (2 x 3H, s, CH_3), 3.49 (1H, d, H3'a, J_{gem} 9.6), 3.52 (1H, d, H3'b, J_{gem} 9.6), 4.32 (1H, d, H4a, J_{gem} 10.4), 4.47 (1H, d, H4b, J_{gem} 10.4), 4.68 (1H, s, H2), 5.34 (1H, s, $Ph_2C\underline{H}$), 7.18 - 7.29 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 27.6, 28.2 (CH_3), 68.6 (C3'), 73.2 (C4), 76.9 (H2), 84.5 ($Ph_2\underline{C}H$), 85.4 (C3), 114.6 ($Me_2\underline{C}$), 126.8, 126.9, 127.9, 128.0, 128.6, 128.7 (Ar$\underline{C}$H), 141.0, 141.1 (ArC), 174.3 (C1); LRMS (ESI +ve): 377 (100% M+Na$^+$).

5-*O*-Benzhydryl-2-*C*-methyl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone, **3.18**



SM: **3.17**, 1.0 g, 5.0 mmol (R_f 0.10)

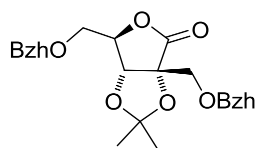
R: 1.5 g, 7.7 mmol

P: 1.7 g, 92% (R_f 0.50)

A: Pale yellow oil

HRMS (ESI +ve): $C_{22}H_{24}NaO_5$ found: 391.1516; ($M+Na^+$) requires: 391.1516; $[\alpha]_D^{21}$ -1.0 (*c* 0.86, $CHCl_3$); ν_{max} (thin film): 1786 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.42, 1.45 (2 x 3H, s, Me), 1.51 (3H, s, H2'), 3.71 (1H, dd, H5a, $J_{5a,4}$ 2.5, J_{gem} 10.9), 3.77 (1H, dd, H5b, $J_{5b,4}$ 3.3, J_{gem} 10.9), 4.53 (1H, s, H3), 4.59 - 4.60 (1H, m, H4), 5.39 (1H, s, $Ph_2C\underline{H}$), 7.25 - 7.38 (10H, m, Ph); δ_c ($CDCl_3$, 100 MHz): 19.9 (C2'), 26.8, 26.8 (Me), 68.3 (C5), 82.0 (C4), 82.7 (C3), 82.8 (C2), 85.0 ($Ph_2\underline{C}H$), 112.9 ($\underline{C}Me_2$), 127.0, 127.2, 127.9, 128.1, 128.5, 128.6 ($Ar\underline{C}H$), 140.5, 140.7 (ArC), 176.3 (C1); LRMS (ESI +ve): 386 (100%, $M+NH_4^+$), 432 (84%, $M+MeCN+Na^+$), 754 (97%, $2M+NH_4^+$), 759 (86%, $2M+Na^+$).

2',5-Di-*O*-benzhydryl-2,3-*O*-isopropylidene-D-hamamelono-1,4-lactone, **3.20**



SM: **3.19**, 218 mg, 1.0 mmol (R_f 0.0)

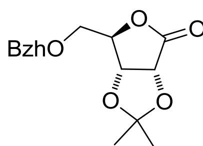
R: 582 mg, 3.0 mmol

P: 503 mg, 91% (R_f 0.25)

A: Pale yellow glass

HRMS (ESI +ve): $C_{35}H_{34}NaO_6$ found: 573.2240; ($M+Na^+$) requires: 573.2248; $[\alpha]_D^{23}$ -9.1 (*c* 1.27, $CHCl_3$); ν_{max} (thin film): 1784 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.46, 1.47 (2 x 3H, s, Me), 3.61 (1H, dd, H5a, $J_{5a,4}$ 3.8, J_{gem} 10.4), 3.66 (1H, d, H2'a, J_{gem} 10.4), 3.70 (1H, dd, H5b, $J_{5b,4}$ 4.8, J_{gem} 10.4), 3.78 (1H, d, H2'b, J_{gem} 10.4), 4.65 (1H, ddd, H4 $J_{4,3}$ 0.7, $J_{4,5a}$ 3.8, $J_{4,5b}$ 4.8), 4.89 (1H, d, H3, $J_{3,4}$ 0.7), 5.05, 5.23 (2 x 1H, s, $Ph_2C\underline{H}$), 7.18 - 7.34 (20H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 26.6, 27.1 (CH_3), 66.9 (C5), 68.1 (C2'), 79.9 (C3), 82.5 (C4), 84.6, 84.7 ($Ph_2\underline{C}H$), 85.3 (C2), 113.5 (Me_2C), 126.7, 127.1, 127.2, 127.3, 127.5, 127.8, 127.8, 128.0, 128.4, 128.5, 128.5, 128.6 ($Ar\underline{C}H$), 140.7, 141.0, 141.6 (ArC), 174.7 (C1); LRMS (ESI +ve): 573 (100%, $M+Na^+$).

5-*O*-Benzhydryl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone, **3.22**



SM: **3.21**, 192 mg, 1.0 mmol (R_f 0.10)

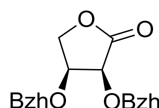
R: 340 mg, 1.75 mmol

P: 352 mg, 97%, (R_f 0.45)

A: Colourless glass

HRMS (ESI +ve): $C_{21}H_{22}NaO_5$ found 377.1355; (M+Na$^+$) requires 377.1359; $[\alpha]_D^{23}$ -27.9 (*c* 0.82, $CHCl_3$). ν_{max} 1785 (s, CO); δ_H ($CDCl_3$ 400 MHz): 1.34, 1.41 (2 x 3H, s, Me), 3.60 (1H, dd, H5a J_{gem} 10.6, $J_{5a,4}$ 1.5), 3.71 (1H, dd, H5b J_{gem} 10.6, $J_{5b,4}$ 2.3), 4.61-4.63 (1H, m, H4), 4.71 (1H, d, H3 $J_{3,2}$ 5.6), 4.81 (1H, d, H2 $J_{2,3}$ 5.6), 5.32 (1H, s, $Ph_2C\underline{H}$), 7.17-7.33 (10H, m, Ph). δ_c ($CDCl_3$ 100 MHz): 25.7, 26.8 (Me), 68.0 (C5), 75.8 (C2), 78.5 (C3), 81.1 (C4), 84.9 ($Ph_2\underline{C}H$), 113.2 ($\underline{C}Me_2$), 126.7, 126.8, 127.9, 128.0, 128.6, 128.7 (aromatic $\underline{C}H$), 140.6, 141.0 (aromatic C), 174.4 (C1); LRMS (ESI +ve): 372 (86%, M+NH_4^+), 418 (74%, M+MeCN+Na$^+$), 726 (100%, 2M+NH_4^+), 731 (88%, 2M+Na$^+$).

2,3-Di-*O*-benzhydryl-L-erythrono-1,4-lactone, **3.24**



SM: **3.23**, 118 mg, 1.0 mmol (R_f 0.0)

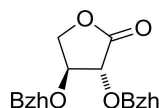
R: 582 mg, 3.0 mmol

P: 346 mg, 77% (R_f 0.45)

A: Colourless glass

HRMS (ESI +ve): $C_{30}H_{26}NaO_4$ found: 473.1726; (M+Na^+) requires: 473.1723; $[\alpha]_D^{18}$ +2.7 (*c* 1.27, $CHCl_3$); ν_{max} (thin film):1790 (s, CO); δ_H ($CDCl_3$, 400 MHz): 4.12 (1H, dd, H4a, J_{gem} 10.4, $J_{4a,3}$ 3.5), 4.16 (1H, d, H2, $J_{2,3}$ 4.8), 4.24 (1H, ddd, H3, $J_{3,2}$ 4.8, $J_{3,4a}$ 3.5, $J_{3,4b}$ 1.0), 4.36 (1H, dd, H4b, J_{gem} 10.4, $J_{4b,3}$ 1.0), 5.73 (1H, s, C<u>H</u>Ph2), 5.95 (1H, s, C<u>H</u>Ph2), 7.27 - 7.43 (10H, m, Ph); δ_c ($CDCl_3$, 100 MHz): 69.9 (C4), 72.6 (C3), 72.8 (C2), 82.5 (<u>C</u>HPh2), 82.7 (<u>C</u>HPh2), 127.1, 127.2, 127.4, 127.6, 127.8, 127.8, 128.0, 128.1, 128.4, 128.4, 128.6, 128.6 (Ar<u>C</u>H), 140.5, 140.9, 141.1, 141.2 (ArC), 173.5 (C1); LRMS (ESI +ve): 468 (100%, M+NH_4^+), 918 (99%, 2M+NH_4^+), 923 (73%, 2M+Na^+).

2,3-Di-*O*-benzhydryl-L-threono-1,4-lactone, **3.26**



SM: **3.25**, 118 mg, 1.0 mmol (R_f 0.0)

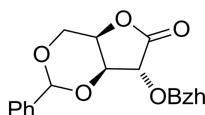
R: 776 mg, 4.0 mmol

P: 448 mg, 97% (R_f 0.65)

A: Colourless glass

HRMS (ESI +ve): $C_{30}H_{26}NaO_4$ found 437.1718; (M+Na^+) requires: 437.1723; $[\alpha]_D^{24}$ +18.0 (*c* 1.49, $CHCl_3$); ν_{max} (thin film): 1794 (s, CO); δ_H ($CDCl_3$, 400 MHz): 3.98 (1H, dd, H4a, $J_{4a,3}$ 6.6, J_{gem} 9.3), 4.16 (1H, dd, H4b, $J_{4b,3}$ 6.6, J_{gem} 9.3), 4.34 (1H, d, H2, $J_{2,3}$ 6.6), 4.43 (1H, q, H3, $J_{3,2}$ = $J_{3,4}$ 6.6), 5.47, 6.16 (2 x 1H, s, Ph2C<u>H</u>), 7.22 - 7.43 (20H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 69.1 (C4), 75.5 (C2), 77.4 (C3), 82.3, 83.3 (Ph2<u>C</u>H), 126.7, 127.1, 127.3, 127.7, 127.8, 127.9, 128.3, 128.3, 128.4, 128.4, 128.7, 128.8 (Ar<u>C</u>H), 139.9, 141.0, 141.1 (ArC), 173.1 (C1); LRMS (ESI +ve): 468 (83%, M+NH_4^+), 918 (100%, 2M+NH_4^+), 923 (77%, 2M+Na^+).

2-*O*-Benzhydryl-4,6-*O*-benzylidene-D-xylono-1,4-lactone, **3.28**



SM: **3.27**, 236 mg, 1 mmol (R_f 0.10, 1:2 EtOAc/CyH)

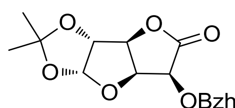
R: 388 mg, 2.0 mmol

P: 387 mg, 96% (R_f 0.45, 1:2 EtOAc/CyH)

A: White crystalline solid

HRMS (ESI +ve): $C_{25}H_{22}NaO_5$ found 425.1361; ($M+Na^+$) requires: 425.1359; $[\alpha]_D^{23}$ +129.1 (*c* 1.02, $CHCl_3$); m.p. 122-124°C; ν_{max} (thin film): 1788 (s, CO); δ_H ($CDCl_3$, 400 MHz): 3.99 (1H, s, H2), 4.11 (1H, dd, H5a, J_{gem} 13.6, $J_{5a,4}$ 2.0), 4.52 (1H, d, H5b, J_{gem} 13.6), 5.55 - 4.58 (2H, m, H3, H4), 5.43 (1H, s, PhC<u>H</u>), 5.79 (1H, s, Ph_2C<u>H</u>), 7.18 - 7.34 (15H, m, Ph); δ_c ($CDCl_3$, 100 MHz): 66.3 (C5), 73.3 (C4), 76.3 (C3), 75.7 (C2), 82.2 (Ph_2<u>C</u>H), 99.6 (Ph<u>C</u>H), 126.2, 126.8, 127.8, 128.4, 128.8, 129.6 (Ar<u>C</u>H), 136.7, 139.5, 140.7 (ArC), 172.7 (C1); LRMS (ESI +ve): 420 (68%, $M+NH_4^+$), 466 (39%, $M+MeCN+Na^+$), 822 (100%, $2M+NH_4^+$), 827 (64%, $2M+Na^+$).

5-*O*-Benzhydryl-1,2-*O*-isopropylidene-D-glucurono-3,6-lactone, **3.30**



SM: **3.29**, 1.08 g, 5.0 mmol (R_f 0.05)

R: 1.46 g, 7.5 mmol

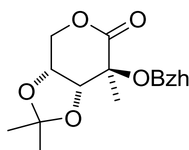
P: 1.47 g, 77% (R_f 0.45)

A: White crystalline solid

HRMS (ESI +ve): $C_{22}H_{22}NaO_6$ found 405.1309; ($M+Na^+$) requires 405.1309; $[\alpha]_D^{18}$ +28.8 (*c* 1.41, $CHCl_3$); m.p. 156-158°C; ν_{max} (thin film): 1803 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.35, 1.52 (2 x 3H, s, Me), 4.28 (1H, d, H5, $J_{5,4}$ 4.3), 4.68 (1H, d, H3, $J_{3,4}$ 3.0), 4.79 (1H, d, H2, $J_{2,1}$ 3.8),

4.84 (1H, dd, H4, $J_{4,3}$ 3.0, $J_{4,5}$ 4.3), 5.96 (1H, s, C<u>H</u>Ph_2), 6.07 (1H, d, H1, $J_{1,2}$ 3.8), 7.26 - 7.47 (20H, m, Ph); δ_c ($CDCl_3$, 100 MHz): 26.5, 26.8 (Me), 73.8 (C5), 77.4 (C4), 81.8 (C3), 82.5 (C2), 83.1 (<u>C</u>HPh_2), 107.0 (C1), 113.1 (<u>C</u>Me_2), 127.3, 127.6, 128.0, 128.2, 128.6, 128.6 (Ar<u>C</u>H), 140.1, 140.2 (ArC), 172.0 (C6); LRMS (ESI +ve): 400 (89%, $M+NH_4^+$), 446 (58%, $M+MeCN+Na^+$), 782 (100%, $2M+NH_4^+$), 787 (95%, $2M+Na^+$).

2-*O*-Benzhydryl-2-*C*-methyl-3,4-*O*-isopropylidene-D-arabinono-1,5-lactone, **3.32**



SM: **3.31**, 100 mg, 0.5 mmol (R_f 0.05)

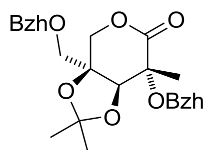
R: 155 mg, 0.8 mmol

P: 173 mg, 95% (R_f 0.65)

A: White crystalline solid

HRMS (ESI +ve): $C_{22}H_{24}NaO_5$ found 391.1516; ($M+Na^+$) requires: 391.1516; $[\alpha]_D^{23}$ -2.5 (*c* 1.02, $CHCl_3$); m.p. 131-133°C; ν_{max} (thin film): 1748 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.35, 1.42 (2 x 3H, s, Me), 1.44 (3H, s, H2’), 4.09 (1H, d, H5a, J_{gem} 12.1), 4.35 (1H, dd, H5b, J_{gem} 12.1, $J_{5b,4}$ 2.0), 4.46 - 4.52 (2H, m, H3, H4), 5.63 (1H, s, Ph_2C<u>H</u>), 7.23 - 7.37 (10H, m, Ph); δ_c ($CDCl_3$, 100 MHz): 18.5 (C2’), 24.0, 26.1 (Me), 68.4 (C5), 72.2 (C4), 78.3 (C2), 78.6 (C3), 79.0 (Ph_2<u>C</u>H), 109.4 (<u>C</u>Me_2), 126.0, 127.4, 127.5, 128.1, 128.4, 128.6 (Ar<u>C</u>H), 141.0, 142.8 (ArC), 169.7 (C1); LRMS (ESI +ve): 386 (89%, $M+NH_4^+$), 432 (92%, $M+MeCN+Na^+$), 754 (98%, $2M+NH_4^+$), 759 (100%, $2M+Na^+$).

2,4'-Di-*O*-benzhydryl-4-*C*-hydroxymethyl-3,4-*O*-isopropylidene-2-*C*-methyl-L-arabinono-1,5-lactone, **3.34**



SM: **3.33**, 232 mg, 1.0 mmol (R_f 0.0)

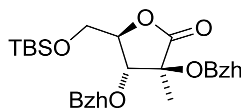
R: 582 mg, 3.0 mmol + 194 mg, 1.0 mmol

P: 449 mg, 80% (R_f 0.70)

A: Colourless glass

HRMS (ESI +ve): $C_{36}H_{36}NaO_6$ found 587.2405; ($M+Na^+$) requires: 587.2404; $[\alpha]_D^{23}$ +1.5 (*c* 1.14, $CHCl_3$); ν_{max} (thin film): 1751 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.39, 1.40 (2 x 3H, s, Me), 1.46 (3H, s, H2'), 3.57 (1H, d, H4'a J_{gem} 10.1), 3.60 (1H, d, H4'b J_{gem} 10.1), 4.04 (1H, d, H5a J_{gem} 11.9), 4.16 (1H, d, H5b J_{gem} 11.9), 4.31 (1H, s, H3), 5.41 (1H, s, 4'-*O*-C<u>H</u>Ph$_2$), 5.62 (1H, s, 2-*O*-C<u>H</u>Ph$_2$), 7.13 - 7.38 (20H, m, Ph); δ_c ($CDCl_3$, 100 MHz): 19.0 (C2'), 26.8, 27.5 (Me), 71.9 (C5), 72.1 (C4'), 78.2 (C2), 79.0 (2-*O*-<u>C</u>HPh$_2$), 80.7 (C3), 81.5 (C4), 84.5 (4'-*O*-<u>C</u>HPh$_2$), 110.5 (<u>C</u>Me$_2$), 125.8, 127.0, 127.1, 127.3, 127.5, 127.7, 127.8, 128.1, 128.4, 128.4, 128.5, 128.7 (Ar<u>C</u>H), 140.8, 141.4, 141.5, 142.8 (ArC), 169.9 (C1); LRMS (ESI +ve): 582 (100%, $M+NH_4^+$), 587 (79%, $M+Na^+$).

2,3-Di-*O*-benzhydryl-5-*O*-*tert*-butyldimethylsilyl-2-*C*-methyl-D-ribono-1,4-lactone, **3.36**



SM: **3.35**, 276 mg, 1.0 mmol (R_f 0.0)

R: 582 mg, 3.0 mmol + 194 mg, 1.0 mmol

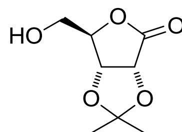
P: 499 mg, 82% (R_f 0.55)

A: Colourless glass

HRMS (ESI +ve): $C_{38}H_{44}NaO_5Si$ found 631.2850; ($M+Na^+$) requires: 631.2850; $[\alpha]_D^{23}$ +59.7 (*c* 1.03, $CHCl_3$); ν_{max} (thin film): 1787 (s, CO); δ_H ($CDCl_3$, 400 MHz): -0.04, -0.01 (2 x 3H, s, Si<u>Me</u>), 0.83 (9H, s, C<u>Me₃</u>), 1.51 (3H, s, H2'), 3.22 (1H, dd, H5a, J_{gem} 11.6, $J_{5a,4}$ 3.3), 3.63 (1H, dd, H5b, J_{gem} 11.6, $J_{5b,4}$ 3.0), 4.13 - 4.17 (1H, m, H4), 4.52 (1H, d, H3, $J_{3,4}$ 6.8), 5.31 (1H, s, 3-*O*-C<u>H</u>Ph_2), 6.03, 1H, s, 2-*O*-C<u>H</u>Ph_2), 7.15 - 7.38 (20H, m, Ph); δ_c ($CDCl_3$, 100 MHz): -5.5, -5.4 (Si<u>C</u>H_3), 18.0 (C2'), 25.8 (C(<u>C</u>H_3)$_3$), 60.8 (C5), 78.0 (C3), 78.5 (2-*O*-<u>C</u>HPh_2), 80.7 (C4), 82.2 (C2), 83.0 (3-*O*-<u>C</u>HPh_2), 126.5, 126.8, 127.0, 127.3, 127.3, 127.4, 127.4, 128.0, 128.3, 128.3, 128.4, 128.5 (Ar<u>C</u>H), 141.5, 141.7, 143.1, 143.6 ArC), 175.1 (C1); LRMS (ESI +ve): 626 (100% $M+NH_4^+$), 672 (70% $M+MeCN+Na^+$).

3.6.2. isoDMDP by Intramolecular S_N2

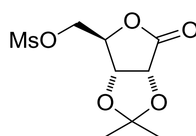
2,3-*O*-Isopropylidene-D-ribono-1,4-lactone, 3.21



Bromine (10.0 mL, 194 mmol) was added dropwise to a solution D-ribofuranose **3.37** (24.6 g, 129 mmol) in water (500 mL) in the presence of barium carbonate (38.5 g, 195 mmol) at 0°C in a covered flask. The reaction mixture was stirred at 0°C for 1.5 h, then at RT for a further 2 h after which TLC analysis (7:3 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.35) and the formation of a single product (R_f 0.50). The mixture was quenched by slow addition of sat. sodium thiosulfate and the aqueous layer extracted with EtOAc (2 x 500 mL, 2 x 250 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was suspended in hot chloroform (500 mL), filtered and concentrated under reduced pressure to afford the title lactone **3.21** (23.3 g, 96%) as a white crystalline solid.

HRMS (ESI +ve): $C_8H_{12}NaO_5$ found 211.0577; ($M+Na^+$) requires 211.0577; $[\alpha]_D^{25}$ -74.0 (*c* 1.21, $CHCl_3$); m.p. 137-140°C; ν_{max} (thin film): 3470 (s, OH), 1775 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.38, 1.47 (2 x 3H, s, Me), 2.87 (1H, s, OH), 3.80 (1H, dd, H5a J_{gem} 12.4, $J_{5a,4}$ 1.5), 4.00 (1H, dd, H5b J_{gem} 12.4, $J_{5b,4}$ 2.3), 4.64 (1H, t, H4 $J_{4,5}$ 2.0), 4.79 (1H, d, H3 $J_{3,2}$ 5.6), 4.84 (1H, d, H2 $J_{2,3}$ 5.6); δ_C ($CDCl_3$, 100 MHz): 25.4, 25.7 (Me), 61.8 (C5), 75.7 (C2), 78.3 (C3), 82.9 (C4), 113.1 ($\underline{C}Me_2$), 175.2 (C1); LRMS (ESI +ve): 211 (68%, $M+Na^+$), 383 (100%, $2M+Li^+$), 399 (92%, $2M+Na^+$); [Lit.[63] m.p. 139°C; $[a]_D^{20}$ -74 (*c* 1, $CHCl_3$)].

2,3-*O*-Isopropylidene-5-*O*-methanesulfonyl-D-ribono-1,4-lactone, 3.38

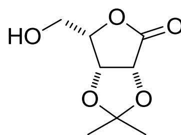


Methanesulfonyl chloride (3.0 mL, 39 mmol) was added dropwise to a solution of lactone **3.21** (4.95 g, 26.3 mmol) in DCM (50 mL) and TEA (5.5 mL, 40 mmol) at 0°C. The reaction mixture was stirred at 0-10°C for 2.5 h after which TLC analysis (3:2 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.45) and the formation of a single product (R_f 0.55). The reaction mixture was diluted with DCM (100 mL) and water (150 mL) added. The organic layer was collected and the aqueous layer extracted with DCM (3 x 100 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:9 to 1:1 EtOAc/CyH) afforded the title mesylate **3.38** (6.72 g, 96%) as a white crystalline solid.

HRMS (ESI +ve): $C_9H_{14}NaO_7S$ found 289.0353; ($M+Na^+$) requires 289.0352; $[\alpha]_D^{25}$ -54.6 (*c* 1.17, $CHCl_3$); m.p. 64-66°C; ν_{max} (thin film): 1788 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.40, 1.49 (2 x 3H, s, $C\underline{Me_2}$), 3.06 (3H, s, $SO_2\underline{Me}$), 4.45 (1H, dd, H5a J_{gem} 11.4, $J_{5a,4}$ 2.3), 4.47 (1H, dd, H5b J_{gem} 11.6, $J_{5b,4}$ 2.3), 4.79-4.81 (2H, m, H3, H4), 4.83 (1H, d, H2 $J_{2,3}$ 5.6); δ_C ($CDCl_3$, 100 MHz): 25.5, 26.6 ($C\underline{Me_2}$), 37.6 ($SO_2\underline{Me}$), 68.2 (C5), 75.0 (C2), 77.4, 79.2 (C3, C4), 114.0 ($\underline{C}Me_2$),

173.2 (C1); LRMS (ESI +ve): 289 (75%, $M+Na^+$), 555 (100%, $2M+Na^+$); Lit.[63] 66-67°C; $[a]_D^{20}$ -52 (*c* 1, $CHCl_3$)].

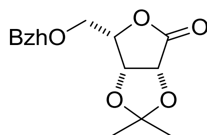
2,3-*O*-Isopropylidene-L-lyxono-1,4-lactone, 3.40



A solution of potassium hydroxide (2.12 g, 37.9 mmol) in water (65 mL) was added to a solution of mesylate **3.38** (3.30 g, 12.4 mmol) in 1,4-dioxane (65 mL) at RT. TLC analysis (EtOAc) after 1 h showed the complete consumption of starting material (R_f 0.90) and baseline material (R_f 0.00); LRMS did not reveal evidence of any mesylated species. The solution was acidified with glacial acetic acid (15 mL) and TLC analysis (5:3:2 n-BuOH/EtOH/H_2O) revealed an intermediate (R_f 0.40), the complete consumption of which was observed after 3 d at RT, accompanied by the formation of a single product (R_f 0.80). The mixture was concentrated under reduced pressure and the crude residue partitioned between water (50 mL) and EtOAc (50 mL). The organic layer was collected and the aqueous extracted with EtOAc (3 x 50 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:4 to 7:3 EtOAc/CyH) afforded the title lactone **3.40** (1.37 g, 59%) as a white crystalline solid.

HRMS (ESI +ve): $C_8H_{12}NaO_5$ found 211.0575; ($M+Na^+$) requires 211.0577; $[\alpha]_D^{25}$ -89.5 (*c* 0.89, Me_2CO); m.p. 98-100°C; ν_{max} (thin film): 3454 (br, m, OH), 1783 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.40, 1.48 (2 x 3H, s, Me), 2.41 (1H, a-t, OH $J_{OH,5}$ 6.3), 3.96 (1H, ddd, H5a J_{gem} 12.4, $J_{5a,4}$ 5.3, $J_{5a,OH}$ 7.3), 4.03 (1H, ddd, H5b J_{gem} 12.4, $J_{5b,4}$ 6.4, $J_{5b,OH}$ 4.9), 4.64 (1H, ddd, H4 $J_{4,3}$ 3.5, $J_{4,5a}$ 5.3, $J_{4,5b}$ 6.6 $J_{4,5}$ 2.0), 4.87 (1H, d, H2 $J_{3,2}$ 5.4), 4.89 (1H, dd, H3 $J_{3,2}$ 5.6, $J_{3,4}$ 3.4); δ_C ($CDCl_3$, 100 MHz): 25.7, 26.6 (Me), 60.8 (C5), 76.0, 76.1 (C2, C3), 79.1 (C4), 114.5 ($\underline{C}Me_2$), 173.5 (C1); LRMS ESI +ve): 211 (57%, $M+Na^+$), 243 (70%, $M+MeOH+Na^+$), 385 (100%), 399 (59%, $2M+Na^+$); [Lit.[54] m.p. 98-99°C, $[\alpha]_D^{20}$ -89 (*c* 1.0, Me_2CO)].

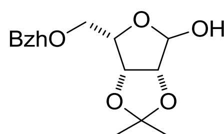
5-*O*-Benzhydryl-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone, 3.41



Diphenyldiazomethane (2.29 g, 11.8 mmol) was added to a refluxing solution of lactone **3.40** (1.37 g, 7.29 mmol) in toluene (150 mL). The reaction mixture was stirred at reflux for 1 h after which no purple colour remained and TLC analysis (1:1 EtOAc/CyH) revealed a trace of starting material (R_f 0.10) and a major product (0.70). The reaction mixture was concentrated under reduced pressure and the residue purified by flash column chromatography (1:49 to 1:3 EtOAc/CyH) to afford the title lactone **3.41** (2.09 g, 81%) as a colourless oil.

HRMS (ESI +ve): $C_{22}H_{26}NaO_6$ found 409.1628; ($M+MeOH+Na^+$) requires 409.1622; $[\alpha]_D^{25}$ -37.9 (*c* 1.80, $CHCl_3$); ν_{max} (thin film): 1789 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.37, 1.41 (2 x 3H, s, Me), 3.81 (1H, dd, H5a J_{gem} 10.8, $J_{5a,4}$ 7.1), 3.87 (1H, dd, H5b J_{gem} 10.8, $J_{5b,4}$ 5.1), 4.71 (1H, ddd, H4 $J_{4,3}$ 3.0, $J_{4,5a}$ 7.1, $J_{4,5b}$ 5.1), 4.79-4.83 (2H, m, H2, H3), 5.47 (1H, s, C<u>H</u>Ph_2), 7.25-7.39 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 25.9, 26.7 (Me), 66.8 (C5), 75.9 (C4), 76.0, 78.2 (C2, C3), 84.4 (<u>C</u>HPh_2), 114.2 (<u>C</u>Me_2), 126.9, 127.2, 127.7, 127.7, 128.5, 128.5 (Ar<u>C</u>H), 141.4, 141.5 (ArC), 173.7 (C1); LRMS: 795 (100%, $2M+2MeOH+Na^+$).

5-*O*-Benzhydryl-2,3-*O*-isopropylidene-L-lyxofuranose, 3.42

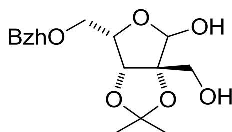


DIBAL solution (1.5 M in toluene, 5.0 mL, 7.5 mmol) was added dropwise to a solution of lactone **3.41** (2.09 g, 5.90 mmol) in DCM (25 mL) at -78°C and the mixture stirred at this temperature for 90 min. TLC analysis (9:1 PhMe/acetone) revealed the complete consumption of starting material (0.55) and the formation of a single product (0.45). The mixture was diluted with DCM (30 mL) and stirred with sat. potassium sodium tartrate (50 mL) at RT for 1 h. The

organic layer was collected and the aqueous extracted with DCM (4 x 40 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:99 to 1:9 acetone/PhMe) afforded the title furanose **3.42** (2.03 g, 97%) as a colourless oil in a 17:1 ratio of anomers, which crystallised on standing.

HRMS (ESI +ve): $C_{21}H_{24}NaO_5$ found 379.1518; ($M+Na^+$) requires 379.1516; $[\alpha]_D^{25}$ +3.1 (*c* 1.56, $CHCl_3$); m.p. 68-71°C; ν_{max} (thin film): 3423 (br, m, OH); δ_H ($CDCl_3$, 400 MHz), major anomer only: 1.30, 1.40 (2 x 3H, s, Me), 2.87 (1H, d, OH $J_{OH,1}$ 1.8), 3.73 (1H, dd, H5a J_{gem} 10.4, $J_{5a,4}$ 7.3), 3.81 (1H, dd, H5b J_{gem} 10.4, $J_{5b,4}$ 4.0), 4.46 (1H, dt, H4 $J_{4,3}$ 3.8, $J_{4,5a}$ 7.3, $J_{4,5b}$ 3.8), 4.59 (1H, d, H2 $J_{2,3}$ 5.8), 4.75 (1H, dd, H3 $J_{3,2}$ 5.8, $J_{3,4}$ 3.8), 5.41 (1H, d, H1 $J_{1,OH}$ 1.8), 5.46 (1H, s, C<u>H</u>Ph$_2$), 7.23-7.40 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 24.8, 26.0 (Me), 67.4 (C5), 79.3 (C4), 80.1 (C3), 84.2 (<u>C</u>HPh$_2$), 85.3 (C2), 101.2 (C1), 112.5 (<u>C</u>Me$_2$), 127.0, 127.2, 127.4, 127.5, 128.3, 128.3 (Ar<u>C</u>H), 141.9, 142.0 (ArC); LRMS (ESI -ve): 355 (100%, $M-H^+$), 711 (95%, $2M-H^+$).

5-*O*-Benzhydryl-2-*C*-hydroxymethyl-2,3-*O*-isopropylidene-L-lyxofuranose, 3.43

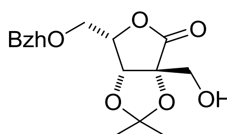


Potassium carbonate (345 mg, 2.49 mmol) was added to a mixture of 37% aqueous formaldehyde (4.0 mL, 53 mmol) and a solution of L-lyxofuranose **3.42** (590 mg, 1.66 mmol) in methanol (6 mL) and heated at reflux. TLC analysis (1:1 EtOAc/CyH) after 4 h revealed a major product (R_f 0.40), trace starting material (R_f 0.70) and a minor component (0.05). The reaction mixture was cooled to RT, neutralised with glacial acetic acid and concentrated under reduced pressure. The crude residue was partitioned between EtOAc (20 mL) and sat. sodium bicarbonate (20 mL). The aqueous was discarded and the organic washed sequentially with sat. sodium bicarbonate (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:9 to 1:1

EtOAc/CyH) afforded the title furanose **3.43** (502 mg, 78%) as a white foam in a 3:1 ratio of anomers.

HRMS (ESI +ve): $C_{22}H_{26}NaO_6$ found 409.1623; (M+Na$^+$) requires 409.1622; $[\alpha]_D^{25}$ +8.3 (*c* 0.54, $CHCl_3$); ν_{max} (thin film): 3445 (br, m, OH); δ_H ($CDCl_3$, 400 MHz): 1.36, 1.39 (2 x 3H, s, Me^A), 1.45, 1.49 (2 x 3H, s, Me^B), 2.06 (1H, dd, OH2'^{B} $J_{OH,2'}$ 5.3, 6.6), 2.64 (1H, dd, OH2'^{A} $J_{OH,2'}$ 6.1, 7.6), 3.70-3.81 (7H, m, H2'a$^{A\&B}$, H2'b^B, H5a$^{A\&B}$, H5b$^{A\&B}$), 3.84-3.87 (1H, m, H4^B), 3.86 (1H, d, OH1^B $J_{OH,1}$ 11.9), 3.91 (1H, dd, H2'b^A J_{gem} 11.9, $J_{2'b,OH}$ 6.1), 3.96 (1H, d, OH1^A $J_{OH,1}$ 2.5), 4.48 (1H, dt, H4 $J_{5,4}$ 3.5, $J_{5,6}$ 3.5, 7.1), 4.59 (1H, d, H3^B $J_{4,5}$ 2.0), 4.61 (1H, d, H3^A $J_{3,4}$ 3.0), 4.88 (1H, d, H1^B $J_{1,OH}$ 11.9), 5.40 (1H, d, H1^A $J_{1,OH}$ 2.5), 5.44 (2H, s, C$\underline{H}$Ph$_2^{A\&B}$), 7.23-7.39 (20H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 27.0, 27.1 (Me^B), 27.3, 27.5 (Me^A), 62.7 (C2'^{B}), 63.6 (C2'^{A}), 66.6 (C5^B), 67.3 (C5^A), 75.0 (C4^B), 80.0 (C4^A), 82.1 (C3^B), 83.2 (C3^A), 84.2 ($\underline{C}$HPh$_2^B$), 84.3 ($\underline{C}$HPh$_2^A$), 89.5 (C2^B), 93.5 (C2^A), 97.4 (C1^B), 103.7 (C1^A), 113.7 ($\underline{C}$Me$_2^A$), 113.9 ($\underline{C}$Me$_2^B$), 127.0, 127.1, 127.1, 127.1, 127.5, 127.5, 127.6, 128.3, 128.4, 128.4 (Ar$\underline{C}$H), 141.7, 141.8, 141.8, 141.9 (ArC); LRMS (ESI +ve): 409 (100%, M+Na$^+$), 795 (99%, 2M+Na$^+$).

5-*O*-Benzhydryl-2-*C*-hydroxymethyl-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone, 3.44

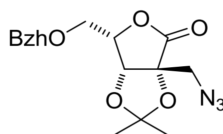


Potassium carbonate (1.1 g, 8.0 mmol) and iodine (2.0 g, 7.9 mmol) were added to a hot solution of L-lyxofuranose **3.43** (1.54 g, 3.99 mmol) in *tert*-butanol (15 mL) and the mixture heated at reflux. TLC analysis (2:3 EtOAc/CyH) after 1 h revealed the complete consumption of starting material (R_f 0.35) and the formation of a single product (R_f 0.50). Heating was ceased and sat. sodium thiosulfate (~5 mL) added dropwise, followed by EtOAc (25 mL). The mixture was stirred vigorously until the iodine was visibly quenched. The organic layer was collected and the remaining aqueous suspension extracted with EtOAc (4 x 25 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification

by flash column chromatography (1:9 to 1:2 EtOAc/CyH) afforded the title lactone **3.44** (1.31 g, 86%) as a white crystalline solid.

HRMS (ESI +ve): $C_{22}H_{24}NaO_6$ found 407.1466; (M+Na$^+$) requires 407.1465; $[\alpha]_D^{25}$ -28.3 (*c* 0.98, $CHCl_3$); m.p. 132-134°C; ν_{max} (thin film): 3478 (br, m, OH), 1786 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.38, 1.40 (2 x 3H, s, Me), 2.30 (1H, dd, OH $J_{OH,2'a}$ 4.5, $J_{OH,2'b}$ 7.6), 3.81 (1H, dd, H5a J_{gem} 10.6, $J_{5a,4}$ 6.8), 3.86 (1H, dd, H5b J_{gem} 10.6, $J_{5b,4}$ 4.9), 3.90 (1H, dd, H2'a J_{gem} 11.5, $J_{2'a,OH}$ 4.2), 3.96 (1H, dd, H2'b J_{gem} 11.5, $J_{2'b,OH}$ 7.6), 4.74 (1H, ddd, H4 $J_{4,3}$ 3.7, $J_{4,5a}$ 6.8, $J_{4,5b}$ 4.8), 4.77 (1H, d, H3 $J_{3,2}$ 3.5), 5.46 (1H, s, C<u>H</u>Ph_2), 7.23-7.37 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 26.3, 26.9 (Me), 61.1 (C2'), 66.6 (C5), 78.4, 78.6 (C2, C3), 84.3 (<u>C</u>HPh_2), 85.9 (C2), 113.7 (<u>C</u>Me_2), 126.9, 127.1, 127.6, 127.7, 128.4, 128.4 (Ar<u>C</u>H), 141.4, 141.5 (ArC), 175.3 (C1); LRMS (ESI +ve): 407 (100%, M+Na$^+$), 791 (78%, 2M+Na$^+$).

2-*C*-Azidomethyl-5-*O*-benzhydryl-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone, 3.45

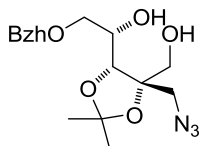


Trifluoromethanesulfonic anhydride (2.0 mL, 12 mmol) was added dropwise to a solution of lactone **3.44** (3.54 g, 9.22 mmol) in DCM (50 mL) and pyridine (2.0 mL, 26 mmol) at -40°C and the mixture stirred at -30°C for 1 h. TLC analysis (2:3 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.45) and a single product (R_f 0.75). The reaction mixture was diluted with DCM (80 mL), washed with 2 M HCl (3 x 100 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the triflate derivative. Sodium azide (750 mg, 11.5 mmol) was added to a solution of the triflate in DMF (50 mL) and the mixture stirred at RT for 1 h. TLC analysis (1:4 EtOAc/CyH) revealed the complete consumption of the triflate (R_f 55) and the formation of a single product (R_f 0.45). The reaction mixture was diluted with EtOAc (120 mL), washed with 1:1 brine/water (3 x 100 mL) and brine (50 mL), dried over $MgSO_4$,

filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:49 to 7:43 EtOAc/CyH) afforded the title azide **3.45** (3.67 g, 97%) as a colourless gum.

HRMS (ESI +ve): $C_{22}H_{23}N_3NaO_5$ found 432.1530; (M+Na$^+$) requires 432.1530; $[\alpha]_D^{25}$ +3.5 (*c* 1.04, $CHCl_3$); ν_{max} (thin film): 2110 (s, N_3), 1786 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.40, 1.44 (2 x 3H, s, Me), 3.55 (1H, d, H2'a J_{gem} 12.9), 3.81 (1H, dd, H5a J_{gem} 10.6, $J_{5a,4}$ 6.6), 3.81 (1H, d, H2'b J_{gem} 12.9), 3.85 (1H, dd, H5b J_{gem} 10.6, $J_{5b,4}$ 5.1), 4.68 (1H, ddd, H4 $J_{4,3}$ 3.5, $J_{4,5a}$ 6.6, $J_{4,5b}$ 5.1), 4.72 (1H, d, H3 $J_{3,4}$ 3.5), 5.46 (1H, s, C<u>H</u>Ph_2), 7.24-7.38 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 26.1, 26.8 (Me), 50.4 (C2'), 66.5 (C5), 78.1 (C4), 78.4 (C3), 84.4 (<u>C</u>HPh_2), 84.9 (C2), 114.3 <u>C</u>Me_2), 126.9, 127.1, 127.7, 127.7, 128.4, 128.4 (Ar<u>C</u>H), 141.3, 141.4 (ArC), 174.0 (C1); LRMS (ESI -ve): 444 (100%, M+Cl$^-$).

2-*C*-Azidomethyl-5-*O*-benzhydryl-2,3-*O*-isopropylidene-L-lyxitol, 3.46

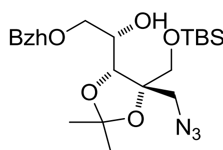


Sodium borohydride (65 mg, 1.7 mmol) was added to a solution of lactone **3.45** (233 mg, 0.570 mmol) in ethanol (5 mL) at 0°C and stirred at RT for 1 h. TLC analysis (1:2 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.65), and the formation of product (R_f 0.00). The reaction mixture was quenched with sat. ammonium chloride, diluted with brine (10 mL) and extracted with EtOAc (3 x 10 mL). TLC analysis (1:2 EtOAc/CyH) at this point revealed a major component (R_f 0.25) in the organic fractions which were combined, dried over $MgSO_4$, filtered and concentrated. Purification by falsh column chromatography (1:9 to 3:2 EtOAc/CyH) afforded the title diol **3.46** (194 mg, 82%) as a colourless oil.

HRMS (ESI +ve): $C_{22}H_{27}N_3NaO_5$ found 436.1842; (M+Na$^+$) requires 436.1843; $[\alpha]_D^{25}$ +71.7 (*c* 0.81, $CHCl_3$); ν_{max} (thin film): 3418 (br, m, OH), 2103 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.43, 1.48 (2 x 3H, s, Me), 2.96 (1H, d, OH4 $J_{OH,4}$ 5.3), 3.18 (1H, t, OH1 $J_{OH,1}$ 7.2), 3.33 (1H, d, H2'a J_{gem}

13.4), 3.50 (1H, dd, H1a J_{gem} 12.1, $J_{1a,OH}$ 7.3), 3.58 (1H, d, H2'b J_{gem} 13.4), 3.59 (1H, dd, H5a J_{gem} 9.5, $J_{5a,4}$ 5.3), 3.68 (1H, dd, H5b J_{gem} 9.3, $J_{5b,4}$ 7.3), 3.77 (1H, dd, H1b J_{gem} 12.1, $J_{1b,OH}$ 7.3), 4.08-4.13 (1H, m, H4), 4.13 (1H, s, H3), 5.43 (1H, s, C<u>H</u>Ph$_2$), 7.27-7.37 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 26.1, 28.2 (Me), 53.2 (C2'), 63.1 (C1), 67.1 (C4), 71.1 (C5), 77.0 (C3), 83.2 (C2), 84.4 (<u>C</u>HPh$_2$), 108.7 (<u>C</u>Me$_2$), 126.9, 126.9, 127.7, 127.8, 128.5, 128.6 (Ar<u>C</u>H), 141.4, 141.6 (ArC); LRMS (ESI +ve): 436 (100%, M+Na$^+$), 849 (99%, 2M+Na$^+$).

2-*C*-Azidomethyl-5-*O*-benzhydryl-1-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-L-lyxitol, 3.47

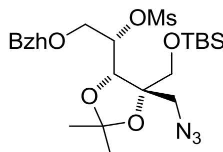


tert-Butyldimethylsilyl chloride (150 mg, 0.995 mmol) was added to a solution of L-lyxitol **3.46** (363 mg, 0.879 mmol) and imidazole (125 mg, 1.84 mmol) in DMF (5 mL) and stirred at RT for 16 h. TLC anaysis (1:2 EtOAc/CyH) revealed a trace of starting material (R_f 0.23) and the formation of major (R_f 0.75) and minor (R_f 0.85) products. The reaction mixture was diluted with EtOAc (20 mL), washed with 1:1 brine/water (3 x 20 mL) and the organic fraction was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:99 to 1:9 EtOAc/CyH) afforded the title silyl ether **3.47** (369 mg, 80%) as a colourless oil, as well as di-silylated material (60 mg, 11%).

HRMS (ESI +ve): $C_{28}H_{41}N_3NaO_5Si$ found 550.2708; (M+Na$^+$) requires 550.2708; $[\alpha]_D^{25}$ +19.5 (*c* 1.13, $CHCl_3$); ν_{max} (thin film): 3475 (br, m, OH), 2104 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.07 (6H, s, Si<u>Me</u>$_2$), 0.87 (9H, s, C<u>Me</u>$_3$), 1.46 (6H, s, C<u>Me</u>$_2$), 3.11 (1H, d, OH $J_{OH,4}$ 5.3), 3.29 (1H, d, H2'a J_{gem} 12.9), 3.44 (1H, d, H2'b J_{gem} 12.9), 3.51 (1H, d, H1a J_{gem} 10.4), 3.60 (1H, dd, H5a J_{gem} 9.6, $J_{5a,4}$ 6.1), 3.65 (1H, dd, H5b J_{gem} 9.6, $J_{5b,4}$ 5.3), 3.85 (1H, d, H1b J_{gem} 10.4), 4.04-4.11 (1H, m, H4), 4.23 (1H, d, H3 $J_{3,4}$ 3.8), 5.41 (1H, s, C<u>H</u>Ph$_2$), 7.24-7.37 (10H, m, Ph); δ_C ($CDCl_3$, 100

MHz): -5.7, -5.7 (Si<u>Me</u>$_2$), 18.1 (<u>C</u>Me$_3$), 25.8 (C<u>Me</u>$_3$), 26.1, 28.4 (C<u>Me</u>$_2$), 53.8 (C2'), 62.8 (C1), 67.3 (C4), 70.3 (C5), 78.2 (C3), 82.9 (C2), 84.3 (<u>C</u>HPh$_2$), 108.3 (<u>C</u>Me$_2$), 126.8, 127.1, 127.4, 127.7, 128.3, 128.4 (Ar<u>C</u>H), 141.8, 141.9 (ArC); LRMS (ESI +ve): 550 (100%, M+Na$^+$).

2-*C*-Azidomethyl-5-*O*-benzhydryl-1-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-4-*O*-methanesulfonyl-L-lyxitol, 3.48

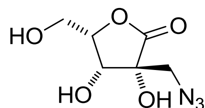


Methanesulfonyl chloride (0.03 mL, 0.4 mmol) was added dropwise to a solution of L-lyxitol **3.47** (122 mg, 0.231 mmol) and triethylamine (0.06 mL, 0.4 mmol) in DCM (1 mL) at 0°C and stirred at RT for 2 h. TLC analysis (9:1 PhMe/acetone) revealed the complete consumption of starting material (R_f 0.70) and the formation of a single product (R_f 0.75). The reaction mixture was diluted with DCM (10 mL) and washed with water (10 mL). The aqueous was extracted with DCM (2 x 5mL) and the combined organic fractions dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (99:1 to 24:1 PhMe/acetone) afforded the title mesylate **3.48** (140 mg, quant.) as a colourless oil.

HRMS (ESI +ve): $C_{29}H_{43}N_3NaO_7SSi$ found 628.2483; (M+Na$^+$) requires 628.2483; $[\alpha]_D^{25}$ +8.2 (*c* 0.85, $CHCl_3$); m.p. 74-76°C; ν_{max} (thin film): 2104 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.01, 0.03 (2 x 3H, s, Si<u>Me</u>$_2$), 0.81 (9H, s, C<u>Me</u>$_3$), 1.43, 1.45 (2 x 3H, s, C<u>Me</u>$_2$), 3.07 (3H, s, SO$_2$<u>Me</u>), 3.22 (1H, d, H2'a J_{gem} 13.1), 3.38 (1H, d, H2'b J_{gem} 13.1), 3.45 (1H, d, H1a J_{gem} 10.6), 3.72 (1H, d, H1b J_{gem} 10.9), 3.73 (1H, d, H5a J_{gem} 11.4), 3.85 (1H, dd, H5b J_{gem} 11.4, $J_{5b,4}$ 3.3), 4.52 (1H, d, H3 $J_{3,4}$ 9.6), 4.99-5.03 (1H, m, H4), 5.37 (1H, s, C<u>H</u>Ph$_2$), 7.23-7.40 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): -5.9, -5.8 (Si<u>Me</u>$_2$), 18.1 (<u>C</u>Me$_3$), 25.7 (C<u>Me</u>$_3$), 26.0, 28.3 (C<u>Me</u>$_2$), 38.8 (SO$_2$<u>Me</u>), 53.3 (C2'), 62.9 (C1), 68.9 (C5), 76.5 (C3), 79.9 (C4), 83.4 (C2), 84.7 (<u>C</u>HPh$_2$), 108.3 (<u>C</u>Me$_2$), 126.6,

127.4, 127.5, 128.0, 128.3, 128.6 (ArCH), 141.1, 141.5 (ArC); LRMS (ESI +ve): 628 (100%, $M+Na^+$).

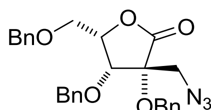
2-*C*-Azidomethyl-L-lyxono-1,4-lactone, 3.53



Lactone **3.45** (978 mg, 2.39 mmol) was dissolved in pre-mixed 20:1 MeOH/AcCl (21 mL) and stirred at 80°C for 18 h. TLC analysis (1:3 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.70), the formation of BzhOMe (R_f 0.80) and baseline material (R_f 0.00). The reaction mixture was concentrated to dryness, agitated with diethyl ether (~5 mL) and the mixture left to stand until crystals had formed. The ether was decanted and the remaining white crystalline solid triturated with ether until BzhOMe could no longer be detected in the organic by TLC anaysis. The crystals were dried under reduced pressure, giving the title lactone **3.53** (447 mg, 92%).

HRMS (ESI +ve): $C_6H_9N_3NaO_5$ found 226.0435; ($M+Na^+$) requires 226.0434; $[\alpha]_D^{25}$ -43.9 (*c* 0.77, MeOH); m.p. 130-132°C; ν_{max} (thin film): 3423 (br, m, OH), 2115 (s, N_3), 1773 (s, CO); δ_H (CD_3OD, 400 MHz): 3.46 (1H, d, H2'a J_{gem} 13.0), 3.55 (1H, d, H2' J_{gem} 13.1), 3.86 (2H, d, H5 $J_{5,4}$ 5.1), 4.33 (1H, d, H3 $J_{3,4}$ 4.8), 4.57 (1H, q, H4 $J_{4,3} = J_{4,5}$ 4.9); δ_C (CD_3OD, 100 MHz): 54.2 (C2'), 61.2 (C5), 71.3 (C3), 78.0 (C2), 82.7 (C4), 177.3 (C1); LRMS (ESI +ve): 226 (100%, $M+Na^+$).

2-*C*-Azidomethyl-2,3,5-tri-*O*-benzyl-L-lyxono-1,4-lactone, 3.54

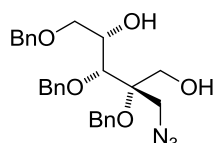


Sodium hydride (60% in mineral oil, 170 mg, 4.25 mmol) was added to a solution of lactone **3.53** (286 mg, 1.41 mmol) and benzyl bromide (1.7 mL, 14.3 mmol) in DMF (10 mL) in the

presence of molecular sieves at 0°C. TLC analysis (1:2 EtOAc/CyH) after 2 h at 0°C revealed the complete consuption of starting materal (R_f 0.0), major (R_f 0.65) and minor (R_f 0.15) products. A further portion of sodium hydride (30 mg, 0.75 mmol) was added, TLC analysis after a further 1 h showed persistence of the minor component, a further portion of sodium hydride (30 mg, 0.75 mmol) was added and TLC analysis after a further 1 h showed near complete consumption of the minor component. The pH was adjusted to ~6 with glacial acetic acid and the mixture diltuted with EtOAc (50 mL), washed with 1:1 brine/water (2 x 50 mL, 2 x 20 mL) and brine (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:49 to 3:22 EtOAc/CyH) afforded the title perbenzylated lactone **3.54** (556 mg, 83%) as a colourless oil.

HRMS (ESI +ve): $C_{27}H_{27}N_3NaO_5$ found 496.1844; ($M+Na^+$) requires 496.1843; $[\alpha]_D^{25}$ +10.6 (*c* 1.73, $CHCl_3$); ν_{max} (thin film): 2106 (s, N_3), 1779 (s, CO); δ_H ($CDCl_3$, 400 MHz): 3.39 (1H, d, H2'a J_{gem} 12.9), 3.68 (1H, d, H2'b J_{gem} 12.9), 3.80 (1H, dd, H5a J_{gem} 10.9, $J_{5a,4}$ 7.1), 3.84 (1H, dd, H5b J_{gem} 10.9, $J_{5b,4}$ 4.8), 4.32 (1H, d, H3 $J_{3,4}$ 5.6), 4.51 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.9), 4.57 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.6), 4.58 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.9), 4.61-4.65 (1H, m, H4), 4.77 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.6), 4.90 (2H, s, C<u>H</u>$_2$Ph), 7.18-7.37 (15H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 52.9 (C2'), 68.1 (C5), 69.7, 73.6, 74.3 (<u>C</u>H$_2$Ph), 77.8 (C3), 79.5 (C4), 81.4 (C2), 127.5, 127.7, 127.8, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5 (Ar<u>C</u>H), 136.8, 137.4. 137.5 (ArC), 171.7 (C1); LRMS (ESI +ve): 491 (54%, $M+NH_4^+$), 496 (100%, $M+Na^+$), 969 (79%, $2M+Na^+$).

2-*C*-Azidomethyl-2,3,5-tri-*O*-benzyl-L-lyxitol, 3.55

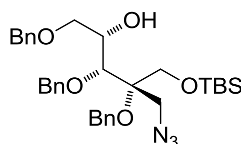


Sodium borohydride (215 mg, 5.69 mmol) was added to a solution of lactone **3.54** (894 mg, 1.89 mmol) in EtOH (12 mL) at 0°C. The reaction mixture was then stirred at RT for 3 h after which

TLC analysis (1:2 EtOAc/CyH) revealed the complete consumption of starting materal (R_f 0.70) and the formation of product (R_f 0-0.45 streak). The reaction mixture was quenched with sat. ammonium chloride, diluted with brine (30 mL) and extracted with EtOAc (3 x 30 mL). TLC analysis (1:2 EtOAc/CyH) of the combined organic fractions at this stage revealed a single product (R_f 0.35). The organic was dried over $MgSO_4$, filtered, concentrated and purified by flash column chromatography (1:9 to 3:7 EtOAc/CyH) to give the title diol **3.55** (839 mg, 93%) as a colourless oil.

HRMS (ESI +ve): $C_{27}H_{31}N_3NaO_5$ found 500.2159; ($M+Na^+$) requires 500.2156; $[\alpha]_D^{25}$ +13.6 (*c* 0.97, $CHCl_3$); ν_{max} (thin film): 3418 (m, OH), 2103 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 2.84 (1H, d, OH4 $J_{OH,4}$ 8.1), 3.01 (1H, dd, OH1 $J_{OH,1a}$ 7.8, $J_{OH,1b}$ 4.5), 3.43 (1H, dd, H5a J_{gem} 9.3, $J_{5a,4}$ 6.8), 3.53 (1H, dd, H5b J_{gem} 9.3, $J_{5b,4}$ 5.8), 3.71 (1H, d, H2'a J_{gem} 13.6), 3.79 (1H, d, H2'b J_{gem} 13.6), 3.81 (1H, dd, H1a J_{gem} 12.1, $J_{1a,OH}$ 7.6), 3.99 (1H, s, H3), 4.02 (1H, dd, H1b J_{gem} 12.1, $J_{1b,OH}$ 4.3), 4.07 (1H, a-q, H4 *J* 6.8), 4.48 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.9), 4.55 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.9), 4.57 (1H, d, C<u>H</u>$_2$Ph J_{gem} 10.9), 4.68 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.1), 4.72 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.1), 4.78 (1H, d, C<u>H</u>$_2$Ph J_{gem} 10.9), 7.21-7.33 (15H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 51.7 (C2'), 61.5 (C1), 65.3 (<u>C</u>H$_2$Ph), 68.1 (C4), 71.9 (C5), 73.4 (<u>C</u>H$_2$Ph), 75.8 (<u>C</u>H$_2$Ph), 78.6 (C3), 81.7 (C2), 127.5, 127.6, 127.9, 127.9, 128.0, 128.2, 128.4, 128.5, 128.5 (Ar<u>C</u>H), 137.6, 137.7, 138.2 (ArC); LRMS (ESI -ve): 512 (96%, $M+^{35}Cl^-$), 522 (74%, $M+HCO_2^-$), 953 (100%, $2M-H^+$).

2-*C*-Azidomethyl-2,3,5-tri-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-L-lyxitol, 3.56

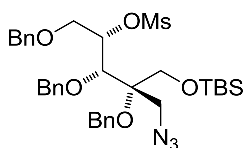


TBSCl (190 mg, 1.26 mmol) was added to a solution of L-lyxitol **3.55** (201 mg, 0.421 mmol) and imidazole (190 mg, 2.79 mmol) in DMF (3 mL) at 0°C. The reaction mixture was stirred at this temperature for 5 h after which TLC anaysis (1:2 EtOAc/CyH revealed complete consumption of

starting materal (R_f 0.35) and the formation of a single product (R_f 0.75). The mixture was diluted with EtOAc and the organic washed with 1:1 brine water (3 x 20 mL), dried over $MgSO_4$ and concentrated under reduced presure. Purification by flash column chromatography (1:99 to 1:9 EtOAc/CyH) afforded the title silyl ether **3.56** (233 mg, 94%) as a colourless oil.

HRMS (ESI +ve): $C_{33}H_{45}N_3NaO_5Si$ found 614.3025; ($M+Na^+$) requires 614.3021; $[\alpha]_D^{25}$ -2.9 (*c* 1.11, $CHCl_3$); ν_{max} (thin film): 3418 (m, OH), 2103 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.08, 0.10 (2 x 3H, s, SiMe2), 0.93 (9H, s, $SiCMe_3$), 3.01 (1H, d, OH $J_{OH,4}$ 6.6), 3.47 (1H, dd, H5a J_{gem} 9.1, $J_{5a,4}$ 7.3), 3.52 (1H, dd, H5b J_{gem} 9.3, $J_{5b,4}$ 5.6), 3.56 (1H, d, H2'a J_{gem} 13.1), 3.83 (1H, d, H2'b J_{gem} 13.1), 3.90 (1H, d, H1a J_{gem} 11.1), 3.99 (1H, d, H1b J_{gem} 11.1), 4.11 (1H, s, H3), 4.20 (1H, a-q, H4 *J* 6.6), 4.47 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.9), 4.55 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.9), 4.62 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.1), 4.69 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.1), 4.71 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.1), 4.81 (1H, d, C<u>H</u>$_2$Ph J_{gem} 11.1), 7.24-7.36 (15H, m, Ph); δ_C ($CDCl_3$, 100 MHz): -5.6, -5.8 (Si<u>Me</u>$_2$), 18.1 (Si<u>C</u>Me$_3$), 25.9 (SiC<u>Me</u>$_3$), 52.1 (C2'), 63.0 (C1), 66.4 (<u>C</u>H$_2$Ph), 67.8 (C4), 71.9 (C5), 73.2 (<u>C</u>H$_2$Ph), 75.2 (<u>C</u>H$_2$Ph), 76.8 (C3), 82.0 (C2), 127.4, 127.7, 127.7, 127.8, 128.3, 128.4, 128.4 (Ar<u>C</u>H), 138.0, 138.0, 138.6 (ArC); LRMS (ESI -ve): 626 (100%, $M+{}^{35}Cl^-$).

2-*C*-Azidomethyl-2,3,5-tri-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-4-*O*-methanesulfonyl-L-lyxitol, 3.57

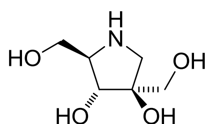


Methanesulfonyl chloride (60 μL, 0.77 mmol) was added dropwise to a solution of L-lyxitol **3.56** (233 mg, 0.394 mmol) and pyridine (0.15 mL, 1.9 mmol) in DCM (3 mL) at room temperature. TLC analysis (19:1 PhMe/acetone) after 7 h revealed the complete consumption of starting material (R_f 0.30) and the formation of a major product (R_f 0.40). The reaction mixture was diluted with DCM (10 mL), washed with 2 M HCl(aq) (3 x 10 mL) and brine (10 mL), dried

over $MgSO_4$, filtered, concentrated under reduced pressure and purified by flash column chromatography (PhMe) to give the title mesylate **3.57** (243 mg, 92%) as a colourless oil.

HRMS (ESI +ve): $C_{34}H_{47}N_3NaO_7SSi$ found 692.2797; (M+Na^+) requires 692.2796; $[\alpha]_D^{25}$ -8.3 (*c* 0.99, $CHCl_3$); ν_{max} (thin film): 2104 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.07, 0.09 (2 x 3H, s, Si$\underline{Me}_2$), 0.91 (9H, s, SiC$\underline{Me}_3$), 2.92 (3H, s, $SO_2\underline{Me}$), 3.65 (1H, d, H2'a J_{gem} 13.3), 3.74 (1H, dd, H5a J_{gem} 10.9, $J_{5a,4}$ 4.4), 3.77 (1H, dd, H5b J_{gem} 10.9, $J_{5a,4}$ 6.6), 3.82 (1H, d, H1a J_{gem} 11.3), 3.83 (1H, d, H2'b J_{gem} 13.3), 3.91 (1H, d, H1b J_{gem} 11.3), 4.08 (1H, d, H3 $J_{3,4}$ 4.8), 4.39 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.6), 4.43 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.6), 4.60 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.3), 4.69 (1H, d, C$\underline{H}_2$Ph J_{gem} 10.9), 4.73 (1H, d, C$\underline{H}_2$Ph J_{gem} 10.9), 4.78 (1H, d, C$\underline{H}_2$Ph J_{gem} 11.3), 5.20 (1H, dt, H4 $J_{4,3}$ = $J_{4,5a}$ 4.8, $J_{4,5b}$ 6.5), 7.23-7.37 (15H, m, Ph); δ_C ($CDCl_3$, 100 MHz): -5.6, -5.6 (Si$\underline{Me}_2$), 18.0 (Si$\underline{C}Me_3$), 25.8 (SiC$\underline{Me}_3$), 38.7 ($SO_2\underline{Me}$), 51.1 (C2'), 62.5 (C1), 66.4 ($\underline{C}H_2$Ph), 70.2 (C5), 73.2 ($\underline{C}H_2$Ph), 75.2 ($\underline{C}H_2$Ph), 77.0 (C3), 80.4 (C4), 81.5 (C2), 127.5, 127.6, 127.8, 127.8, 127.9, 127.9, 128.3, 128.4, 128.4 (Ar$\underline{C}$H), 137.5, 137.7, 138.3 (ArC); LRMS (ESI +ve): 687 (84%, M+NH_4^+), 692 (100%, M+Na^+), 708 (96%, M+K^+).

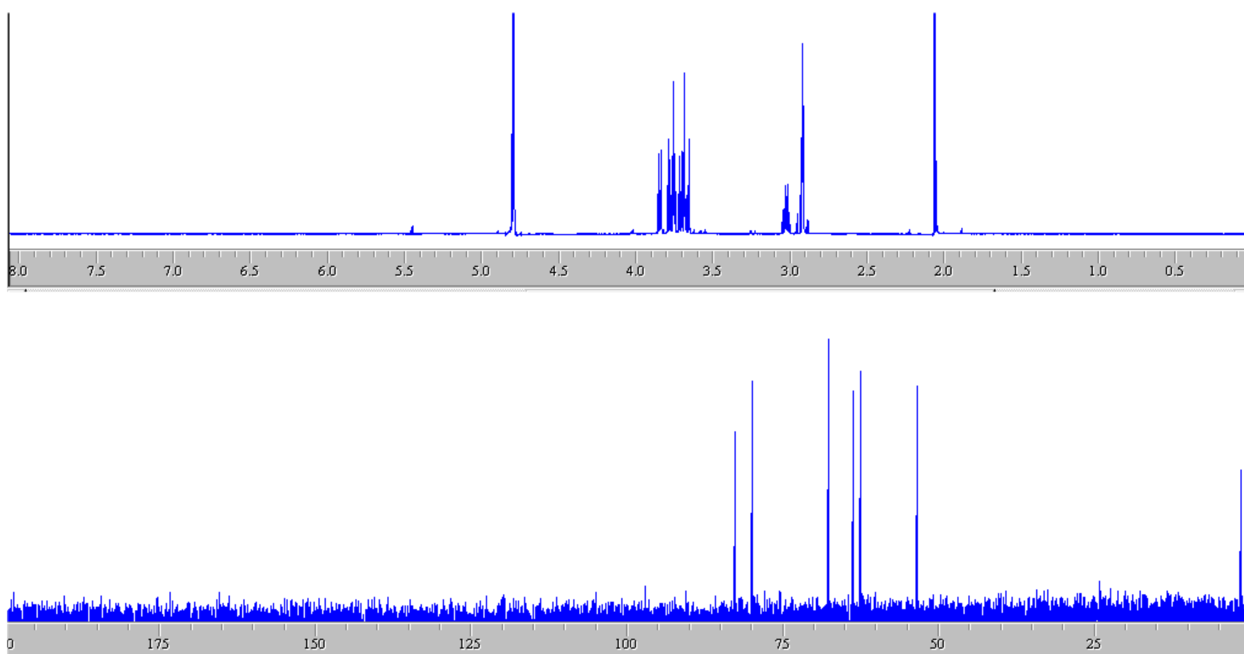
1,4-Dideoxy-2-*C*-hyroxymethyl-1,4-imino-D-arabinitol [isoDMDP], 3.14D



Sodium acetate (100 mg, 1.22 mmol) and Pd (10% on C, 30 mg, 28 μmol) were added to a solution of azido-mesylate **3.57** (243 mg, 0.363 mmol) in 1:5 water/1,4-dioxane (6 mL) and the vessel degassed and flushed with Ar before charging with hydrogen. The mixture was stirred under hydrogen at room temperature for 14 h before acidifying with 2 M HCl(aq) (2 mL), recharging with hydrogen and stirring under hydrogen for a further 24 h. The catalyst was removed by filtration and the filtrate loaded directly onto a column of Dowex (50W-X8, H^+) and the resin washed with EtOH and then water until neutral fractions were obtained. The product was liberated with 2 M NH_3(aq) and the ammoniacal fractions concentrated under reduced

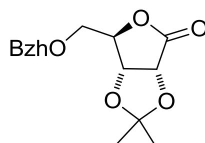
pressure, assisted by coevaporation with EtOH, to give isoDMDP **3.14D** (57 mg, 96%) as a white solid.

HRMS (ESI +ve): $C_6H_{14}NO_4$ found 164.0925; (M+H$^+$) requires 164.0917; $[\alpha]_D^{25}$ -9.3 (*c* 0.96 H_2O); m.p. 138-140°C; ν_{max} (thin film, Ge): 3356 (s, br, OH/NH); δ_H (D_2O, 400 MHz): 2.90 (1H, d, H1a J_{gem} 12.6), 2.94 (1H, d, H1b J_{gem} 12.5), 3.03 (1H, dt, H4 $J_{4,3}$ 5.1, $J_{4,5a}$ 6.1, $J_{4,5b}$ 5.1), 3.67 (1H, d, H2'a J_{gem} 11.9), 3.70 (1H, dd, H5a J_{gem} 11.6, $J_{5a,4}$ 6.3), 3.77 (1H, dd, H5b J_{gem} 11.6, $J_{5b,4}$ 5.0), 3.77 (1H, d, H2'b J_{gem} 12.1), 3.84 (1H, d, H3 $J_{3,4}$ 5.3); δ_C (D_2O, 400 MHz): 53.6 (C1), 62.6 (C5), 63.7 (C2'), 67.7 (C4), 79.9 (C3), 82.8 (C2); LRMS (ESI +ve): 164 (100%, M+H$^+$).



3.6.3. isoDMDP by Intramolecular Reductive Amination

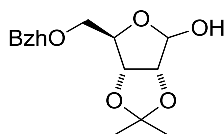
5-*O*-Benzhydryl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone, 3.22



Diphenyldiazomethane (3.00 g, 15.5 mmol) was added to a solution of ribonolactone acetonide **3.21** (1.83 g, 9.73 mmol) in refluxing PhMe (200 mL). The reaction mixture was stirred at reflux

for 1 h until the absence of purple colour, indicating the complete decomposition of diphenyldiazomethane, was observed. TLC analysis (3:1 CyH/EtOAc), revealed the complete consumption of starting material (R_f 0.10) and the formation of a major product (R_f 0.45). The mixture was concentrated under reduced pressure and purification by flash column chromatography (1:99 to 7:43 EtOAc/CyH) afforded benzhydryl ether **3.22** (3.24 g, 94%) as a colourless glass. See **Section 3.6.1** for data.

5-*O*-Benzhydryl-2,3-*O*-isopropylidene-D-ribofuranose, 3.64

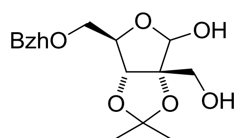


DIBAL solution (1.5 M in toluene, 8.0 mL, 12 mmol) was added dropwise to a solution of protected D-ribonolactone **3.22** (3.11 g, 8.79 mmol) in DCM (30 mL) at -78°C and the mixture stirred at this temperature for 1 h. TLC analysis (9:1 PhMe/acetone) revealed the complete consumption of starting material (0.55) and the formation of a single product (0.50). The mixture was diluted with DCM (100 mL) and stirred with sat. potassium sodium tartrate (150 mL) at RT for 1 h. The organic layer was collected and the aqueous extracted with DCM (2 x 100 mL), the combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:99 to 6:94 acetone/PhMe) afforded the title furanose **3.64** (3.04 g, 97%) as a colourless oil in a 3:1 ratio of anomers, which crystallised on standing.

HRMS (ESI +ve): $C_{21}H_{24}NaO_5$ found 379.1518; (M+Na$^+$) requires 379.1516; $[\alpha]_D^{25}$ -2.1 (*c* 0.66, $CHCl_3$); m.p. 68-70°C; ν_{max} (thin film): 3424 (s, br, OH); δ_H ($CDCl_3$, 400 MHz): 1.35, 1.50 (2 x 3H, s, Me^A), 1.40, 1.57 (2 x 3H, s, Me^B), 3.49 (1H, dd, H5a^B J_{gem} 10.2, $J_{5a,4}$ 2.5), 3.62 (1H, dd, H5a^A J_{gem} 10.2, $J_{5a,4}$ 2.5), 3.63 (1H, dd, H5b^B J_{gem} 10.1, $J_{5b,4}$ 2.8), 3.71 (1H, dd, H5b^A J_{gem} 10.2, $J_{5b,4}$ 2.5), 3.95 (1H, d, OH^B $J_{OH,1}$ 11.5), 4.23 (1H, t, H4^B $J_{4,5}$ 2.5), 4.41 (1H, t, H4^A $J_{4,5}$ 2.5), 4.44 (1H, d, OH^A $J_{OH,1}$ 11.4), 4.59 (1H, d, H2^A $J_{2,3}$ 5.9), 4.66 (1H, dd, H2^B $J_{2,1}$ 3.9, $J_{2,3}$ 6.2), 4.80 (1H,

d, H3^B $J_{3,2}$ 6.2), 4.85 (1H, d, H3^A $J_{3,2}$ 5.8), 5.31 (1H, d, H1^A $J_{1,OH}$ 11.4), 5.34 (1H, s, C$\underline{H}$Ph$_2$^{B}), 5.45 (1H, s, C$\underline{H}$Ph$_2$^{A}), 5.59 (1H, dd, H1^B $J_{1,2}$ 3.9, $J_{1,OH}$ 11.4), 7.25-7.40 (20H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 24.7, 26.1 (Me^B), 25.0, 26.5 (Me^A), 70.6 (C5^A), 75.0 (C5^B), 79.4 (C2^B), 79.7 (C4^B), 82.1 (C3$^{A\&B}$), 84.7 ($\underline{C}$HPh$_2$^{B}), 85.5 ($\underline{C}$HPh$_2$^{A}), 85.6 (C4^A), 87.5 (C2^A), 98.0 (C1^B), 103.9 (C1^A), 112.1 ($\underline{C}$Me$_2$^{A}), 113.0 ($\underline{C}$Me$_2$^{B}), 126.6, 126.8, 126.9, 126.9, 127.6, 127.7, 128.2, 128.2, 128.5, 128.6, 128.8, 128.8 (Ar$\underline{C}$H), 140.1, 140.2, 141.5, 141.6 (ArC); LRMS (ESI +ve): 735 (100%, 2M+Na$^+$).

5-*O*-Benzhydryl-2-*C*-hydroxymethyl-2,3-*O*-isopropylidene-D-ribofuranose, 3.65

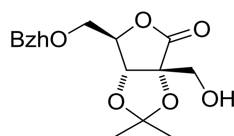


Potassium carbonate (600 mg, 4.35 mmol) was added to a mixture of 39.5% aqueous formaldehyde (7.0 mL, 93 mmol) and a solution of D-ribofuranose **3.64** (1.02 g, 2.87 mmol) in methanol (10 mL) and heated at reflux. TLC analysis (1:2 EtOAc/CyH) after 2 h revealed a major product (R_f 0.20), trace starting material (R_f 0.40) and a minor component (0.05). The reaction mixture was cooled to RT, neutralised with glacial acetic acid and concentrated under reduced pressure. The crude residue was partitioned between EtOAc (50 mL) and 1:1 sat. sodium bicarbonate/brine (50 mL). The aqueous was discarded and the organic washed sequentially with 1:1 sat. sodium bicarbonate/brine (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:9 to 2:3 EtOAc/CyH) afforded the title furanose **3.65** (832 mg, 75%) as a colourless oil in a 6:5 ratio of anomers.

HRMS (ESI +ve): $C_{22}H_{26}NaO_6$ found 409.1624; (M+Na$^+$) requires 409.1622; $[\alpha]_D^{25}$ +15.2 (*c* 0.68, $CHCl_3$); ν_{max} (thin film): 3418 (s, br, OH); δ_H (C_6D_6, 400 MHz): 1.26, 1.39 (2 x 3H, s, Me^B), 1.31, 1.49 (2 x 3H, s, Me^A), 1.45 (1H, dd, OH2'^{B} $J_{OH,2'a}$ 5.6, $J_{OH,2'b}$ 6.8), 2.17 (1H, dd, OH2'^{A} $J_{OH,2'a}$ 6.3, $J_{OH,2'b}$ 7.3), 3.20 (1H, dd, H5a^B J_{gem} 10.4, $J_{5a,4}$ 3.3), 3.25 (1H, dd, H5a^A J_{gem}

10.1, $J_{5a,4}$ 3.5), 3.40 (1H, dd, H5b^B J_{gem} 10.4, $J_{5b,4}$ 4.0), 3.43 (1H, dd, H5b^A J_{gem} 10.1, $J_{5b,4}$ 4.5), 3.49 (1H, dd, H2'a^B J_{gem} 11.9, $J_{2'a,OH}$ 5.3), 3.58 (1H, dd, H2'b^B J_{gem} 11.9, $J_{2'b,OH}$ 7.1), 3.79 (1H, dd, H2'a^A J_{gem} 12.1, $J_{2'a,OH}$ 6.1), 3.84 (1H, dd, H2'b^A J_{gem} 12.1, $J_{2'b,OH}$ 7.6), 3.87 (1H, d, OH1^B $J_{OH,1}$ 10.4), 4.09 (1H, d, OH1^A $J_{OH,1}$ 9.1), 4.28 (1H, ddd, H4^B $J_{4,3}$ 1.5, $J_{4,5a}$ 3.5, $J_{4,5b}$ 4.3), 4.36 (1H, ddd, H4^A $J_{4,3}$ 1.3, $J_{4,5a}$ 3.3, $J_{4,5b}$ 4.5), 4.71 (1H, d, H3^B $J_{3,4}$ 1.3), 4.80 (1H, d, H3^A $J_{3,4}$ 1.0), 5.06 (1H, s, C<u>H</u>Ph$_2^B$), 5.08 (1H, s, C<u>H</u>Ph$_2^A$), 5.41 (1H, d, H1^B $J_{1,OH}$ 10.6), 5.49 (1H, d, H1^A $J_{1,OH}$ 9.1), 6.98-7.04 (4H, m, Ph), 7.06-7.12 (8H, m, Ph), 7.20-7.25 (8H, m, Ph); δ_C (C_6D_6, 100 MHz): 27.3, 27.5 (Me^B), 27.9, 28.3 (Me^A), 62.8 (C2'^{B}), 62.9 (C2'^{A}), 70.5 (C5^B), 70.6 (C5^A), 81.5 (C4^B), 84.3 (C3^B), 85.0 (<u>C</u>HPh$_2^B$), 85.3 (<u>C</u>HPh$_2^A$), 85.4 (C3^A), 86.3 (C4^A), 92.2 (C2^B), 95.1 (C2^A), 99.3 (C1^B), 105.5 (C1^A), 113.3 (<u>C</u>Me$_2^A$), 114.1 (<u>C</u>Me$_2^B$), 127.3, 127.4, 127.4, 127.5, 127.8, 127.9, 128.2, 128.3, 128.7, 128.7, 128.9, 128.9 (Ar<u>C</u>H), 141.0, 141.2, 142.0, 142.2 (ArC); LRMS (ESI +ve): 795 (100%, 2M+Na$^+$).

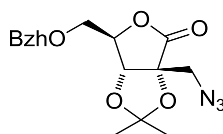
5-*O*-Benzhydryl-2-*C*-hydroxymethyl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone, 3.66



Potassium carbonate (570 mg, 4.13 mmol) and iodine (1.05 g, 4.13 mmol) were added to a hot solution of furanose **3.65** (800 mg, 2.07 mmol) in *tert*-butanol (8 mL) and the mixture heated at reflux. TLC analysis (1:2 EtOAc/CyH) after 90 min revealed the complete consumption of starting material (R_f 0.25) and the formation of a single product (R_f 0.40). Heating was ceased and sat. sodium thiosulfate (~5 mL) added dropwise, followed by EtOAc (10 mL). The mixture was stirred vigorously until the iodine was visibly quenched, the organic layer was collected and the remaining aqueous suspension extracted with EtOAc (3 x 10 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:9 to 7:13 EtOAc/CyH) afforded the title lactone **3.66** (683 mg, 86%) as a colourless gum.

HRMS (ESI +ve): $C_{22}H_{24}NaO_6$ found 407.1467; (M+Na$^+$) requires 407.1465; $[\alpha]_D^{25}$ +10.3 (*c* 1.01, $CHCl_3$); ν_{max} (thin film): 3584 (s, br, OH), 1779 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.46, 1.47 (2 x 3H, s, Me), 1.99-2.03 (1H, m, OH), 3.73 (1H, dd, H5a J_{gem} 10.9, $J_{5a,4}$ 2.8), 3.81 (1H, dd, H5b J_{gem} 10.9, $J_{5b,4}$ 3.3), 3.81-3.83 (2H, m, H2'), 2.67 (1H, t, H4 $J_{4,5}$ 3.0), 4.79 (1H, s, H3), 5.40 (1H, s, C<u>H</u>Ph_2), 7.26-7.39 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 26.8, 27.1 (Me), 60.5 (C2'), 68.2 (C5), 79.2 (C3), 82.9 (C4), 85.0 (<u>C</u>HPh_2), 86.1 (C2), 113.4 (<u>C</u>Me_2), 127.0, 127.2, 128.0, 128.1, 128.6, 128.7 (Ar<u>C</u>H), 140.4, 140.5 (ArC), 174.7 (C1); LRMS (ESI +ve): 791 (100%, 2M+Na$^+$).

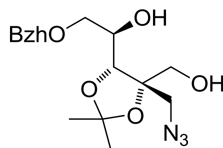
2-*C*-Azidomethyl-5-*O*-benzhydryl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone, 3.67



Trifluoromethanesulfonic anhydride (0.43 mL, 2.6 mmol) was added dropwise to a solution of lactone **3.66** (653 mg, 1.70 mmol) in DCM (10 mL) and pyridine (0.41 mL, 5.1 mmol) at -40°C and the mixture was stirred at -30°C for 1 h. TLC analysis (1:2 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.40) and the formation of a single product (R_f 0.65). The reaction mixture was diluted with DCM (10 mL), washed with 2 M HCl (3 x 10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the triflate derivative. Sodium azide (165 mg, 2.54 mmol) was added to a solution of the triflate derivative in DMF (10 mL) and the mixture stirred at RT for 90 min. TLC analysis (1:3 EtOAc/CyH) revealed the complete consumption of the triflate (R_f 50) and the formation of a single product (R_f 0.55). The reaction mixture was diluted with EtOAc (20 mL), washed with 1:1 brine/water (3 x 15 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:49 to 1:9 EtOAc/CyH) afforded the title azide **3.67** (667 mg, 96%) as a colourless oil.

HRMS (ESI +ve): $C_{22}H_{23}N_3NaO_5$ found 432.1527; (M+Na$^+$) requires 432.1530; $[\alpha]_D^{25}$ +58.4 (*c* 1.13, $CHCl_3$); m.p. 74-76°C; ν_{max} (thin film): 2106 (s, N_3), 1783 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.46, 1.53 (2 x 3H, s, Me), 3.33 (1H, d, H2'a J_{gem} 13.4), 3.63 (1H, d, H2'b J_{gem} 13.4), 3.71 (1H, dd, H5a J_{gem} 10.9, $J_{5a,4}$ 2.0), 3.79 (1H, dd, H5b J_{gem} 10.9, $J_{5b,4}$ 2.8), 4.64 (1H, ddd, H4 $J_{4,3}$ 0.8, $J_{4,5a}$ 2.0, $J_{4,5b}$ 2.8), 4.79 (1H, d, H3 $J_{3,4}$ 0.8), 5.37 (1H, s, $CHPh_2$), 7.22-7.40 (10H, m, Ph); δ_C ($CDCl_3$, 100 MHz): 26.5, 27.1 (Me), 49.7 (C2'), 68.2 (C5), 79.5 (C3), 82.9 (C4), 85.2 ($CHPh_2$), 85.7 (C2), 114.0 (CMe_2), 127.1, 127.2, 128.1, 128.2, 128.6, 128.7 (ArCH), 140.1 (2 x ArC), 173.9 (C1); LRMS (ESI +ve): 432 (59%, M+Na$^+$), 841 (100%, 2M+Na$^+$).

2-*C*-Azidomethyl-5-*O*-benzhydryl-2,3-*O*-isopropylidene-D-ribitol, 3.68

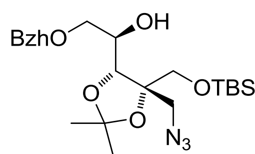


Sodium borohydride (180 mg, 4.76 mmol) was added to a solution of lactone **3.67** (652 mg, 1.59 mmol) in 13:2 EtOH/^{t}BuOH (7.5 mL) and stirred at RT for 90 min. TLC analysis (1:2 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.65) and a major product (R_f 0-0.40 streak). The reaction mixture was quenched with sat. ammonium chloride, diluted with brine (20 mL) and extracted with EtOAc (3 x 30 mL). TLC analysis (1:2 EtOAc/CyH) of the combined organic fractions at this stage revealed a single product (R_f 0.35). The organic was dried over $MgSO_4$, filtered, concentrated and purified by flash column chromatography (1:9 to 7:13 EtOAc/CyH) to give the title diol **3.68** (637 mg, 97%) as a colourless gum.

HRMS (ESI +ve): $C_{22}H_{27}N_3NaO_5$ found 436.1843; (M+Na$^+$) requires 436.1843; $[\alpha]_D^{25}$ +48.4 (*c* 0.73, $CHCl_3$); ν_{max} (thin film): 3385 (s, br, OH); 2103 (s, N_3); δ_H (C_6D_6, 400 MHz): 1.19, 1.28 (2 x 3H, s, Me), 2.42 (1H, t, OH1 $J_{OH,1}$ 5.7), 3.07 (1H, d, OH4 $J_{OH,4}$ 3.9), 3.13 (1H, d, H2'a J_{gem} 13.1), 3.46 (1H, d, H2'b J_{gem} 13.1), 3.49-3.53 (2H, m, H1a, H5a), 3.62 (1H, dd, H1b J_{gem} 11.4,

$J_{1b,OH}$ 5.2), 3.72 (1H, dd, H5b J_{gem} 9.6, $J_{5b,4}$ 2.3), 3.94-4.01 (1H, m, H4), 4.02 (1H, d, H3 $J_{3,4}$ 9.5), 5.25 (1H, s, C<u>H</u>Ph_2), 7.01-7.06 (2H, m, Ph), 7.10-7.16 (4H, m, Ph), 7.28-7.32 (4H, m, Ph); δ_C (C_6D_6, 400 MHz): 26.1, 28.5 (Me), 54.6 (C2'), 62.7 (C1), 69.6 (C4), 71.6 (C5), 77.8 (C3), 84.7 (<u>C</u>HPh_2), 85.0 (C2), 109.0 (<u>C</u>Me_2), 127.4, 127.8, 128.3, 128.7 (Ar<u>C</u>H), 142.3, 142.4 (ArC); LRMS (ESI +ve): 436 (93%, M+Na^+), 849 (100%, 2M+Na^+).

2-*C*-Azidomethyl-5-*O*-benzhydryl-1-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-D-ribitol, 3.69

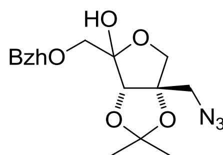


TBSCl (330 mg, 2.19 mmol) was added to a solution of diol **3.68** (461 mg, 1.12 mmol) and imidazole (300 mg, 4.41 mmol) in DMF (6 mL) at 0°C. The mixture was stirred at this temperature for 5 h after which TLC analysis (1:3 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.20) and the formation of a major product (R_f 0.70). The reaction mixture was diluted with EtOAc (20 mL), washed with 1:1 brine/water (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. Purifiaction by flash column chromatography (1:99 to 1:19 EtOAc/CyH) afforded the title silyl ether **3.69** (576 mg, 98%) as a colourless oil.

HRMS (ESI +ve): $C_{28}H_{41}N_3NaO_5Si$ found 550.2708; (M+Na^+) requires 550.2708; $[\alpha]_D^{25}$ +12.7 (*c* 0.90, $CHCl_3$); ν_{max} (thin film): 3474 (m, br, OH), 2103 (s, N_3); δ_H ($CHCl_3$, 400 MHz): 0.14, 0.15 (2 x 3H, s, Si<u>Me</u>$_2$), 0.93 (9H, s, C<u>Me</u>$_3$), 1.38, 1.41 (2 x 3H, s, C<u>Me</u>$_2$), 3.43 (1H, d, H2'a J_{gem} 12.9), 3.60 (1H, d, H1a J_{gem} 10.4), 3.61 (1H, dd, H5a J_{gem} 10.4, $J_{5a,4}$ 5.0), 3.65 (1H, d, H2'b J_{gem} 12.9), 3.66 (1H, d, OH $J_{OH,4}$ 4.0), 3.75 (1H, d, H1b J_{gem} 10.4), 3.78 (1H, dd, H5b J_{gem} 10.4, $J_{5b,4}$ 2.3), 4.01 (1H, ddd, H4 $J_{4,3}$ 9.6, $J_{4,5a}$ 5.0, $J_{4,5b}$ 2.1, $J_{4,OH}$ 4.0), 4.07 (1H, d, H3 $J_{3,4}$ 9.6), 5.49 (1H, s, C<u>H</u>Ph_2), 7.22-7.39 (10H, m, Ph); δ_C ($CHCl_3$, 100 MHz): -5.8, -5.7 (Si<u>Me</u>$_2$), 18.2 (<u>C</u>Me_3), 25.8 (C<u>Me</u>$_3$), 25.9, 28.3 (C<u>Me</u>$_2$), 55.2 (C2'), 62.7 (C1), 69.4 (C4), 70.8 (C5), 78.0 (C3), 83.8 (C2),

84.2 (CHPh$_2$), 108.5 (CMe$_2$), 127.0, 127.1, 127.3, 127.3, 128.2, 128.3 (ArCH), 142.1, 142.2 (ArC); LRMS (ESI +ve): 545 (91%, M+NH$_4^+$), 550 (100%, M+Na$^+$).

4-*C*-Azidomethyl-1-*O*-benzhydryl-2,3-*O*-isopropylidene-L-ribulofuranose, 3.71



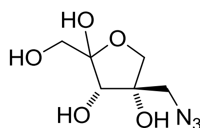
Dess-Martin periodinane (675 mg, 1.59 mmol) was added to a solution of ribitol **3.69** (576 mg, 1.09 mmol) in DCM (10 mL) and the mixture stirred at RT. TLC analysis (PhMe) after 2 h revealed the complete consumption of starting materal (R_f 0.20) and the formation of a single product (R_f 0.25). The mixture was diluted with EtOAc (30 mL) and stirred with thiosulfate-bicarbonate solution (30 mL, 8% w/v aqueous sodium thiosulfate saturated with sodium bicarbonate) for 30 min, then the aqueous was discarded and the organic washed with thiosulfate-bicarbonate solution (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude ketone **3.70** (573 mg, 100%) as a colourless oil.

TBAF (1 M in THF, 0.80 mL, 0.80 mmol) was added to a solution of the crude ketone **3.70** (361 mg, 0.688 mmol) in THF (5 mL) at 0°C. The mixture was stirred at 0°C for 1 h and then RT for 1 h after which TLC analysis (1:3 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.70) and the formation of a major product (R_f 0.50). The reaction mixture was concentrated under reduced pressure and purification by flash column chromatography (1:49 to 1:4 EtOAc/CyH) afforded the title ribulose **3.71** (262 mg, 92%) as a colourless, partially crystalline material, in an 11:2 ratio of anomers.

HRMS (ESI +ve): $C_{22}H_{25}N_3NaO_5$ found 434.1684; (M+Na$^+$) requires 434.1686; $[\alpha]_D^{25}$ +79.3 (*c* 1.06, $CHCl_3$); ν_{max} (thin film): 3474 (m, br, OH), 2103 (s, N_3); δ_H ($CHCl_3$, 400 MHz): 1.43, 1.44 (2 x 3H, s, Me^A), 1.54, 1.60 (2 x 3H, s, Me^B), 3.35 (1H, d, H4'^{B}a J_{gem} 13.0), 3.42 (1H, d, H4'^{B}b J_{gem} 12.9), 3.50 (1H, s, OH^A), 3.50 (1H, d, H4'^{A}a J_{gem} 12.9), 3.60 (1H, d, H1^{B}a J_{gem} 10.2), 3.62

(1H, d, H4'^{B}a J_{gem} 12.9), 3.67 (1H, d, H1^{B}b J_{gem} 10.2), 3.72 (1H, d, H1^{A}a J_{gem} 10.4), 3.76 (1H, d, H1^{A}b J_{gem} 10.5), 3.85 (1H, d, H5^{B}a J_{gem} 9.9), 3.95 (1H, d, H5^{A}a J_{gem} 10.1), 3.96 (1H, d, H5^{B}b J_{gem} 9.9), 4.02 (1H, d, H5^{A}b J_{gem} 10.1), 4.26 (1H, s, OH^{B}), 4.27 (1H, s, H3^{A}), 4.56 (1H, s, H3^{B}), 5.42 (1H, s, C<u>H</u>Ph$_2$^{B}), 5.54 (1H, s, C<u>H</u>Ph$_2$^{A}), 7.24-7.38 (20H, m, Ph); δ_C ($CHCl_3$, 125 MHz): 27.4, 27.5 (Me^{A}), 27.5, 27.9 (Me^{B}), 53.6 (C4'^{B}), 55.0 (C4'^{A}), 69.6 (C1^{A}), 72.6 (C1^{B}), 74.0 (C5^{B}), 75.1 (C5^{A}), 83.0 (C3^{B}), 84.6 (<u>C</u>HPh$_2$^{A}), 84.9 (<u>C</u>HPh$_2$^{B}), 87.0 (C3^{A}), 91.9 (C4^{A}), 91.9 (C4^{B}), 103.5 (C2^{B}), 105.2 (C2^{A}), 114.4 (<u>C</u>Me$_2$^{A}), 115.5 (<u>C</u>Me$_2$^{B}), 126.5, 126.9, 127.0, 127.1, 127.5, 127.6, 127.8, 127.8, 128.4, 128.5, 128.5, 128.6 (Ar<u>C</u>H), 141.1, 141.2, 141.2, 141.4 (ArC); LRMS (ESI +ve): 434 (100%, M+Na$^+$), 845 (77%, 2M+Na$^+$).

4-*C*-Azidomethyl-L-ribulofuranose, 3.62

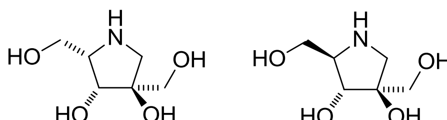


*p*TSA (100 mg) was added to a solution of ribulofuranose **3.71** (203 mg, 0.494 mmol) in 2:3 water/1,4-dioxane (5 mL) at 85°C. The reaction mixture was stirred at this temperature for 18 h after which TLC analysis (1:9 acetone/PhMe) revealed the complete consumption of starting material (R_f 0.60) and the formation of BzhOH (R_f 0.55) and a major product (R_f 0.00; 0.50 in 45:5:1 EtOAc/EtOH/H_2O). The mixture was neutralised with sat $NaHCO_3$, preadsorbed on silica gel and purified by flash column chromatography (1:2 to 1:0 EtOAc/CyH, then 45:5:1 to 7:2:1 EtOAc/IPA/H_2O) to give the title free ribulose **3.62** (64 mg, 63%) as a colourless oil. NMR analysis revealed a 5:1 ratio of anomers in aqueous solution.

HRMS (ESI +ve): $C_6H_{11}N_3NaO_5$ found 228.0593; (M+Na$^+$) requires 228.0591; $[\alpha]_D^{25}$ Equilibrium: -18.8 (*c* 0.97, H_2O); ν_{max} (thin film, Ge): 3399 (s, br, OH), 2111 (s, N_3); δ_H (D_2O, 400 MHz): 3.43 (1H, d, H4'a^{A} J_{gem} 12.9), 3.55 (1H, d, H4'a^{B} J_{gem} 13.1), 3.56 (1H, d, H1a^{A} J_{gem} 12.1), 3.58 (1H, d, H4'b^{A} J_{gem} 13.0), 3.59 (1H, d, H1b^{A} J_{gem} 12.0), 3.61 (1H, d, H4'b^{B} J_{gem} 13.0), 3.67 (1H, d, H1a^{B} J_{gem} 12.0), 3.70 (1H, d, H1b^{B} J_{gem} 12.0), 3.89 (1H, d, H5a^{B} J_{gem} 10.2), 3.92

(1H, d, H5a^{A} J_{gem} 10.2), 3.98 (1H, s, H3^{A}), 3.99 (1H, d, H5b^{A} J_{gem} 10.2), 4.02 (1H, s, H3^{B}), 4.03 (1H, d, H5b^{B} J_{gem} 10.2); δ_C (D_2O, 125 MHz): 55.8 (C4'^{B}), 56.3 (C4'^{A}), 63.2 (C1^{A}), 63.4 (C1^{B}), 72.2 (C3^{A}), 73.8 (C5^{B}), 74.1 (C5^{A}), 78.6 (C4^{A}), 79.7 (C3^{B}), 80.0 (C4^{B}), 103.8 (C2^{A}), 106.3 (C2^{B}); LRMS (ESI +ve): 228 (100%, M+Na^{+}).

1,4-Dideoxy-2-*C*-hydroxymethyl-1,4-imino-L-xylitol [isoDGDP], 3.63, and isoDMDP, 3.14D

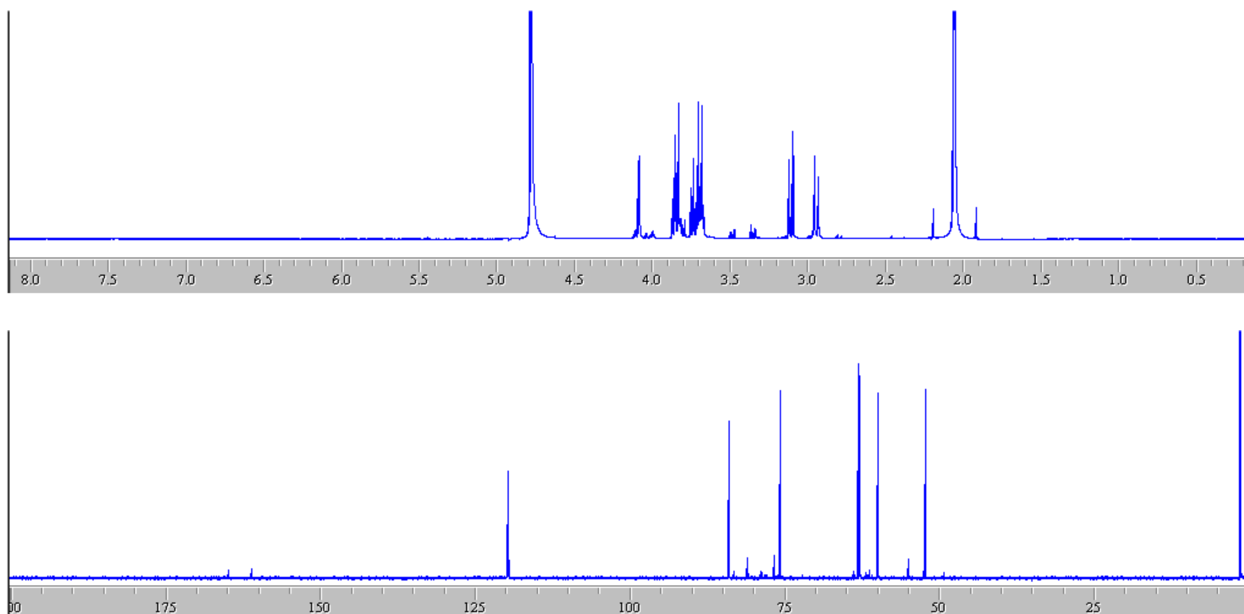


Pd (10% on C, 30 mg, 28 μmol) was added to a solution of ribulofuranose **3.62** (64 mg, 0.31 mmol) in water (5 mL) and the vessel degassed and flushed with Ar before charging with hydrogen. The mixture was stirred under hydrogen at room temperature for 18 h before removal of the catalyst by filtration. The filtrate was concentrated under reduced pressure and the crude mixture loaded onto a column of Dowex (50W-X8, H^{+}). The resin was washed with EtOH and then water until neutral fractions were obtained, and the product was then liberated with 2 M NH_3(aq). The ammoniacal fractions were concentrated under reduced pressure, assisted by coevaporation with EtOH, to give a mixture of isoDGDP **3.63** and isoDMDP **3.14D** (43 mg, 84%) in a ratio of approximately 3:2, as judged by relative integrations in the ^{1}H NMR spectrum, as a brown foam. Separation of a sample of the isomeric mixture (32 mg, 0.20 mmol) was achieved by ion exchange chromatography (Atsushi Kato, Univ. Toyama), giving isoDGDP **3.63** (6.6 mg, 21%) and isoDMDP **3.14D** (5.6 mg, 18%) as colourless oils.

Data for isoDGDP **3.63**

HRMS (ESI +ve): $C_6H_{14}NO_4$ found 164.0916; (M+H^{+}) requires 164.0917; $[\alpha]_D^{25}$ -16.6 (*c* 0.47, H_2O); ν_{max} (thin film, Ge): 3331 (s, br, OH); δ_H (D_2O, 500 MHz): 2.95 (1H, d, H1a J_{gem} 12.6), 3.11 (1H, d, H1b J_{gem} 12.6), 3.67-3.71 (1H, m, H4), 3.70 (1H, d, H2'a J_{gem} 12.0), 3.74 (1H, dd, H5a J_{gem} 11.0, $J_{5a,4}$ 7.3), 3.85 (1H, d, H2'b J_{gem} 12.0), 3.86 (1H, dd, H5b J_{gem} 11.0, $J_{5b,4}$ 6.0),

4.09 (1H, d, H3 $J_{3,4}$ 3.8); δ_C (D_2O, 125 MHz): 52.4 (C1), 60.0 (C2'), 63.1 (C5), 63.1 (C4), 75.9 (C3), 84.1 (C2); LRMS (ESI +ve): 164 (100%, $M+H^+$).



NMR data for isoDMDP **3.13D** was identical to that obtained previously (**Section 3.6.2**).

3.7. References

1. Jespersen, T. M.; Bols, M.; Sierks, M. R.; Skrydstrup, T. *Tetrahedron* **1994,** *50*, 13449-13460.
2. Jespersen, T. M.; Dong, W. L.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew Chem Int Ed* **1994,** *33*, 1778-1779.
3. Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhara, Y. *J Am Chem Soc* **1998,** *120*, 3007-3018.
4. Zhu, X. X.; Sheth, K. A.; Li, S. H.; Chang, H. H.; Fan, J. Q. *Angew Chem Int Ed* **2005,** *44*, 7450-7453.
5. Jakobsen, P.; Lundbeck, J. M.; Kristiansen, M.; Breinholt, J.; Demuth, H.; Pawlas, J.; Candela, M. P. T.; Andersen, B.; Westergaard, N.; Lundgren, K.; Asano, N. *Bioorg Med Chem* **2001,** *9*, 733-744.
6. Asano, N.; Ikeda, K.; Yu, L.; Kato, A.; Takebayashi, K.; Adachi, I.; Kato, I.; Ouchi, H.; Takahata, H.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2005,** *16*, 223-229.
7. Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishim.H; Hamada, M.; Takeuchi, T. *J Antibiot* **1974,** *27*, 963-969.
8. Kudo, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J Antibiot* **1992,** *45*, 1662-1668.
9. Kudo, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J Antibiot* **1992,** *45*, 954-962.
10. Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T. *J Antibiot* **1992,** *45*, 963-970.
11. Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T. *J Org Chem* **2000,** *65*, 2-11.
12. Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *J Antibiot* **1999,** *52*, 348-350.

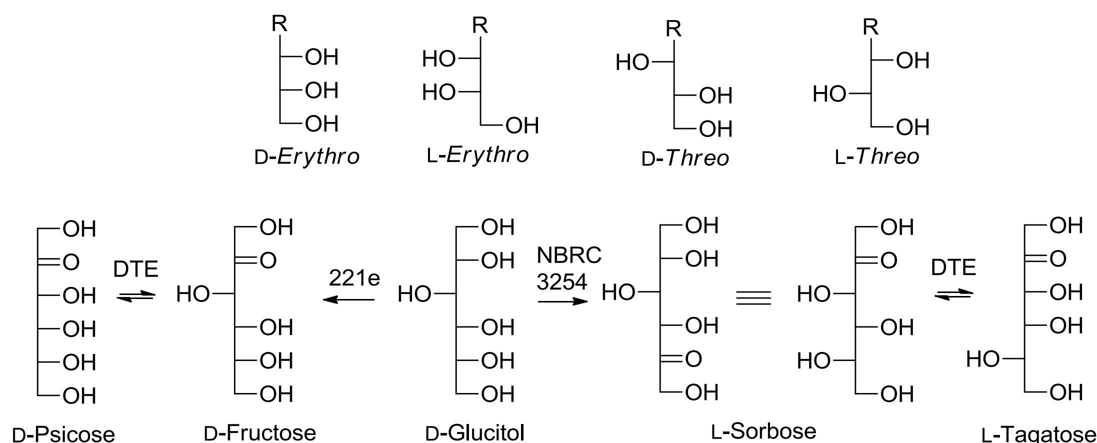
13. Nishimura, Y.; Satoh, T.; Kudo, T.; Kondo, S.; Takeuchi, T. *Bioorg Med Chem* **1996,** *4*, 91-96.
14. Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *Bioorg Med Chem* **2000,** *8*, 343-352.
15. Håkansson, A. E.; van Ameijde, J.; Horne, G.; Nash, R. J.; Wormald, M. R.; Kato, A.; Besra, G. S.; Gurcha, S.; Fleet, G. W. J. *Tetrahedron Letters* **2008,** *49*, 179-184.
16. Pearson, W. H.; Hembre, E. J. *Tetrahedron Letters* **2001,** *42*, 8273-8276.
17. Clinch, K.; Evans, G. B.; Fleet, G. W. J.; Furneaux, R. H.; Johnson, S. W.; Lenz, D. H.; Mee, S. P. H.; Rands, P. R.; Schramm, V. L.; Taylor Ringia, E. A.; Tyler, P. C. *Org Biomol Chem* **2006,** *4*, 1131-1139.
18. Lewandowicz, A.; Ringia, E. A. T.; Ting, L.-M.; Kim, K.; Tyler, P. C.; Evans, G. B.; Zubkova, O. V.; Mee, S.; Painter, G. F.; Lenz, D. H.; Furneaux, R. H.; Schramm, V. L. *J Biol Chem* **2005,** *280*, 30320-30328.
19. Bols, M.; Persson, M. P.; Butt, W. M.; Jørgensen, M.; Christensen, P.; Hansen, L. T. *Tetrahedron Letters* **1996,** *37*, 2097-2100.
20. Hotchkiss, D. J.; Kato, A.; Odell, B.; Claridge, T. D. W.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2007,** *18*, 500-512.
21. Kati, W. M.; Montgomery, D.; Maring, C.; Stoll, V. S.; Giranda, V.; Chen, X. Q.; Laver, W. G.; Kohlbrenner, W.; Norbeck, D. W. *Antimicrob Agents Ch* **2001,** *45*, 2563-2570.
22. daCruz, F. P. DPhil, University of Oxford, 2010.
23. Yasuda, K.; Kizu, H.; Yamashita, T.; Kameda, Y.; Kato, A.; Nash, R. J.; Fleet, G. W. J.; Molyneux, R. J.; Asano, N. *J Nat Prod* **2002,** *65*, 198-202.
24. Lillelund, V. H.; Jensen, H. H.; Liang, X. F.; Bols, M. *Chem Rev* **2002,** *102*, 515-553.
25. Best, D.; Jenkinson, S. F.; Saville, A. W.; Alonzi, D. S.; Wormald, M. R.; Butters, T. D.; Norez, C.; Becq, F.; Bleriot, Y.; Adachi, I.; Kato, A.; Fleet, G. W. J. *Tetrahedron Letters* **2010,** *51*, 4170-4174.
26. Wuts, P. G. M. G., T. W. *Greene's Protective Groups in Organic Synthesis*; John Wiley & Sons: New York, 2006.
27. Crich, D.; Ritchie, T. J. *Carbohydr Res* **1990,** *197*, 324-326.
28. Friesen, R. W.; Daljeet, A. K. *Tetrahedron Letters* **1990,** *31*, 6133-6136.
29. Wuts, P. G. M.; Bigelow, S. S. *J Org Chem* **1988,** *53*, 5023-5034.
30. Seela, F.; Frohlich, T. *Helv Chim Acta* **1994,** *77*, 399-408.
31. Yu, C. Y.; Asano, N.; Ikeda, K.; Wang, M. X.; Butters, T. D.; Wormald, M. R.; Dwek, R. A.; Winters, A. L.; Nash, R. J.; Fleet, G. W. J. *Chem Commun* **2004**, 1936-1937.
32. Clinch, K.; Evans, G. B.; Fleet, G. W. J.; Furneaux, R. H.; Johnson, S. W.; Lenz, D. H.; Mee, S. P. H.; Rands, P. R.; Schramm, V. L.; Ringia, E. A. T.; Tyler, P. C. *Org Biomol Chem* **2006,** *4*, 1131-1139.
33. Stanescu, M. A.; Varma, R. S. *Tetrahedron Letters* **2002,** *43*, 7307-7309.
34. Jackson, G.; Jones, H. F.; Petursson, S.; Webber, J. M. *Carbohydr Res* **1982,** *102*, 147-157.
35. Miller, J. B. *J Org Chem* **1959,** *24*, 560-561.
36. Ko, K. Y.; Kim, J. Y. *B Kor Chem Soc* **1999,** *20*, 771-772.
37. Best, D.; Jenkinson, S. F.; Rule, S. D.; Higham, R.; Mercer, T. B.; Newell, R. J.; Weymouth-Wilson, A. C.; Fleet, G. W. J.; Petursson, S. *Tetrahedron Letters* **2008,** *49*, 2196-2199.
38. Bethell, D.; Newall, A. R.; Stevens, G.; Whittake.D. *J Chem Soc B* **1969**, 749-754.
39. Bethell, D.; Newall, A. R.; Whittake.D. *J Chem Soc B* **1971**, 23-31.
40. Kirmse, W.; Kund, K. *J Am Chem Soc* **1989,** *111*, 1465-1473.
41. Warner, P. M.; Chu, I. S. *J Am Chem Soc* **1984,** *106*, 5366-5367.
42. Belt, S. T.; Bohne, C.; Charette, G.; Sugamori, S. E.; Scaiano, J. C. *J Am Chem Soc* **1993,** *115*, 2200-2205.
43. Chateauneuf, J. E. *Res Chem Intermediat* **1994,** *20*, 249-263.

44. Kirmse, W.; Kilian, J.; Steenken, S. *J Am Chem Soc* **1990,** *112*, 6399-6400.
45. Dix, E. J.; Goodman, J. L. *J Phys Chem* **1994,** *98*, 12609-12612.
46. Pliego, J. R.; De Almeida, W. B. *J Phys Chem A* **1999,** *103*, 3904-3909.
47. Peon, J.; Polshakov, D.; Kohler, B. *J Am Chem Soc* **2002,** *124*, 6428-6438.
48. Mercer, T. B. Part II thesis, University of Oxford, 2008.
49. Mercer, T. B.; Jenkinson, S. F.; Bartholomew, B.; Nash, R. J.; Miyauchi, S.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2009,** *20*, 2368-2373.
50. Rule, S. D. Part II thesis, University of Oxford, 2008.
51. Higham, R. Part II thesis, University of Oxford, 2008.
52. Newell, R. J. Part II thesis, University of Oxford, 2008.
53. Humphlett, W. J. *Carbohyd Res* **1967,** *4*, 157-164.
54. Batra, H.; Moriarty, R. M.; Penmasta, R.; Sharma, V.; Stanciuc, G.; Staszewski, J. P.; Tuladhar, S. M.; Walsh, D. A. *Org Process Res Dev* **2006,** *10*, 484-486.
55. Ho, P. T. *Can J Chem* **1979,** *57*, 381-383.
56. Mori, N.; Togo, H. *Tetrahedron* **2005,** *61*, 5915-5925.
57. Jenkinson, S. F.; Kato, A. *Unpublished results*.
58. Card, P. J.; Hitz, W. D. *J Org Chem* **1985,** *50*, 891-893.
59. Legler, G.; Korth, A.; Berger, A.; Ekhart, C.; Gradnig, G.; Stutz, A. E. *Carbohydr Res* **1993,** *250*, 67-77.
60. Liu, K. K. C.; Kajimoto, T.; Chen, L. R.; Zhong, Z. Y.; Ichikawa, Y.; Wong, C. H. *J Org Chem* **1991,** *56*, 6280-6289.
61. Booth, K. V.; Jenkinson, S. F.; Best, D.; Nieto, F. F.; Estevez, R. J.; Wormald, M. R.; Weymouth-Wilson, A. C.; Fleet, G. W. J. *Tetrahedron Letters* **2009,** *50*, 5088-5093.
62. Garrison, A. W.; Boozer, C. E. *J Am Chem Soc* **1968,** *90*, 3486-3494.
63. Herdeis, C.; Waibel, D. *Arch Pharm* **1991,** *324*, 269-274.

4. Modified Hexitols as Izumoring Substrates

4.1. Introduction

The concept of Izumoring was introduced in **Chapter 1**, here some of the details of the approach will be examined more closely, and application of the strategy to deoxygenated and fluorinated monosaccharide systems will be presented. At the core of the Izumoring strategy is the combination of microbial oxidation of polyols to their corresponding ketoses, and subsequent epimerisation by D-tagatose-3-epimerase [DTE].[1,2] All of the bacterial polyol dehydrogenases [PDHs] are active at the secondary alcohol closest to the chain terminus of a carbohydrate polyol, e.g. C2 or C5 of a hexitol. Furthermore, the stereochemical arrangement is recognised, with oxidases from different microorganisms displaying different configurational requirements for oxidation. It is not solely the configuration of the oxidised centre that is significant, but the configuration of the vicinal diol composed of the oxidised centre and the adjacent secondary alcohol, e.g. C2/C3 or C5/C4 of a hexitol. In addition, it is not merely the *erythro*/*threo* relative configuration of this diol, but the absolute configuration that is recognised. Therefore a system has been developed whereby four different microorganisms are employed that recognise the D-*erythro* (*Gluconobacter thailandicus* NBRC 3254),[3] L-*erythro* (*Enterobacter aerogenes* IK7),[4] D-*threo* (*Klebsiella pneumoniae* 40b)[5] and L-*threo* (*Enterobacter agglomerans* 221e)[6] motifs (**Scheme 4.1**). These reactions are reversed if oxygen is limited.



Scheme 4.1: Recognised diol motifs and the four ketoses available from D-glucitol

For example, D-glucitol bears L-*threo* and D-*erythro* motifs and can therefore be oxidised to D-fructose by 221e, or alternatively to L-sorbose by NBRC 3254. DTE catalyses epimerisation of the stereogenic centre adjacent to the carbonyl functionality of a ketose with complete regioselectivity, to give an equilibrium mixture of C3 epimeric ketoses. Despite its name, DTE[7-9] is a highly promiscuous enzyme which appears to catalyse the epimerisation of any ketose; thus D-fructose is equilibrated with D-psicose,[10] and L-sorbose with L-tagatose;[11] this equilibration can also be performed for the enantiomeric pairs.[11,12] Aldoses may then be generated from these ketoses by the action of aldose isomerases,[13-19] reduction of these aldoses generates new hexitols and the process can be repeated to gain access to new aldoses. Thus all the monosaccharides are linked in a web of biotechnological transformations, dubbed Izumoring (**Figure 4.1**).

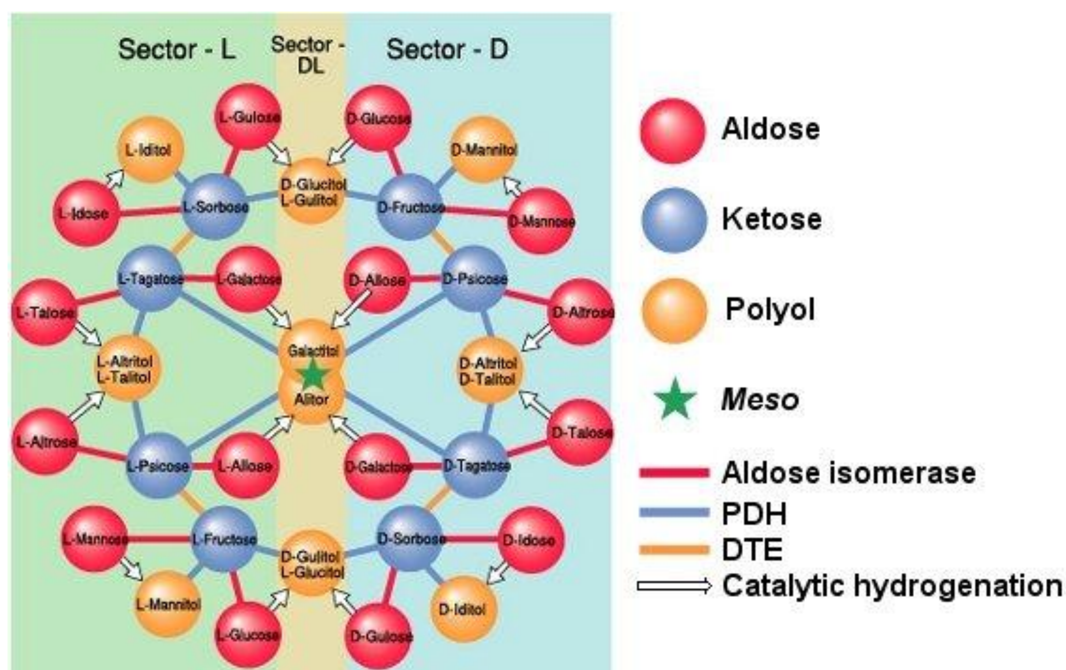


Figure 4.1: The hexitol Izumoring

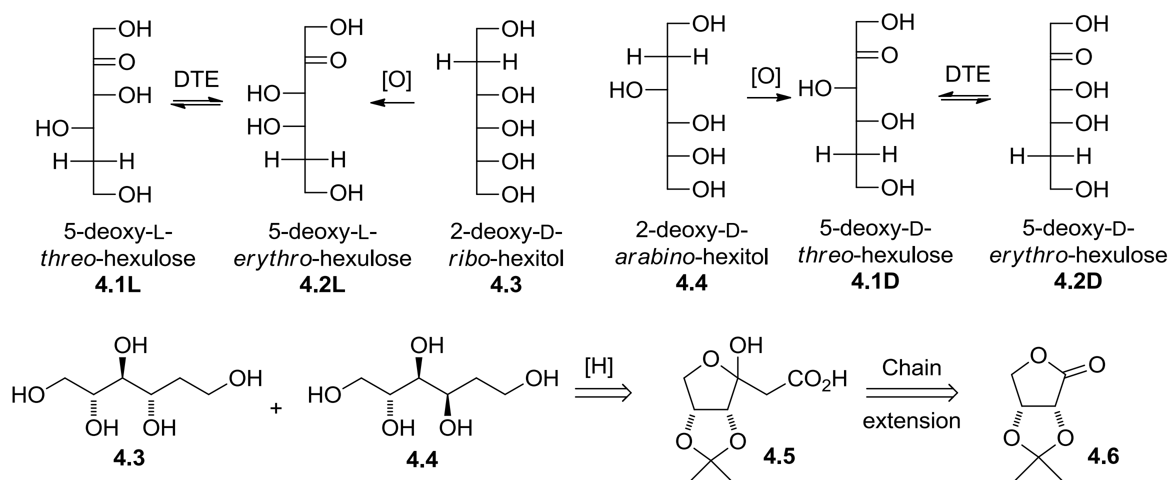
Beyond the vicinal diol motif, the microorganisms exploited by Izumoring appear to tolerate any stereochemical configuration in the remainder of the substrate, and the complete regioselectivity of these processes coupled with the seemingly boundless substrate tolerance creates a harmony of immense flexibility. While the approach was first found to be effective for unmodified monosaccharides, it has since been shown applicable to 1/6-deoxy,[20,21,4] methyl-branched[22,23]

and azido-substituted sugars;[24-26] challenging the limits of the process is an ongoing objective of the Fleet-Izumori collaboration. In this chapter the applicability of the Izumoring process to the interconversion of 5-deoxy[3] and terminally fluoro-substituted hexoses is explored.

Beyond the predominantly academic exercise of challenging the generality of the Izumoring process, deoxy- and fluoro-carbohydrates are interesting molecules in their own right, having biological applications as well as being potential carbohydrate building blocks. Apart from the obvious examples of the common and biologically critical 6-deoxy-L-galactose [fucose], 6-deoxy-L-mannose [rhamnose] and 2-deoxy-D-ribose, deoxy-sugar motifs are common in antibacterial macrolides[27] and modification of these motifs may provide new antibiotics to fight resistant bacterial strains.[28] The biochemical applications of fluorosugars are varied and important,[29] including antiviral activity as a result of their incorporation into oligosaccharides where they act as chain terminators and prevent formation of viral envelopes; this is a mode of action that is also demonstrated by deoxy sugars.[30] The replacement of a hydroxyl substituent with fluorine is a generally well tolerated modification, resulting in little conformational perturbation and retaining hydrogen bond acceptor capability, but fluorosugars are rarely metabolised in animals. This makes fluorosugars excellent candidates for the probing of enzymatic mechanisms and the nature of active sites,[31,32] and the loss of hydrogen bond donor ability associated with fluorocarbohydrates can also lead to their biological recognition as deoxy sugars.[33] Since fluorinated natural products are very rare, fluorinated metabolites are easily detected by ^{19}F NMR. This allows the tracking of metabolites through various biological pathways[34,35] and biosynthetic routes.[36,37] In addition, ^{18}F can be tracked by positron emission topography [PET],[38] in particular 6-deoxy-6-fluoro-D-fructose has recently been identified as a candidate for the PET imaging of breast cancer[39,40] and a newly developed synthesis of [^{18}F]-2-deoxy-2-fluoro-D-allose may facilitate the probing of allose metabolism by this method.[41]

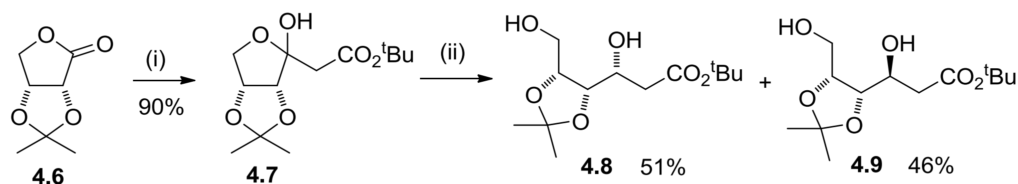
4.2. 5-Deoxy Izumoring

It was envisaged that application of the Izumoring strategy could provide access to all twelve 5-deoxy-hexoses from only two epimeric 2-deoxy hexitols: 2-deoxy-D-*ribo*-hexitol [2-deoxy-D-allitol] **4.3** and 2-deoxy-D-*arabino*-hexitol **4.4** [2-deoxy-D-glucitol] (**Scheme 4.2**). The 5-deoxy-aldoses would be accessed by the catalytic action of an aldose isomerase on the two pairs of 5-deoxy-ketoses shown.



Scheme 4.2: The 5-deoxy Izumoring concept and retrosynthesis of a pair of epimeric deoxy-hexitols

Retrosynthetic analysis of the target hexitol suggested that both could be obtained by a two-carbon chain extension of D-erythronolactone **4.6**, which is readily available by Humphlett oxidation of D-arabinose[42] or peroxide oxidation of isoascorbic acid followed by acetonation.[43] Although such chain extensions have been reported under various Reformatsky conditions,[44,45] a convenient lithium enolate chain extension procedure was utilised that proved highly efficient on multigram scale. A lithium *tert*-butyl acetate solution was prepared by deprotonation of *tert*-butyl acetate with LDA[46] which was then added slowly *via* cannula to a solution of erythronolactone **4.6** at -78°C in THF. The best results were obtained by slow addition of the enolate solution over a period of 1 h, giving intermediate **4.7** in 90% yield (**Scheme 4.3**).



Reagents: (i) AcO^tBu, LDA, THF; (ii) $NaBH_4$, MeOH.
Scheme 4.3: Formation and reduction of intermediate **4.7**

Chemoselective reduction of the ketone functionality with sodium borohydride gave a mixture of the two separable epimeric hexonic acid esters **4.8** and **4.9** in high yield with virtually no stereoselectivity. The relative configuration of the crystalline isomer **4.8**, which had a longer retention time on silica gel, was firmly established as *arabino* by X-ray crystallography (**Figure 4.2**),[47] from which the configuration of the *ribo* epimer **4.9** was inferred. In this case the lack of diastereoselectivity in the reduction step was a distinct advantage as both epimers were desired.

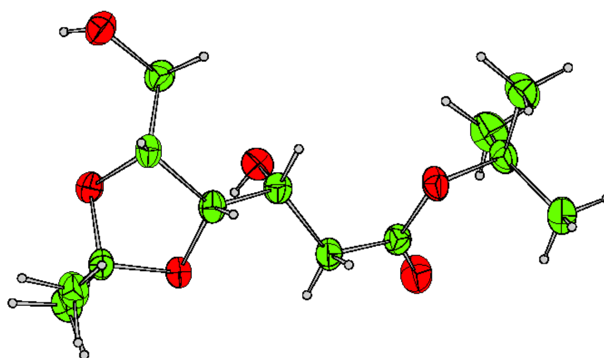
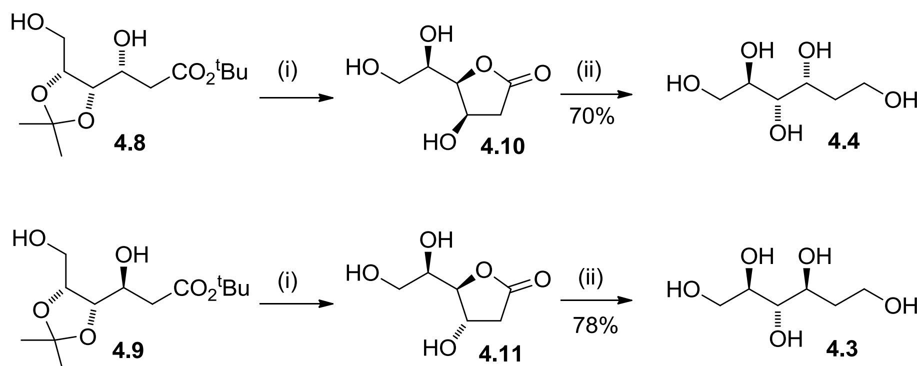


Figure 4.2: The structure of **4.8** confirmed by X-ray crystallography.

With the 2-deoxy-hexonates **4.8/4.9** in hand, access to the corresponding hexitols was first attempted by a reduction-hydrolysis sequence. While the final hydrolysis was straightforward, the preceding reduction of esters **4.8** /**4.9** was cumbersome and low yielding (reducing agents screened were $LiBH_4$, $LiAlH_4$, and DIBAL), which may be a consequence of the bulk associated with the *tert*-butyl ester, and an alternative strategy was sought.

Hydrolysis of *arabino*-hexonate **4.8** was achieved with 10% v/v trifluoroacetic acid [TFA] in aqueous dioxane to give the corresponding 1,4-lactone **4.10**. Ring size was confirmed by HMBC NMR, which showed a correlation between H4 and C1 but not H5 and C1, and is consistent with the literature.[48] A procedure for the reduction of esters with $NaBH_4$ in mixed solvents at elevated

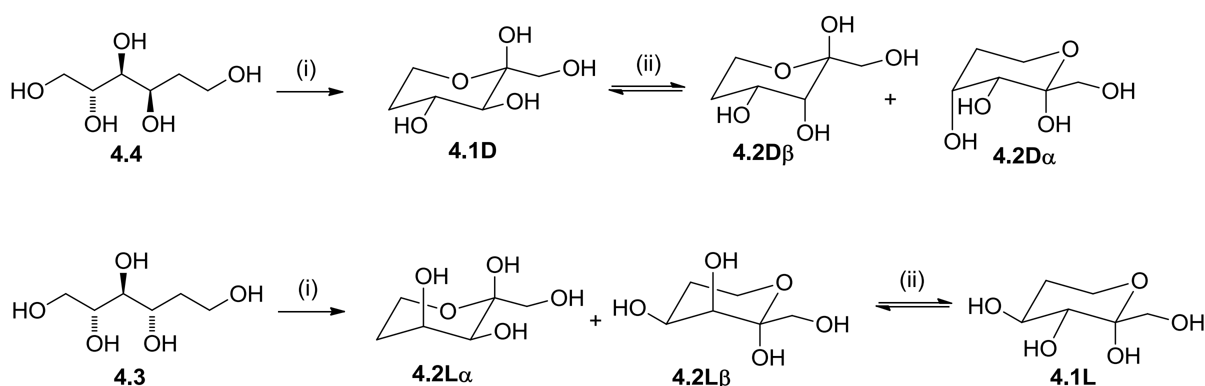
temerature[49] was employed for reduction of lactone **4.10**, furnishing 2-deoxy-D-*arabino*-hexitol **4.4** in 70% yield over the two steps (**Scheme 4.4**). This reduction procedure gave cleaner products than other conventional reductive conditions with more efficient conversion.



Reagents: (i) TFA, water, 1,4-dioxane; (ii) $NaBH_4$, MeOH, ^tBuOH.
Scheme 4.4: Synthesis of 2-deoxy-hexitols

Similar treatment of *ribo*-hexonate **4.9** gave 2-deoxy-D-*ribo*-hexitol **4.3** in 78% yield over two steps, thus providing an efficient synthesis of the 2-deoxy-hexitols **4.3** and **4.4**, in a combined yield of 65% from erythronolactone acetonide **4.6**.

In collaboration with Prof. Ken Izumori, the epimeric hexitols **4.3** and **4.4** were transformed into all four 5-deoxy-ketohexoses by a combination of enzymatic oxidation and epimerisation (**Scheme 4.5**).[3]



Reagents: (i) *G. thailandicus* NBRC 3254; (ii) DTE
Scheme 4.5: Biotechnological manipulation of two deoxy-hexitols

2-Deoxy-D-*arabino*-hexitol = 5-deoxy-D-*lyxo*-hexitol **4.4**, which bears a D-*erythro* motif at C2-C3, was oxidised by *Gluconobacter thailandicus* NBRC 3254 to give 5-deoxy-D-*threo*-hexulose

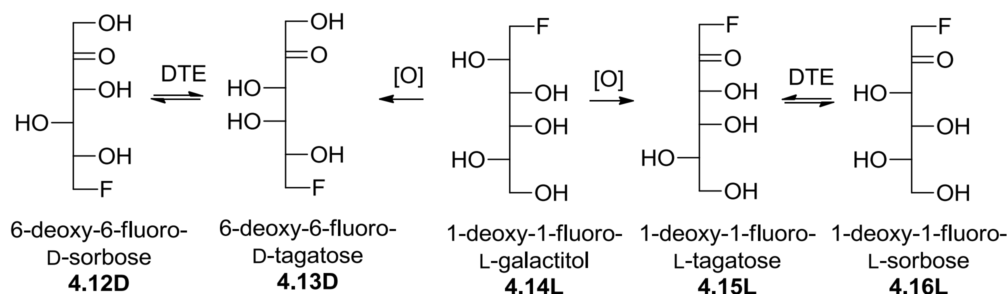
4.1D. Subsequent equilibration with DTE gave a separable mixture of the C3 epimers 5-deoxy-D-*threo*-hexulose **4.1D** and -D-*erythro*-hexulose **4.2D** in a 4:1 ratio. Similarly, oxidation of 2-deoxy-D-*ribo*-hexitol = 5-deoxy-L-*ribo*-hexitol **4.3**, which also bears a D-*erythro* motif at C2-C3, *G. thailandicus* NBRC 3254 gave 5-deoxy-L-*erythro*-hexulose **4.2L** and equilibration with DTE gave 5-deoxy-L-*erythro*-hexulose **4.2L** and -L-*threo*-hexulose **4.1L** in a 1:4 ratio. Conformational analysis by Dr. Mark R. Wormald (Univ. Oxford) indicated that 5-deoxy-*threo*-hexulose **4.1** exists exclusively in the β form, while the *threo* isomer **4.2** exists as a 1:4 mixture of α and β forms in aqueous solution.

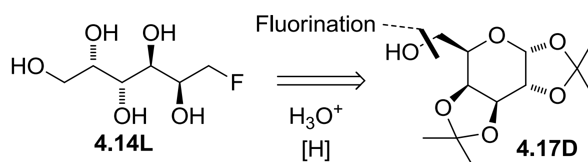
With the biotechnological attainment of all four 2-deoxy-ketohexoses comprehensively demonstrated, one can envisage access to the remaining eight 2-deoxy-aldohexoses by aldose isomerases. The generality of the Izumoring process has been further exemplified and shown as a powerful tool for the synthesis of rare and novel monosaccharides.

4.3. 1/6-Fluoro Izumoring

With Izumoring methodology demonstrated for the 5-deoxy hexoses, the applicability of the methodology to fluorosugars was investigated.

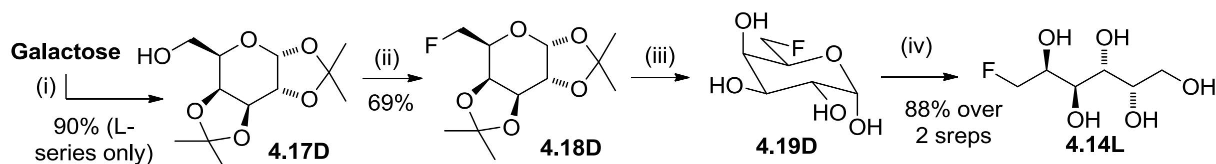
Of the terminal fluoro-hexitols, the most readily available is 1-deoxy-1-fluoro-L-galactitol [6-deoxy-6-fluoro-D-galactitol] **4.14L** by introduction of fluorine to the readily available diacetone-D-galactose **4.17D** at C6 (**Scheme 4.6**). For the preparation of the enantiomeric fluoro-galactitol **4.14D**, L-galactose was obtained biotechnologically by the Izumoring process. Microbial oxidation of fluoro-galactitol **4.14L** would provide access to the 1-fluoro-tagatose **4.15L** or 6-fluoro-tagatose **4.13D**. These ketoses would then be equilibrated with their C3 epimers by the action of DTE, and new fluoro-aldoses could then be accessed with the application of isomerases.





Scheme 4.6: The fluoro Izumoring concept

Fluorine was introduced at C6 of the diacetonide **4.17D** by nucleophilic fluoride displacement of the corresponding triflate, giving **4.18D** in 69% yield (72% for the enantiomer **4.18L**).[50] While not the most efficient of transformations, the reagent combination is economical in comparison to other fluorinating agents such as DAST and TASF, allowing convenient preparation of fluoro-galactose acetonide **4.18** on multigram scale (**Scheme 4.7**).



Reagents and conditions: (i) Me_2CO, H_2SO_4, $CuSO_4$, RT, 18 h; (ii) Tf_2O, Py, DCM, -30°C, 30 min; then TBAF, THF, RT, 24 h; (iii) Dowex (H^+), H_2O, 1,4-dioxane, 60°C, 18 h; (iv) $NaBH_4$, H_2O, RT, 1 h

Scheme 4.7: Synthesis of 1-deoxy-1-fluoro-galactose

The protecting groups were hydrolysed in the presence of a Dowex resin catalyst in aqueous dioxane to give the free fluoro-galactose **4.19D**, which could be crystallised as the α-anomer from aqueous ethanol. Reduction of fluoro-galactose **4.19D** with sodium borohydride in water afforded the highly crystalline fluoro-gatactitol **4.14L**, isolated in 88% yield by recrystallisation over the two steps (91% for the enantiomer **4.14D**). The relative configurations of the unprotected fluororinated species **4.19D** and **4.14L** were confirmed unequivocally by X-ray crystallography (**Figure 4.3**).[51,52]

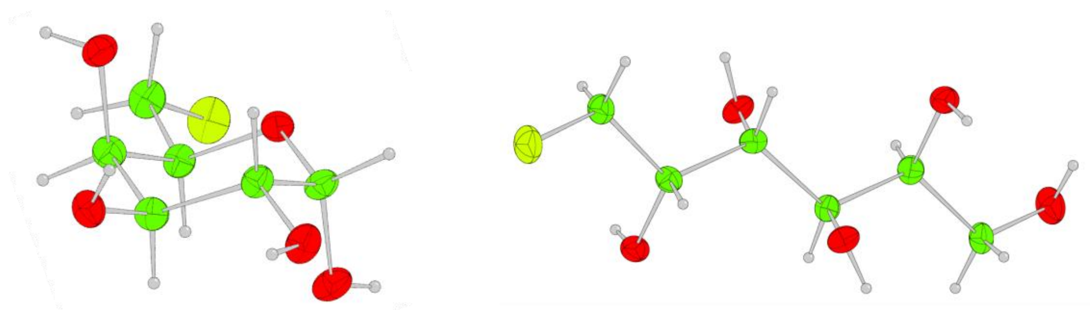
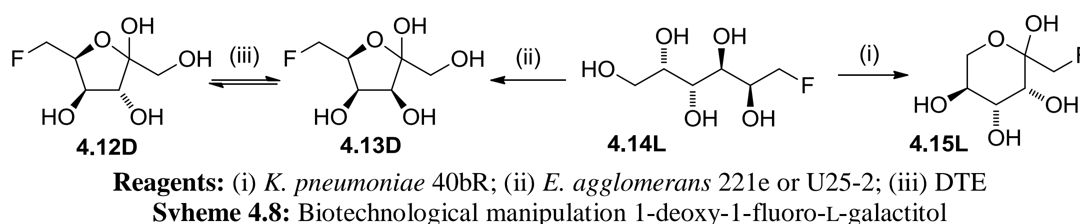


Figure 4.3: X-ray crystal structure of 6-deoxy-6-fluoro-galactose and 1-deoxy-1-fluoro-galactitol

Fluoro-galactitol **4.14L** was indeed found to be a suitable substrate for Izumoring biotransformation (**Scheme 4.8**).



Reagents: (i) *K. pneumoniae* 40bR; (ii) *E. agglomerans* 221e or U25-2; (iii) DTE
Svheme 4.8: Biotechnological manipulation 1-deoxy-1-fluoro-L-galactitol

Oxidation of 1-deoxy-1-fluoro-L-galactitol **4.14L** could be achieved with *Klebsiella pneumoniae* 40bR, which recognises the D-*threo* configured diol at the fluoro terminus, to give 1-deoxy-1-fluoro-L-tagatose **4.15L**. The NMR spectra are complex, showing a 38:9:3:1 mixture of four hemiacetal species in aqueous solution, and detailed conformation analysis is currently underway (Mark R. Wormald, Univ. Oxford). Alternatively, recognition of the L-*threo* hydroxy terminus of fluoro-galactitol **4.14L** by *Enterobacter agglomerans* 221e, provided 6-deoxy-6-fluoro-D-tagatose **4.13D**. The NMR spectra are, once again, complex; two furanose forms, in a 2:1 ratio, accounted for 92% of the equilibrium mixture in aqueous solution, with the open chain ketone form as a minor constituent at 8%. Detailed NMR analysis is underway (Mark R. Wormald Univ. Oxford). According to preliminary communications (Ken Izumori, Univ. Kagawa), DTE was effective in the interconversion 6-deoxy-6-fluoro-D-tagatose **4.13D** and 6-deoxy-6-fluoro-D-sorbose **4.14D**.

Following these promising initial reports, a fluoro-Izumoring can be constructed to illustrate an approach to all the 1/6-fluoroketoses (**Figure 4.4**).

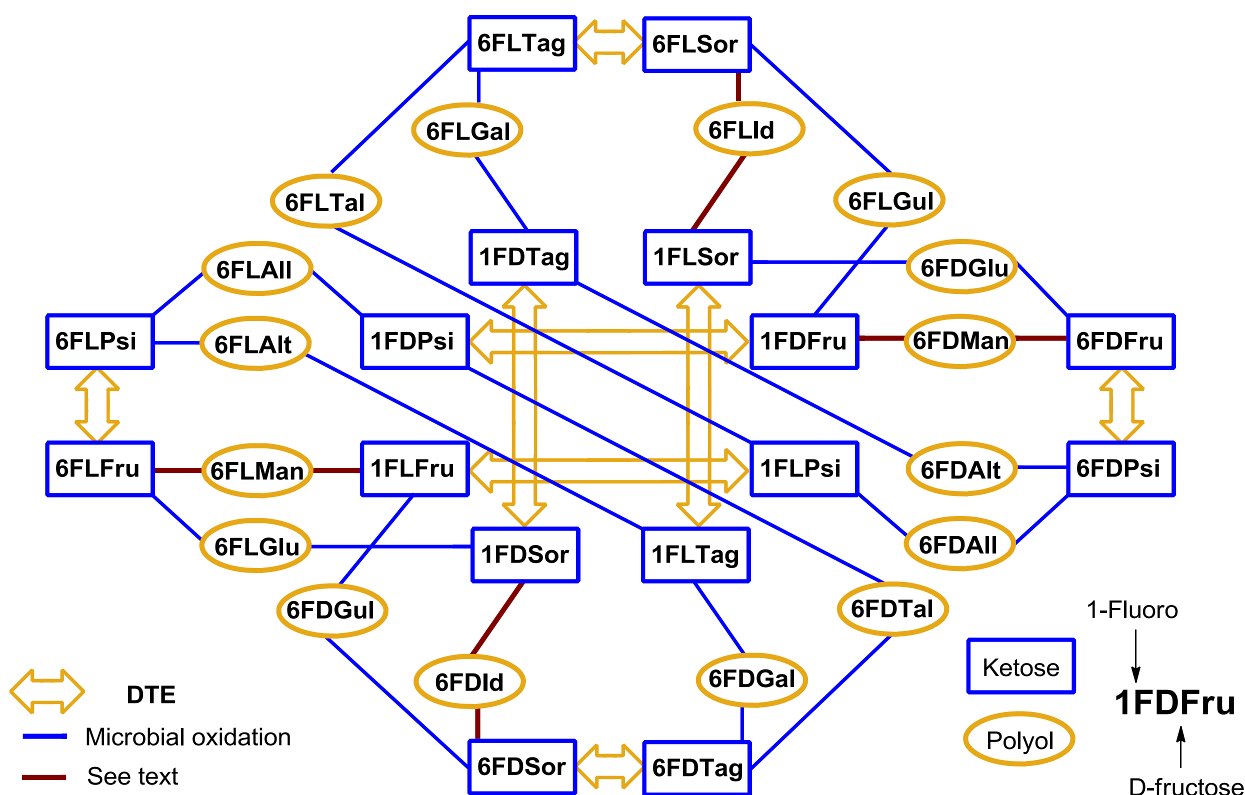


Figure 4.4: The biotechnological network of fluoro Izumoring (fluoro-aldoses omitted for clarity)

The conceivable network of enzymatic transformations is quite complex, and for clarity the 6-fluoro-aldoses have been omitted; they would be accessed by isomerisation of 6-fluoro-ketoses with an aldose isomerase. The red microbial oxidation connections deserve additional comment; oxidation of mannitol or iditol systems would provide mixtures, since both ends of the polyol bear the same *erythro* or *threo* motif for the purposes of molecular recognition and may not be distinguished by microbial oxidation (**Figure 4.5**).

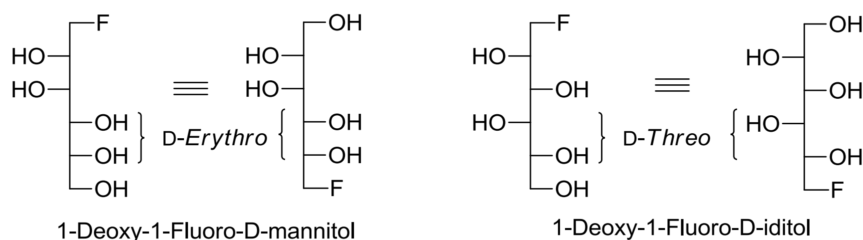


Figure 4.5: A single microbe may oxidise either end of *manno* and *ido* systems as both ends bear diols of the same configuration

The next targets for chemical synthesis in this project are both enantiomers of 6-deoxy-6-fluoro-allitol [1-deoxy-1-fluoro-allitol]. These fluorohexitols (6FDAll and 6FLAll in **Figure 4.4**) provide the most direct access to the remaining *psico* and *fructo* fluoro-ketoses.

4.4. Conclusions and further work

In conclusion, a concise approach to the synthesis of all twelve 5-deoxy hexoses has been explored with the synthesis of all four of the 5-deoxy ketohexoses. The next step in this project should be demonstration of the enzymatic isomerisation of these ketohexoses to their corresponding aldoses.

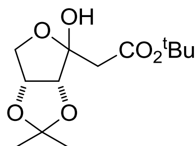
The interconversion of fluorosugars by the Izumoring strategy is an exciting discovery that could allow access to a range of novel fluorinated carbohydrates. Although the fluoro-Izumoring is very much in its infancy, these preliminary results are highly encouraging and particularly exciting in light of the import roles played by fluorosugars in chemical and biochemical research. Besides further investigation of the enzymatic oxidation of 1-deoxy-1-fluoro-D-galactitol **4.14D** and confirmation of DTE epimerisation of the resultant fluoroketoses, successful synthesis of both enantiomers of 1-deoxy-1-fluoro-allitol should provide access to the *fructo* and *psico* systems. Completion of such a project would further demonstrate the power and generality of Izumoring.

4.5. Experimental

For a general experimental refer to **Section 2.6**.

4.5.1. Deoxy-hexitols

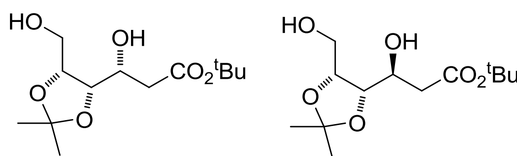
***tert*-Butyl 2-deoxy-4,5-*O*-isopropylidene-D-*erythro*-hex-3-ulofuranosonate, 4.7**



tert-Butyl acetate (15.5 mL, 116 mmol) was added dropwise to a solution of LDA (1.8 M in THF, 60 mL, 108 mmol) at -78°C and stirred for 1 h to afford a solution of the corresponding lithium enolate. The enolate solution was then added dropwise *via* cannula over a period of 1 h to a solution of lactone **4.6** (11.5 g, 72.8 mmol) in THF (100 mL) at -78°C. TLC analysis after a further 15 min (1:2 EtOAc/CyH) revealed the complete consumption of starting material (R_f 0.35) and the formation of a major product (R_f 0.60). The reaction was quenched with sat. NH_4Cl(aq) (80 mL) and water (20 mL) added. The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic fractions dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography afforded **3.7** (18.0 g, 90%) as a colourless oil which crystallised on standing.

HRMS (ESI +ve): $C_{13}H_{22}NaO_6$ found 297.1309; (M+Na$^+$) requires 297.1309; $[\alpha]_D^{18}$ -63.7 (*c* 0.91, $CHCl_3$); mp 23-25°C; ν_{max} (thin film): 3447 (m, OH), 1707 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.30, 1.46 (2 x 3H, s, C$\underline{Me}_2$), 1.48 (9H, s, C$\underline{Me}_3$), 2.70 (1H, d, H2a, J_{gem} 16.7), 2.78 (1H, d, H2b, J_{gem} 16.9), 3.94 (1H, d, H6a, J_{gem} 10.4), 4.04 (1H, dd, H6b, J_{gem} 10.4, $J_{6b,5}$ 3.8), 4.42 (1H, d, H4, $J_{4,5}$ 6.1), 4.84 (1H, dd, H5, $J_{5,4}$ 6.1, $J_{5,6b}$ 3.8), 5.06 (1H, s, OH); δ_C ($CDCl_3$, 100 MHz): 24.8, 26.2 (C$\underline{Me}_2$), 28.1 (C$\underline{Me}_3$), 39.1 (C2), 71.2 (C6), 80.5 (C5), 82.3 ($\underline{C}Me_3$), 85.4 (C4), 104.6 (C3), 112.5 ($\underline{C}Me_2$), 171.9 (C1); LRMS (ESI -ve): 273 (100% M-H$^+$), 333 (87% M+AcO$^-$); [Lit.[45] oil; $[\alpha]_D^{25}$ -45.7 (*c* 1.046, $CHCl_3$)].

tert-Butyl 2-deoxy-4,5-*O*-isopropylidene-D-*arabino*-hexonate, 4.8, and –*ribo*-hexonate, 4.9



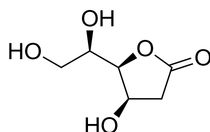
$NaBH_4$ (70 mg, 1.85 mmol) was added portion wise to a solution of **4.7** (511 mg, 1.86 mmol) in methanol (5 mL) at -10°C and the reaction mixture allowed to warm to RT. TLC analysis (1:2 EtOAc/CyH) after 2 h revealed the complete consumption of starting material (R_f 0.60) and the formation of two products (R_f 0.25, 0.30). The reaction mixture was neutralised with glacial acetic acid and concentrated under reduced pressure. Purification by flash column chromatography afforded the more mobile *ribo* component **4.9** (237 mg, 46%) as a colourless oil, the configuration of which was inferred by the confirmation of the structure of the white crystalline less mobile *arabino* component **4.8** (260 mg, 51%) by X-ray crystallography.

Data for **4.9**: HRMS (ESI +ve): $C_{13}H_{24}NaO_6$ found 299.1459; $(M+Na^+)$ requires 299.1465; $[\alpha]_D^{22}$ -41.5 (*c* 0.97, $CHCl_3$); ν_{max} (thin film): 3419 (br, m, OH), 1731 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.33, 1.39 (2 x 3H, s, C<u>Me</u>$_2$), 1.47 (9H, s, C<u>Me</u>$_3$), 2.40 (1H, dd, H2a, J_{gem} 16.9, $J_{2a,3}$ 9.1), 2.74 (1H, dd, H2b, J_{gem} 16.8, $J_{2b,3}$ 2.8), 3.34 (1H, s, OH3), 3.77 (1H, dd, H6a, J_{gem} 11.6, $J_{6a,5}$ 4.8), 3.88 (1H, dd, H6b, J_{gem} 11.6, $J_{6b,5}$ 7.8), 3.99 (1H, dd, H4, $J_{4,3}$ 9.6, $J_{4,5}$ 5.8), 4.13 (1H, dt, H3, $J_{3,2a}$ 9.3, $J_{3,2b}$ 2.5, $J_{3,4}$ 9.3), 4.22-4.26 (1H, m, OH6), 4.33-4.38 (1H, m, H5); δ_C ($CDCl_3$, 100 MHz): 25.2, 27.8 (C<u>Me</u>$_2$), 28.2 (C<u>Me</u>$_3$), 39.1 (C2), 60.7 (C6), 66.7 (C3), 77.4 (C5). 78.7 (C4), 81.8 (<u>C</u>Me$_3$), 108.5 (<u>C</u>Me$_2$), 172.8 (C1); LRMS (ESI +ve): 299 (52% $M+Na^+$), 575 (100% $2M+Na^+$).

Data for **4.8**: HRMS (ESI +ve): $C_{13}H_{24}NaO_6$ found 299.1468; $(M+Na^+)$ requires 299.1465; $[\alpha]_D^{22}$ +10.7 (*c* 0.99, $CHCl_3$); m.p. 72-74°C; ν_{max} (thin film): 3441 (br, m, OH), 1728 (s, CO); δ_H ($CDCl_3$, 400 MHz): 1.37, 1.52 (2 x 3H, s, C<u>Me</u>$_2$), 1.46 (9H, s, C<u>Me</u>$_3$), 2.50 (1H, dd, H2a, J_{gem} 16.2, $J_{2a,3}$ 4.5), 2.74 (1H, dd, H2b, J_{gem} 16.2, $J_{2b,3}$ 8.3), 3.05 (1H, s, OH6), 3.60 (1H, d, OH3, $J_{OH3,3}$ 4.3), 3.81-3.85 (2H, m, H6), 4.08 (1H, dd, H4, $J_{4,3}$ 2.5, $J_{4,5}$ 6.8), 4.18-4.22 (1H, m, H3),

4.24 (1H, dt, H5, $J_{5,4}$ 6.8, $J_{5,6a}$ = $J_{5,6b}$ 4.9); δ_C ($CDCl_3$, 100 MHz): 25.0, 27.0 (C<u>Me</u>$_2$), 28.1 (C<u>Me</u>$_3$), 40.3 (C2), 61.0 (C6), 66.1 (C3), 77.3 (C5). 78.5 (C4), 81.5 (<u>C</u>Me$_3$), 108.5 (<u>C</u>Me$_2$), 171.5 (C1); LRMS (ESI +ve): 299 (57% M+Na^+), 575 (100% 2M+Na^+).

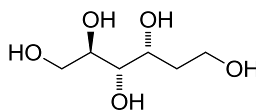
2-Deoxy-D-*arabino*-hexono-1,4-lactone, 4.10



Hexonate **4.8** (5.92 g, 25.0 mmol) was dissolved in 4:25:15 TFA/water/1,4-dioxane (88 mL). TLC analysis after 24 h (9:1 EtOAc/MeOH) revealed the complete consumption of starting material (R_f 0.85) and the formation of a major product (R_f 0.40). The reaction mixture was then concentrated under reduced pressure to afford crude lactone **4.10** (4.89 g, assumed quant.) as a pale yellow oil. A small quantity of this crude was purified by flash column chromatography, to give a white crystalline solid, for the purposes of characterisation.

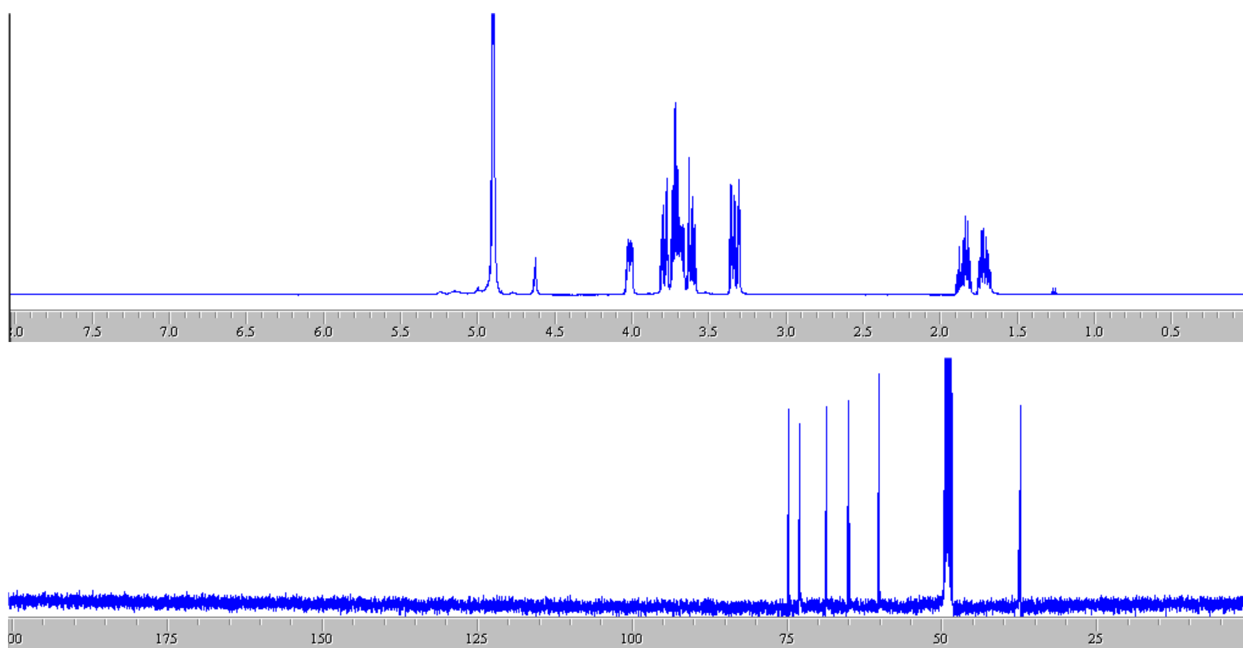
HRMS (ESI +ve): $C_6H_{10}NaO_5$ found 185.0420; (M+Na^+) requires 185.0420; $[\alpha]_D^{25}$ +70.4 (*c* 0.49, H_2O); m.p. 92-94°C; ν_{max} (thin film): 3385 (br, s, OH), 1762 (s, CO); δ_H (D_2O, 400 MHz): 2.55 (1H, d, H2a, J_{gem} 18.2), 3.05 (1H, dd, H2b, J_{gem} 18.2, $J_{2b,3}$ 5.3), 3.69 (1H, dd, H6a, J_{gem} 12.4, $J_{6a,5}$ 5.3), 3.82 (1H, dd, H6b, J_{gem} 12.4, $J_{6b,5}$ 2.8), 4.00 (1H, ddd, H5, $J_{5,4}$ 9.3, $J_{5,6a}$ 5.3, $J_{5,6b}$ 2.8), 4.46 (1H, dd, H4, $J_{4,3}$ 3.5, $J_{4,5}$ 9.3), 4.69 (1H, dd, H3, $J_{3,2b}$ 5.3, $J_{3,4}$ 3.5); δ_C (D_2O, 100 MHz): 39.0 (C2), 63.2 (C6), 67.8, 68.1 (C3, C4), 83.0 (C5), 179.6 (C1); LRMS (ESI –ve): 179 (68% M+OH^-), 275 (74% M+$CF_3CO_2^-$), 323 (95% 2M-H^+), 341 (100% 2M+OH^-); [Lit.[48] m.p. 95-96°C; $[\alpha]_D^{25}$ +73 (*c* 0.5, H_2O)].

2-Deoxy-D-*arabino*-hexitol, 4.4

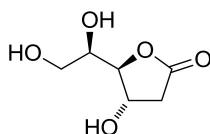


MeOH (16 mL) was added over a period of 1 h to $NaBH_4$ (1.65 g, 44 mmol) suspended in a solution of crude lactone **4.10** (3.36 g, assumed 17.2 mmol) in refluxing *tert*-butanol (60 mL) and stirred at reflux for a further 16 h. The reaction mixture was cooled on ice and 50% aqueous MeOH cautiously added until all precipitates had dissolved and no further evolution of hydrogen gas was observed. TLC analysis (9:1 EtOAc/MeOH) revealed the complete consumption of starting material (R_f 0.40) and the formation of a major product (R_f 0.05). The resultant solution was concentrated under reduced pressure, dissolved in MeOH and stirred with excess Dowex (50W-X8, H^+) for 2 h at RT. The Dowex was then removed by filtration and the filtrate concentrated under reduced pressure. Purification of the crude residue by flash column chromatography (5:2:1 EtOAc/IPA/water) afforded polyol **4.4** (2.00 g, 70%) as a white crystalline solid.

HRMS (ESI +ve): $C_6H_{14}NaO_5$ found 189.0732; ($M+Na^+$) requires 189.0733; $[\alpha]_D^{20}$ +17.2 (*c* 1.02, H_2O); m.p. 100-102°C; ν_{max} (thin film): 3385 (br, s, OH); δ_H (CD_3OD, 400 MHz): 1.64-1.72 (1H, m, H2a), 1.78-1.86 (1H, m, H2b), 3.32 (1H, dd, H4, $J_{4,3}$ 1.8, $J_{4,5}$ 8.1), 3.58 (1H, dd, H6a, J_{gem} 11.0, $J_{6a,5}$ 5.9), 3.63-3.78 (3H, m, H1, H5), 3.76 (1H, dd, H6b, J_{gem} 11.0, $J_{6b,5}$ 3.4), 3.99 (1H, ddd, H3, $J_{3,2}$ 9.1, 6.1, $J_{3,4}$ 1.8); δ_C (CD_3OD, 100 MHz): 36.5 (C2), 59.2 (C1), 64.1 (C6), 67.8 (C3), 72.1 (C5), 73.8 (C4); LRMS (ESI +ve): 189 (98% $M+Na^+$), 230 (100% $M+MeCN+Na^+$), 355 (76% $2M+Na^+$); [Lit.[53] m.p. 103.5-105°C; $[\alpha]_D^{20}$ +17.5 (*c* 1, H_2O)].



2-Deoxy-D-*ribo*-hexono-1,4-lactone, 4.11

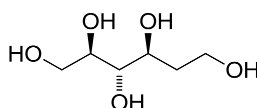


Hexonate **4.9** (2.60 g, 9.42 mmol) was dissolved in 4:25:15 TFA/water/1,4-dioxane (44 mL) and stirred at RT. TLC analysis after 24 h (9:1 EtOAc/MeOH) revealed the complete consumption of starting material (R_f 0.95) and the formation of a major product (R_f 0.30). The reaction mixture was then concentrated under reduced pressure to afford crude lactone **4.11** (1.60 g, assumed quant.) as an off white crystalline solid. A small quantity of this crude was purified by flash column chromatography for the purposes of characterisation.

HRMS (ESI +ve): $C_6H_{10}NaO_5$ found 185.0423; (M+Na$^+$) requires 185.0420; $[\alpha]_D^{20}$ -38.1 (*c* 1.49, EtOH); m.p. 66-68°C; ν_{max} (thin film): 3385 (br, OH), 1772 (s, CO); δ_H (D_2O, 400 MHz): 2.54 (1H, dd, H2a, J_{gem} 18.8, $J_{2a,3}$ 2.4), 3.04 (1H, dd, H2b, J_{gem} 18.8, $J_{2b,3}$ 7.2), 3.64 (1H, dd, H6a, J_{gem} 11.9, $J_{6a,5}$ 6.5), 3.72 (1H, dd, H6b, J_{gem} 11.9, $J_{6b,5}$ 4.1), 3.87 (1H, ddd, H5, $J_{5,4}$ 5.1, $J_{5,6a}$ 6.5, $J_{5,6b}$ 4.1), 4.49 (1H, dd, H4, $J_{4,3}$ 2.4, $J_{4,5}$ 5.1), 4.64 (1H, dt, H3, $J_{3,2a}$ 2.4, $J_{3,2b}$ 7.2, $J_{3,4}$ 2.4); δ_C (D_2O,

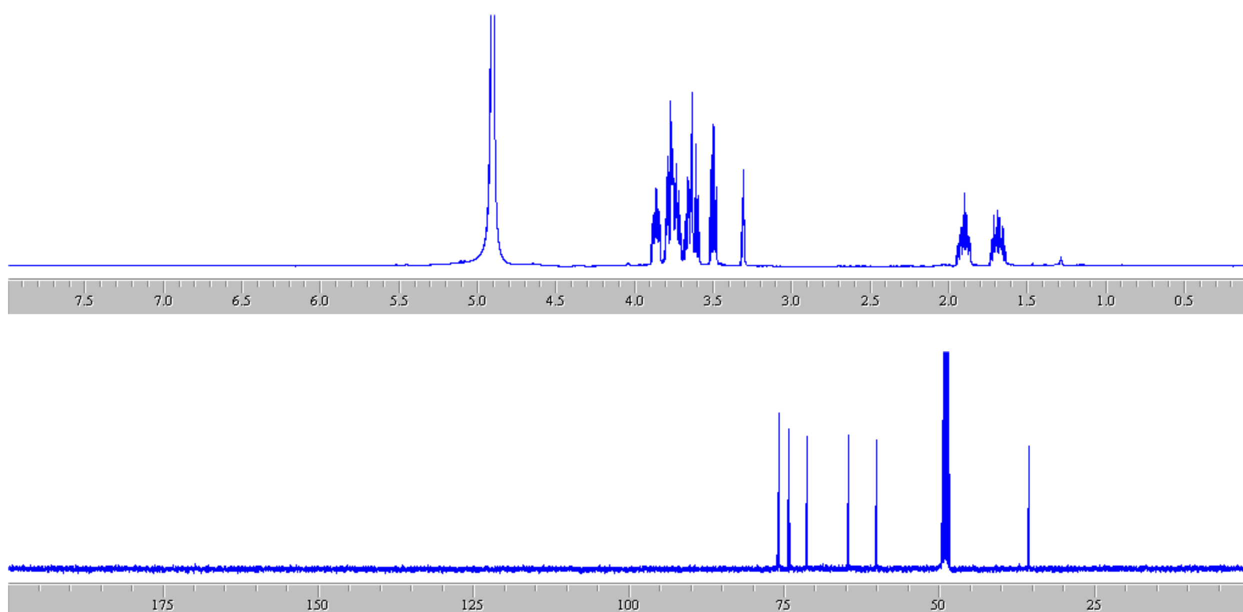
100 MHz): 37.7 (C2), 62.0 (C6), 67.6, 70.9 (C3, C4), 88.5 (C5), 179.6 (C1); LRMS (ESI –ve): 197 (47% $M+Cl^-$), 221 (44% $M+AcO^-$), 275 (46% $M+CF_3CO_2^-$), 323 (100% $2M-H^+$); [Lit.[54] m.p. 71-73°C; $[\alpha]_D$ -42 (EtOH)].

2-Deoxy-D-*ribo*-hexitol, 4.3



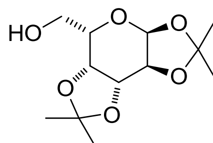
MeOH (7 mL) was added over a period of 1 h to $NaBH_4$ (850 mg, 22.5 mmol) suspended in a solution of crude lactone **4.11** (1.49 g, assumed 8.77 mmol) in refluxing *tert*-butanol (35 mL) and stirred at reflux for a further 16 h. The reaction mixture was cooled on ice and 50% aqueous MeOH cautiously added until all precipitates had dissolved and no further evolution of hydrogen gas was observed. TLC analysis (4:1 EtOAc/MeOH) revealed the complete consumption of starting material (R_f 0.45) and the formation of a major product (R_f 0.20). The resultant solution was concentrated under reduced pressure, dissolved in MeOH and stirred with excess Dowex (50W-X8, H^+) for 2 h at RT. The Dowex was then removed by filtration and the filtrate concentrated under reduced pressure. Purification of the crude residue by flash column chromatography (6:2:1 EtOAc/IPA/water) afforded polyol **4.3** (1.14 g, 78%) as a white crystalline solid.

HRMS (ESI +ve): $C_6H_{14}NaO_5$ found 189.0730; ($M+Na^+$) requires 189.0733; $[\alpha]_D^{25}$ -17.8 (*c* 1.62, MeOH); m.p. 82-84°C; ν_{max} (thin film): 3356 (br, s, OH); δ_H (CD_3OD, 400 MHz): 1.69 (1H, ddt, H2a, J_{gem} 14.1, $J_{2a,1}$ 5.6, $J_{2a,3}$ 9.3), 1.91 (1H, ddt, H2b, J_{gem} 14.1, $J_{2b,1}$ 7.0, $J_{2b,3}$ 2.8), 3.50 (1H, dd, H4, $J_{4,3}$ 5.8, $J_{4,5}$ 6.8), 3.59-3.80 (5H, m, H1, H5, H6), 3.87 (1H, ddd, H3, $J_{3,2a}$ 9.3, $J_{3,2b}$ 2.8, $J_{3,4}$ 5.8); δ_C (CD_3OD, 100 MHz): 35.6 (C2), 60.2 (C1), 64.7 (C6), 71.4 (C3), 74.2 (C5), 76.0 (C4); LRMS (ESI -ve): 165 (100% $M-H^+$), 331 (52% $2M-H^+$); [Lit.[55] m.p. 84-85°C; $[a]_D^{26}$ -20.4 (*c* 1.6, MeOH)].



4.5.2. Fluoro-hexitols

1,2;3,4-Di-*O*-isopropylidene-L-α-galactopyranose, 4.17L



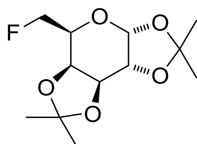
Concentrated sulfuric acid (1.5 mL, cat) was added to a stirring suspension of L-galactose (15.2 g, 84.4 mmol) and anhydrous copper(II) sulfate (33 g, 210 mmol) in acetone (300 mL) at RT. TLC analysis (EtOAc) after 18 h revealed a trace of starting material (R_f 0.00) and the formation of a major product (R_f 0.65). The mixture was neutralised with $NaHCO_3$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:19 to 1:1 EtOAc/CyH) afforded the title diacetonide **4.17L** (19.8 g, 90%) as a colourless oil.

HRMS (ESI +ve): $C_{12}H_{20}NaO_6$ found 283.1151; ($M+Na^+$) requires 283.1152; $[\alpha]_D^{25}$ +50.9 (*c* 1.20, $CHCl_3$); ν_{max} (thin film): 3492 (s, br, OH); δ_H ($CDCl_3$, 400 MHz): 3.33 (6H, s, 2 x Me), 1.45, 1.52 (2 x 3H, s, Me), 2.11 (1H, s, OH), 3.70-3.76 (1H, m, H6a), 3.82-3.89 (2H, m, H6b, H5), 4.26 (1H, d, H4 $J_{4,3}$ 8.0), 4.33 (1H, dd, H2 $J_{2,1}$ 4.9, $J_{2,3}$ 2.3), 4.60 (1H, dd, H3 $J_{3,2}$ 2.3, $J_{3,4}$ 8.0), 5.56 (1H, d, H1 $J_{1,2}$ 4.9); δ_C ($CDCl_3$, 100 MHz): 24.3, 24.9, 25.9, 26.0 (Me), 62.3 (C6), 68.1

(C5), 70.5 (C2), 70.7 (C3), 71.6 (C4), 96.3 (C1), 108.6, 109.4 (CMe$_2$); LRMS (ESI +ve): 283 (94%, M+Na$^+$), 299 (87%, M+K$^+$), 543 (100%, 2M+Na$^+$); Lit.[50] $[\alpha]_D^{23}$ +54.3 (*c* 1.0, $CHCl_3$).

The enantiomer **4.17D** was used as commercially supplied.

6-Deoxy-6-fluoro-1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose, 4.18D



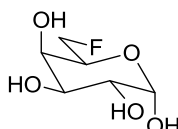
Trifluoromethanesulfonic anhydride (20 mL, 119 mmol) was added slowly to a solution of galactopyranose **4.17D** (23.5 g, 90.4 mmol) in 10:1 DCM/pyridine (220 mL) at -50°C. The reaction mixture was then stirred at -30°C for 30 min after which TLC analysis (2:1 CyH/EtOAc) revealed the complete consumption of starting material (R_f 0.15) and the formation of a major product (R_f 0.70). The reaction mixture was diluted with DCM (250 mL) and washed with HCl(aq) (1 M, 2 x 250 mL). The organic fraction was dried over $MgSO_4$, filtered and concentrated to give the crude triflate derivative, which was suspended in THF (100 mL) and TBAF solution (1 M in THF, 150 mL, 150 mmol) added. The reaction mixture was stirred at RT for 24 h, concentrated under reduced pressure, dissolved in ethyl acetate (400 mL) and washed with 1:1 brine/water (3 x 400 mL). The organic phase was dried over $MgSO_4$, filtered, concentrated and purified by flash column chromatography to afford the title fluoride **4.18D** (16.2 g, 68%) as a yellow oil.

HRMS (ESI +ve): $C_{12}H_{19}FNaO_5$ found 285.1112; (M+Na$^+$) requires 285.1109; $[\alpha]_D^{25}$ -44.2 (*c* 1.00, $CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 1.33 (6H, s, 2 x Me), 1.44, 1.54 (2 x 3H, s, Me), 4.07 (1H, dddd, H5, $J_{5,4}$ 2.0, $J_{5,6a}$ 7.0, $J_{5,6b}$ 5.3, $J_{5,F}$ 13.1), 4.26 (1H, dd, H4 $J_{4,3}$ 8.1, $J_{4,5}$ 2.0), 4.34 (1H, dd, H2 $J_{2,1}$ 5.1, $J_{2,3}$ 2.5), 4.52 (1H, ddd, H6a J_{gem} 9.6, $J_{6a,5}$ 6.9, $J_{6a,F}$ 47.9), 4.56 (1H, ddd, H6b J_{gem} 9.6, $J_{6b,5}$ 5.3, $J_{6b,F}$ 46.0), 4.63 (1H, ddd, H3 $J_{3,2}$ 2.4, $J_{3,4}$ 7.9, J 0.9), 5.55 (1H, d, H1 $J_{1,2}$ 5.1); δ_C ($CDCl_3$, 100 MHz): 24.4, 24.9, 25.9, 26.0 (Me), 66.6 (d, C5 $J_{5,F}$ 22.4), 70.4, 70.5 [d, J 6.4], 70.5

(C2, C3, C4), 82.0 (d, C6 $J_{6,F}$ 167.8), 96.2 (C1), 108.8, 109.6 (<u>C</u>Me$_2$); δ_F ($CDCl_3$, 400 MHz): -231.1 (F); LRMS (ESI +ve): 285 (73%, M+Na$^+$), 547 (100%, 2M+Na$^+$); Lit.[56] $[\alpha]_D^{20}$ -51.4 (*c* 1.284, $CHCl_3$).

Similar treatment of galactopyranose **4.17L** (13.0 g, 30.0 mmol) in a suitably scaled procedure gave fluoride **4.18L** (9.43 g, 72%); $[\alpha]_D^{25}$ +48.0 (*c* 1.29, $CHCl_3$).

6-Deoxy-6-fluoro-D-α-galactopyranose, 4.19D



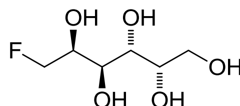
A solution of protected fluoro-galactose **4.18D** (1.59 g, 6.07 mmol) in 4:1 water/1,4-dioxane (50 mL) was stirred in the presence of Dowex (50W-X8, H$^+$) resin (5 g) at 50°C for 18 h. TLC analysis (EtOAc) revealed the complete consumption of starting material (R_f 0.90) and a single product (R_f 0.00). The resin was removed by filtration and the filtrate concentrated under reduced pressure, dissolved in water (30 mL) and washed with chloroform (3 x 30 mL). The aqueous phase was concentrated under reduced pressure, assisted by co-evaporation with ethanol, to give the title free fluorosugar **4.19D** (1.08 g, 98%) as a white crystalline solid. NMR analysis revealed a 7:4 mixture of anomers in aqueous solution.

HRMS (ESI +ve): $C_6H_{11}FNaO_5$ found 205.0486; (M+Na$^+$) requires 205.0483; $[\alpha]_D^{25}$ Initial: +119.8; Equilibrium: +69.4 (*c* 1.12, H_2O); m.p. 158-160°C (EtOH/MeOH); ν_{max} (thin film, Ge): 3385 (s, br, OH); δ_H (D_2O, 400 MHz): 3.50 (1H, dd, H2^A $J_{2,1}$ 8.1, $J_{2,3}$ 9.9), 3.66 (1H, dd, H3^A $J_{3,2}$ 10.1, $J_{3,4}$ 3.3), 3.80 (1H, dd, H2^B $J_{2,1}$ 3.5, $J_{2,3}$ 10.4), 3.87 (1H, dd, H3^B $J_{3,2}$ 10.4, $J_{3,4}$ 3.0), 3.97 (1H, d, H4^A $J_{4,3}$ 3.5), 3.97 (1H, ddd, H5^A $J_{5,6}$ 4.0, 7.1, $J_{5,F}$ 14.9), 4.03 (1H, d, H4^B $J_{4,3}$ 3.0), 4.33 (1H, ddd, H5^B $J_{5,6}$ 3.8, 7.1, $J_{5,F}$ 16.4), 4.50-4.73 (4H, m, H6$^{A\&B}$), 4.61 (1H, d, H1^A $J_{1,2}$ 8.1), 5.27 (1H, d, H1^B $J_{1,2}$ 3.5); δ_C (D_2O, 100 MHz): 68.8 (C2^B), 69.0 (d, C4^A $J_{4,F}$ 7.2), 69.4 (C3^B), 69.6 (d, C4^B $J_{4,F}$ 7.2), 69.6 (d, C5^B $J_{5,F}$ 20.0), 72.3 (C2^A), 73.1 (C3^A), 74.0 (d, C5^A $J_{5,F}$ 20.0), 83.7 (d, C6^A $J_{6,F}$ 165.4), 84.1 (d, C6^B $J_{6,F}$ 164.6), 93.0 (C1^B), 97.0 (C1^A); δ_f (D_2O, 400 MHz): -229.8 (F^B),

-229.6 (F^A); LRMS (ESI +ve): 205 (100%, $M+Na^+$); [Lit.[56] m.p. 160°C; $[a]_D^{20}$ Initial: +135, Equlibrium: +76.5 (*c* 0.967, H_2O)].

For the enantiomer **4.19L**: $[\alpha]_D^{25}$ Initial: -125.7; Equilibrium: -74.1 (*c* 1.23, H_2O); m.p. 156-158°C (EtOH/MeOH).

1-Deoxy-1-fluoro-L-galactitol, 4.14L

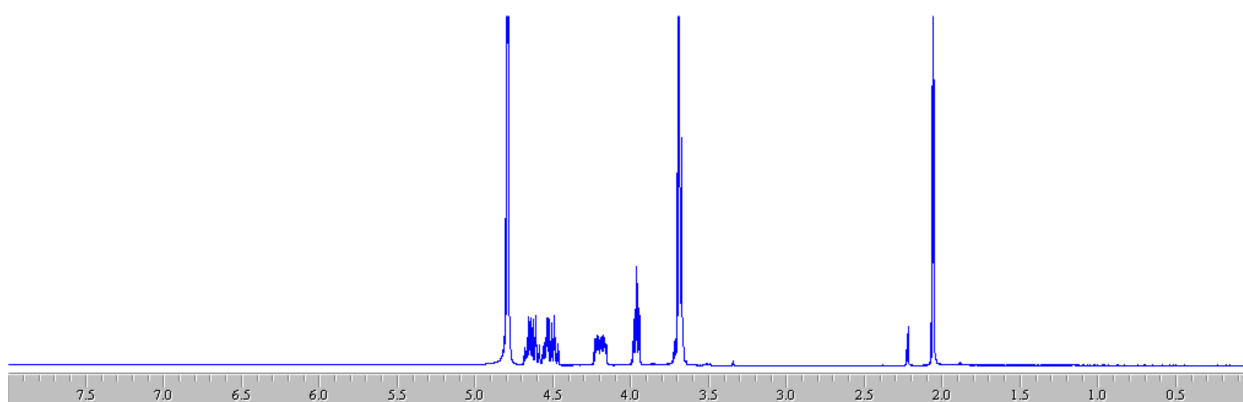


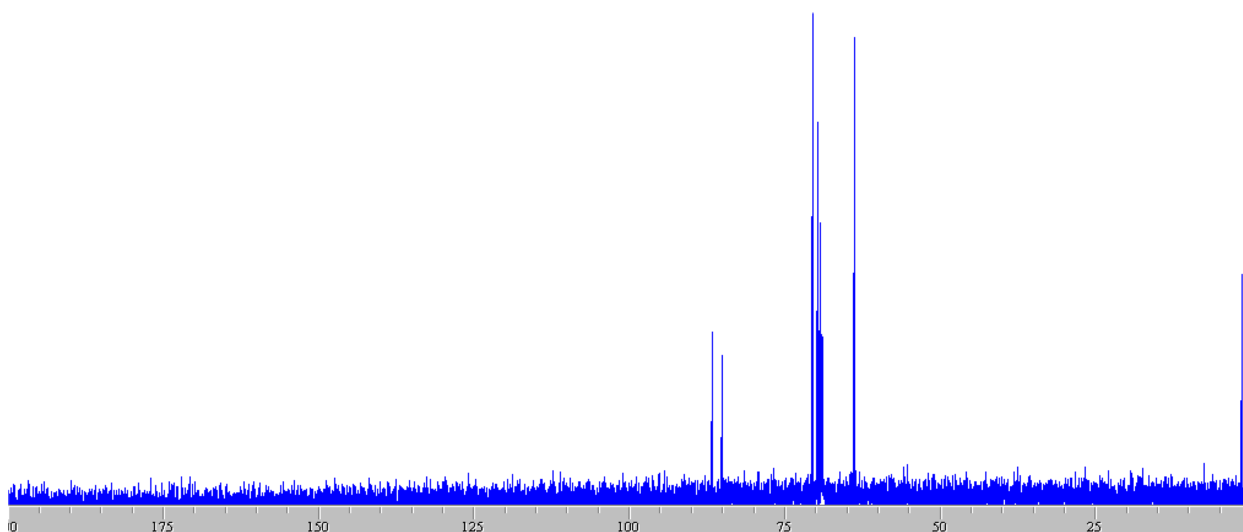
A solution of protected fluoro-galactopyranose **4.18D** (16.2 g, 61.8 mmol) in 4:1 water/1,4-dioxane (150 mL) was stirred in the presence of Dowex (50W-X8, H^+) resin (40 g) at 60°C for 18 h. TLC analysis (EtOAc) revealed the complete consumption of starting material (R_f 0.90) and a single product (R_f 0.00). The resin was removed by filtration and the filtrate concentrated under reduced pressure to give crude fluoro-galactopyranose **4.19D** (12.1 g, assumed quant.). Crude **4.19D** (11.6 g, assumed 59.2 mmol) was dissolved in water (120 mL), sodum borohydride (4.5 g, 120 mmol) added and the reaction mixture stirred at RT for 1 h. TLC analysis (5:3:2 *n*-BuOH/EtOH/H_2O) revealed the complete consumption of starting material (R_f 0.55) and the formation of a single product (R_f 0.35). The mixture was concentrated to dryness under reduced pressure, dissolved in hot methanol (250 mL), neutralised with Dowex 50W-X8 (H^+) resin and stirred at 60°C for 90 min. The resin was removed by filtration and the filtrate concentrated under reduced pressure to give the crude title fluoro-polyol **4.14L** as a pale yellow crystalline solid. Recrystallisation from refluxing ethanol/methanol gave the title fluoro-polyol **4.14L** (9.55 g, 88%) as a white crystalline solid.

HRMS (ESI +ve): $C_6H_{13}FNaO_5$ found 207.0641; ($M+Na^+$) requires 207.0639; $[\alpha]_D^{25}$ +4.1 (*c* 1.06, H_2O); m.p. 172-174°C; ν_{max} (thin film, Ge): 3577 (s, OH), 3315 (s, br, OH); δ_H (D_2O, 400 MHz): 3.67-3.73 (4H, m, H3, [H4 or H5], H6a, H6b), 3.98 (1H, t, H4 or H5 *J* 6.6), 4.20 (1H, ddd, H2 $J_{2,1a}$ 7.3, $J_{2,1b}$ 4.5, $J_{2,F}$ 15.7), 4.55 (1H, ddd, H1a J_{gem} 9.6, $J_{1a,2}$ 7.3, $J_{1a,F}$ 47.7), 4.61 (1H,

ddd, H1b J_{gem} 9.6, $J_{1b,2}$ 4.5, $J_{1a,F}$ 46.0); δ_C (D_2O, 100 MHz): 63.8 (C6), 69.2 (d, C2 $J_{2,F}$ 19.2), 69.4 (d, C3 $J_{3,F}$ 6.4), 69.8, 70.6 (C4, C5), 85.9 (d, C1 $J_{1,F}$ 166.2); δ_F (D_2O, 400 MHz): -228.2 (F); LRMS (ESI +ve): 207 (100%, $M+Na^+$); [Lit.[57] m.p. 173-174°C; $[a]_D^{21}$ +4.2 (*c* 0.5, H_2O)].

Similar treatment of **4.18L** (13.1 g, 50.0 mmol) in a suitably scaled procedure gave **4.14D** (8.33 g, 91%); $[\alpha]_D^{25}$ -4.4 (*c* 1.06, H_2O); m.p. 172-174°C.





4.6. References

1. Izumori, K. *J Biotechnol* **2006,** *124*, 717-722.
2. Granstrom, T. B.; Takata, G.; Tokuda, M.; Izumori, K. *J Biosci Bioeng* **2004,** *97*, 89-94.
3. Rao, D.; Best, D.; Yoshihara, A.; Gullapalli, P.; Morimoto, K.; Wormald, M. R.; Wilson, F. X.; Izumori, K.; Fleet, G. W. J. *Tetrahedron Letters* **2009,** *50*, 3559-3563.
4. Gullapalli, P.; Shiji, T.; Rao, D.; Yoshihara, A.; Morimoto, K.; Takata, G.; Fleet, G. W. J.; Izumori, K. *Tetrahedron: Asymmetry* **2007,** *18*, 1995-2000.
5. Shimonishi, T.; Okumura, Y.; Izumori, K. *J Ferment Bioeng* **1995,** *79*, 620-622.
6. Muniruzzaman, S.; Tokunaga, H.; Izumori, K. *J Ferment Bioeng* **1995,** *79*, 323-327.

7. Yoshida, H.; Yamada, M.; Nishitani, T.; Takada, G.; Izumori, K.; Kamitori, S. *Acta Crystallogr F* **2007,** *63*, 123-125.
8. Itoh, H.; Okaya, H.; Khan, A. R.; Tajima, S.; Hayakawa, S.; Izumori, K. *Biosci Biotech Bioch* **1994,** *58*, 2168-2171.
9. Ishida, Y.; Kamiya, T.; Itoh, H.; Kimura, Y.; Izumori, K. *J Ferment Bioeng* **1997,** *83*, 529-534.
10. Itoh, H.; Sato, T.; Izumori, K. *J Ferment Bioeng* **1995,** *80*, 101-103.
11. Itoh, H.; Izumori, K. *J Ferment Bioeng* **1996,** *81*, 351-353.
12. Itoh, H.; Sato, T.; Takeuchi, T.; Khan, A. R.; Izumori, K. *J Ferment Bioeng* **1995,** *79*, 184-185.
13. Sultana, I.; Rahman Mizanur, M. D.; Takeshita, K.; Takada, G.; Izumori, K. *J Biosci Bioeng* **2003,** *95*, 342-347.
14. Menavuvu, B. T.; Poonperm, W.; Takeda, K.; Morimoto, K.; Granström, T. B.; Takada, G.; Izumori, K. *J Biosci Bioeng* **2006,** *102*, 436-441.
15. Menavuvu, B. T.; Poonperm, W.; Leang, K.; Noguchi, N.; Okada, H.; Morimoto, K.; Granström, T. B.; Takada, G.; Izumori, K. *J Biosci Bioeng* **2006,** *101*, 340-345.
16. Leang, K.; Takada, G.; Fukai, Y.; Morimoto, K.; Granström, T. B.; Izumori, K. *Biochim. Biophys Acta* **2004,** *1674*, 68-77.
17. Bhuiyan, S. H.; Itami, Y.; Takada, G.; Izumori, K. *J Biosci Bioeng* **1999,** *88*, 567-570.
18. Bhuiyan, S. H.; Itami, Y.; Rokui, Y.; Katayama, T.; Izumori, K. *J Ferment Bioeng* **1998,** *85*, 539-541.
19. Bhuiyan, S. H.; Itami, Y.; Izumori, K. *J Ferment Bioeng* **1997,** *84*, 558-562.
20. Yoshihara, A.; Haraguchi, S.; Gullapalli, P.; Rao, D.; Morimoto, K.; Takata, G.; Jones, N.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J.; Izumori, K. *Tetrahedron: Asymmetry* **2008,** *19*, 739-745.
21. Gullapalli, P.; Yoshihara, A.; Morimoto, K.; Rao, D.; Akimitsu, K.; Jenkinson, S. F.; Fleet, G. W. J.; Izumori, K. *Tetrahedron Letters* **2010,** *51*, 895-898.
22. Rao, D.; Yoshihara, A.; Gullapalli, P.; Morimoto, K.; Takata, G.; da Cruz, F. P.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J.; Izumori, K. *Tetrahedron Letters* **2008,** *49*, 3316-3321.
23. Jones, N. A.; Rao, D.; Yoshihara, A.; Gullapalli, P.; Morimoto, K.; Takata, G.; Hunter, S. J.; Wormald, M. R.; Dwek, R. A.; Izumori, K.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2008,** *19*, 1904-1918.
24. Booth, K. V.; Jenkinson, S. F.; Rao, D.; Simonisi, T.; Fleet, G. W. J.; Izumori, K.; Watkin, D. J. *Acta Crystallogr E* **2008,** *64*, o1568-o1569.
25. Jenkinson, S. F.; Booth, K. V.; Newberry, S.; Fleet, G. W. J.; Izumori, K.; Morimoto, K.; Nash, R. J.; Jones, L.; Watkin, D. J.; Thompson, A. L. *Acta Crystallogr E* **2009,** *65*, o1755-o1756.
26. Jenkinson, S. F.; Lenagh-Snow, G. M. J.; Izumori, K.; Fleet, G. W. J.; Watkin, D. J.; Thompson, A. L. *Acta Crystallogr E* **2010,** *66*, o525-o526.
27. Schitter, G.; Wrodnigg, T. M. *Expert Opin Drug Dis* **2009,** *4*, 315-356.
28. Romero, A.; Liang, C. H.; Chiu, Y. H.; Yao, S. L.; Duffield, J.; Sucheck, S. J.; Marby, K.; Rabuka, D.; Leung, P. Y.; Shue, Y. K.; Ichikawa, Y.; Hwang, C. K. *Tetrahedron Letters* **2005,** *46*, 1483-1487.
29. Cheng, Y.; Guo, A. L.; Guo, D. S. *Curr Org Chem* **2010,** *14*, 977-999.
30. Kent, P. W. In *Fluorinated Carbohydrates*; American Chemical Society, 1988; pp. 1-12.
31. Withers Stephen, G.; Street Ian, P.; Percival Michael, D. In *Fluorinated Carbohydrates*; American Chemical Society, 1988; pp. 59-77.
32. Hadwiger, P.; Mayr, P.; Nidetzky, B.; Stutz, A. E.; Tauss, A. *Tetrahedron: Asymmetry* **2000,** *11*, 607-620.

33. Caravano, A.; Field, R. A.; Percy, J. M.; Rinaudo, G.; Roig, R.; Singh, K. *Org Biomol Chem* **2009,** *7*, 996-1008.
34. Taylor, N. F.; Sbrissa, D.; Squire, S. T.; D'Amore, T.; McIntosh, J. M. In *Fluorinated Carbohydrates*; American Chemical Society, 1988; pp. 109-137.
35. Hitz William, D. In *Fluorinated Carbohydrates*; American Chemical Society, 1988; pp. 138-155.
36. Cobb, S. L.; Deng, H.; Hamilton, J. T. G.; McGlinchey, R. P.; O'Hagan, D.; Schaffrath, C. *Bioorg Chem* **2005,** *33*, 393-401.
37. Cobb, S. L.; Deng, H.; Hamilton, J. T. G.; McGlinchey, R. P.; O'Hagan, D. *Chem Commun* **2004**, 592-593.
38. Gatley, S. J.; Holden James, E.; DeGrado Timothy, R.; Ng Chin, K.; Halama James, R.; Koeppe Robert, A. In *Fluorinated Carbohydrates*; American Chemical Society, 1988; pp. 156-175.
39. Trayner, B. J.; Grant, T. N.; West, F. G.; Cheeseman, C. I. *Bioorg Med Chem* **2009,** *17*, 5488-5495.
40. Grant, T. N.; Trayner, B.; Cheeseman, C. I.; West, F. G.; Mercer, J. *J Labelled Compd Rad* **2009,** *52*, S190-S190.
41. Ashique, R.; Chirakal, R. V.; Hughes, D. W.; Schrobilgen, G. J. *Carbohydr Res* **2006,** *341*, 457-466.
42. Humphlett, W. J. *Carbohydr Res* **1967,** *4*, 157-164.
43. Cohen, N.; Banner, B. L.; Laurenzano, A. J.; Carozza, L. *Org. Synth.* **1985,** *63*, 127.
44. Hanessian, S.; Girard, C. *Synlett* **1994**, 865-7.
45. Orsini, F.; Di, T. E. *Tetrahedron: Asymmetry* **2003,** *14*, 2521-2528.
46. Nacro, K.; Baltas, M.; Escudier, J. M.; Gorrichon, L. *Tetrahedron* **1996,** *52*, 9047-9056.
47. Jenkinson, S. F.; Booth, K. V.; Best, D.; Fleet, G. W. J.; Watkin, D. J. *Acta Crystallogr E* **2008,** *64*, o2011-o2012.
48. Kawakish.S; Namiki, M. *Carbohydr Res* **1973,** *26*, 252-254.
49. Soai, K.; Oyamada, H.; Takase, M.; Ookawa, A. *B Chem Soc Jpn* **1984,** *57*, 1948-1953.
50. Brackhagen, M.; Boye, H.; Vogel, C. *J Carbohydr Chem* **2001,** *20*, 31-43.
51. Jenkinson, S. F.; Best, D.; Izumori, K.; Wilson, F. X.; Weymouth-Wilson, A. C.; Fleet, G. W. J.; Thompson, A. L. *Acta Crystallogr E* **2010,** *66*, o1315.
52. Jenkinson, S. F.; Best, D.; Izumori, K.; Wilson, F. X.; Weymouth-Wilson, A. C.; Fleet, G. W. J.; Thompson, A. L. *Acta Crystallogr E* **2010,** *66*, o1330.
53. Regeling, H.; Chittenden, G. J. F. *Carbohydr Res* **1990,** *205*, 261-268.
54. Zorbach, W. W.; Bhat, C. C.; Bhat, K. V. *Adv Chem Ser* **1968**, 1-14.
55. Jarrell, H. C.; Szarek, W. A. *Carbohydr Res* **1978,** *67*, 43-54.
56. Taylor, N. F.; Kent, P. W. *J Chem Soc* **1958**, 872-876.
57. Kent, P. W.; Wright, J. R. *Carbohydr Res* **1972,** *22*, 193-&.

Appendix: Publications Arising from this Thesis

Related to **Chapter 2**:

Daniel Best, Chen Wang, Alexander C. Weymouth-Wilson, Robert A. Clarkson, Francis X. Wilson, Robert J. Nash, Saori Miyauchi, Atsushi Kato, George W.J. Fleet **Looking glass inhibitors: scalable syntheses of DNJ, DMDP, and (3*R*)-3-hydroxy-L-bulgecinine from D-glucuronolactone and of L-DNJ, L-DMDP, and (3*S*)-3-hydroxy-D-bulgecinine from L-glucuronolactone. DMDP inhibits β-glucosidases and β-galactosidases whereas L-DMDP is a potent and specific inhibitor of α-glucosidases** *Tetrahedron: Asymmetry* **2010**, *21,* 311-319

Daniel Best, Phoom Chairatana, Andreas F.G. Glawar, Elizabeth Crabtree, Terry D. Butters, Francis X. Wilson, Chu-Yi Yu, Wu-Bao Wang, Yue-Mei Jia, Isao Adachi, Atsushi Kato, George W.J. Fleet **Synthesis of 2-acetamido-1,2-dideoxy-D-*galacto*-nojirimycin [DGJNAc] from D-glucuronolactone: the first sub-micromolar inhibitor of α-*N*-acetylgalactosaminidases** *Tetrahedron Letters* **2010**, *17*, 2222-2224

Alexander C. Weymouth-Wilson, Robert A. Clarkson, Nigel A. Jones, Daniel Best, Francis X. Wilson, Maria-Soledad Pino-González, George W.J. Fleet **Large scale synthesis of the acetonides of L-glucuronolactone and of L-glucose: easy access to L-sugar chirons** *Tetrahedron Letters* **2009**, *50,* 6307-6310

Daniel Best, Sarah F. Jenkinson, Amber L. Thompson, David J. Watkin, Francis X. Wilson, Robert J. Nash and George W. J. Fleet **(2*S*,3*R*,4*R*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)pyrrolidine-2-carboxylic acid [(2*S*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)proline]** *Acta Cryst.* **2009**, E*65*, o2418-o2419

Related to **Chapter 3**:

Daniel Best, Sarah F. Jenkinson, Sebastian D. Rule, Rosemary Higham, Thomas B. Mercer, Richard J. Newell, Alexander C. Weymouth-Wilson, George W.J. Fleet, Sigthor Petursson **High yield protection of alcohols, including tertiary and base sensitive alcohols, as benzhydryl ethers by heating with diphenyldiazomethane in the absence of any other reagent** *Tetrahedron Letters*, **2008,** *14*, 2196-2199

Related to **Chapter 4**:

Devendar Rao, Daniel Best, Akihide Yoshihara, Pushpakiran Gullapalli, Kenji Morimoto, Mark R. Wormald, Francis X. Wilson, Ken Izumori, George W.J Fleet **A concise approach to the synthesis of all twelve 5-deoxyhexoses: D-tagatose-3-epimerase—a reagent that is both specific and general** Tetrahedron Letters **2009**, *50*, 3559-3563

Sarah F. Jenkinson, K. Victoria Booth, Daniel Best, George W. J. Fleet and David J. Watkin
***tert*-Butyl 2-deoxy-4,5-*O*-isopropylidene-D-gluconate** *Acta Cryst.* **2008**, E*64*, o2011-o2012

Sarah F. Jenkinson, Daniel Best, Ken Izumori, Francis X. Wilson, Alexander C. Weymouth-Wilson, George W. J. Fleet and Amber L. Thompson **6-Deoxy-6-fluoro-D-galactose** *Acta Cryst.* **2010**, E*66*, o1315

Sarah F. Jenkinson, Daniel Best, Ken Izumori, Francis X. Wilson, Alexander C. Weymouth-Wilson, George W. J. Fleet and Amber L. Thompson **1-Deoxy-1-fluoro-L-galactitol** *Acta Cryst.* **2010**, E*66*, o1330