

The synthesis and biological evaluation of novel *N*-acetylhexosaminidase inhibitors

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Abstract

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Iminosugars are known to behave as carbohydrate mimics in biological systems by virtue of their similar structures. However as the ring nitrogen prevents metabolism it means that iminosugars have the potential to become inhibitors of these systems. It is known, for example, that iminosugars can behave as mimics in the hydrolysis mechanism. This leads to possible medicinal applications of iminosugars. One such case is lysosomal storage disorders which arise as a result of a genetic defect which causes missense mutations coding for the *N*-acetylhexosaminidase enzymatic protein. *N*-Acetylhexosaminidases are a sub-member of the class of glycosidase enzymes. They are responsible for the cleavage of *N*-acetylhexosamine residues from glycoconjugates in the lysosome. Mutations in the gene coding for this protein lead to a deficiency in the enzymatic activity resulting in accumulation of unhydrolysed substrate in the lysosome. Lysosomal storage disorders have a phenotype of poor motor development and neurological problems. The infantile form usually leads to death before the age of five. An iminosugar mimic could give rise to a possible treatment for lysosomal storage disorders by acting as a molecular chaperone during protein folding, promoting correct folding by its intrinsic affinity for the native fold of the enzyme. Likewise in the treatment of cancer, the inhibitory ability of iminosugars has potential applications. In cancer, extracellular hydrolysis occurs which favours cancer cell survival. Macrophages, which attack and eliminate cancer cells, can be activated by macrophage activating factor (MAF) which displays an α -*N*-acetylgalactosamine residue that appears essential for the activation cascade. Cancer cells secrete an α -*N*-acetylgalactosaminidase enzyme that acts to decrease the potency of MAF, thus promoting cancer cell survival. Inhibition of cancer cell α -*N*-acetylgalactosaminidase may restore macrophage activation and generate potential therapeutics.

Chapter 1 of this thesis contains extended discussion of the aforementioned, and related, diseases and the therapeutic applications of iminosugars. Some historically and biologically important iminosugars are described along with some current iminosugar drugs.

Chapter 2 describes the synthetic strategies explored in an attempt to synthesise all the members of the 2-acetamido pyrrolidine iminosugars. An overview of the compounds synthesised towards this end by a past group member is given along with the work performed as part of this thesis to complete this goal. Both enantiomers with *arabino*- and *ribo*- stereochemistry and *D-lyxo*- were previously synthesised. The syntheses of both enantiomers with *xylo*- stereochemistry along with the *L-lyxo*- compound were completed as part of this thesis, from either *D*- or *L*-glucuronolactone and *D*-ribose, respectively.

Chapter 3 details the synthetic strategy adopted to synthesise the enantiomer of *D*-DNJNAc, the first potent α -*N*-acetylgalactosaminidase inhibitor to be found. The synthesis towards another piperidine iminosugar, 6-deoxy DGJNAc, is presented in the second half of this chapter, along with two related compounds.

Acknowledgements

I would not be in a position to be even writing this if it were not for George agreeing to take me on for a DPhil nearly four years ago, for which I extend huge gratitude. George's supervision throughout my time in the group has been invaluable. Thank you very much for being so patient and supportive and for allowing me to work on the projects I have. Likewise Terry has been a great source of help for which I am very grateful. It has been a privilege to be able to work with his group and partake in some biological work. This point leads me to also thank Andreas for his help with the enzyme assays. Everything became a lot easier once I had someone to discuss this with and thank you for putting up with all the 'plastic saving' and me always getting confused about the concentrations.

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I will mention the music at this point. My time in the lab opened my eyes to popular music, some less to my taste than others perhaps but that was character building. I won't forget all the Seb mixes, key changes and manic Mondays! I am also thankful for the music that got me through writing up (thanks Cate and JJ :p).

I never thought I would be sad to see the back of a lab. Thank you to all the part IIs that have passed through. All of you have been great fun, despite potential fears when you all turned up on the first day.

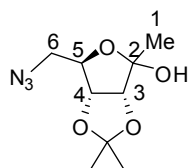
Finally thank you Mum, Dad and Cate for your never ending support. Perhaps all the 'in the lab..' stories will stop now. I owe you a lot and I love you very much. Rinny and Little I've not forgotten you either.

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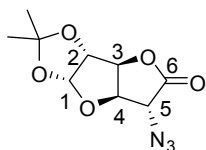
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Note Concerning Nomenclature

The numbering convention used in this report for NMR data is derived from the carbohydrate recommendations according to IUPAC.[§] Examples for reference are given below.

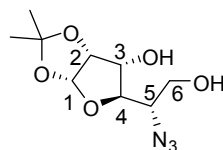


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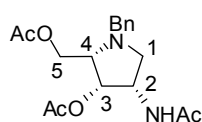
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- β -L-idurono-3,6-lactone



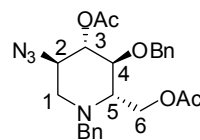
5-Azido-5-deoxy-1,2-*O*-isopropylidene

- β -L-talofuranose



2-Acetamido-3,5-di-*O*-acetyl-*N*-benzyl

-1,2,4-trideoxy-1,4-imino-L-lyxitol



3,6-Di-*O*-acetyl-2-azido-1-*N*,4-*O*-dibenzyl

-1,2,5-trideoxy-1,5-imino-L-glucitol

[§]McNaught A.D.: Nomenclature of carbohydrates. *Carbohydrate Research*, **1997**, 297, 1-92 i

Abbreviations

a-	apparent
Ac	acetyl
Ar	aromatic
Asp	aspartic acid
BBB	blood brain barrier
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
br	broad
Bzh	benzhydryl
<i>c</i>	concentration (in α_D)
CMT	chaperone mediated therapy
CNS	central nervous system
d	double/doublet
DAB	1,4-dideoxy-1,4-imino-D-arabinitol
DCM	dichloromethane
DGJ	1-deoxygalactonojirimycin
DGJNAc	2-acetamido-1,5-imino-1,2,5-trideoxy-D-galactitol
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminium hydride
DIM	1,4-dideoxy-1,4-imino-D-mannitol
dioxane	1,4-dioxane
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-(<i>N,N</i> -dimethylamino)-pyridine
DMDP	2,5-dideoxy-2,5-imino-D-mannitol
DMF	<i>N,N</i> -dimethylformamide
DMP	Dess Martin periodinane
DNJ	1-deoxynojirimycin
DNJNAc	2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol
DPPA	diphenyl phosphoryl azide
DRB	1,4-dideoxy-1,4-imino-D-ribitol
DRBNAc	2-acetamido-1,2,4-trideoxy-1,4-imino-D-ribitol
ER	endoplasmic reticulum
ES+/-	electrospray ionisation
ERT	enzyme replacement therapy
eq	equivalents
FI	field ionisation
GAG	glycosaminoglycan
Gal	galactose
GalNAc	<i>N</i> -acetylgalactosamine
GCase	glucosylceramidase
Glc	glucose
GlcCer	glucosylceramide
GlcNAc	<i>N</i> -acetylglucosamine
Glu	glutamic acid

GSL	glycosphingolipids
h	hours
HIV	human immunodeficiency virus
HRMS	high resolution mass spectrometry
IC ₅₀	half maximal inhibitory concentration
IFG	isofagomine
IR	infrared
IPA	isopropyl alcohol
K _i	inhibition constant
LAB	1,4-dideoxy-1,4-imino-L-arabinitol
LABNAc	2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol
Lit.	literature value
LRBNAc	2-acetamido-1,2,4-trideoxy-1,4-imino-L-ribitol
LRMS	low resolution mass spectrometry
LSD	lysosomal storage disorder
LyxNAc	2-acetamido-1,2,4-trideoxy-1,4-imino-D-lyxitol
m	multiplet (in NMR), medium (in IR)
MAF	macrophage activating factor
Me	methyl
mesylate	methanesulfonyl
min	minute(s)
m.p.	melting point
Ms	methanesulfonyl
MS	mass spectrometry
NAc	<i>N</i> -acetyl
NANA	<i>N</i> -acetylneuraminic acid
NBn	<i>N</i> -benzyl
NB-DNJ	<i>N</i> -butyl-1-deoxynojirimycin
ND	not determined
NGT	NAG-thiazoline
NMR	nuclear magnetic resonance
PC	pharmacological chaperone
PCC	pyridinium chlorochromate
Ph	phenyl
PUGNAc	<i>O</i> -(2-acetamido-2-deoxy-D-glucopyranosylidene)amino- <i>N</i> -phenylcarbamate
pyr	pyridine
q	quartet
quant	quantitative
quin	quintet
R _f	retention factor
s	singlet (in NMR), strong (in IR)
SRT	substrate reduction therapy
t	triplet
TBAF	tetrabutylammonium fluoride
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBSCl	<i>tert</i> -butyldimethylsilyl chloride

Abbreviations

TEA	triethylamine
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	threonine
t.l.c.	thin layer chromatography
tosylate	<i>p</i> -toluenesulfonyl
TPAP	tetrapropylammonium perruthenate
triflate	trifluoromethanesulfonyl
Ts	<i>p</i> -toluenesulfonyl
TS	transition state
w	weak
XylNAc	2-acetamido-1,2,4-trideoxy-1,4-imino-D-xylitol

Chapter 1: General Introduction

Glycosidases

Biologically, carbohydrates are very important molecules. They are involved in a variety of processes including cell signalling and inflammation. Glycosidase enzymes are used to catalyse the cleavage of the glycosidic bond, the ester linkage between two sugar units. There are two proposed mechanisms for hydrolysis, which are given in figure 1.

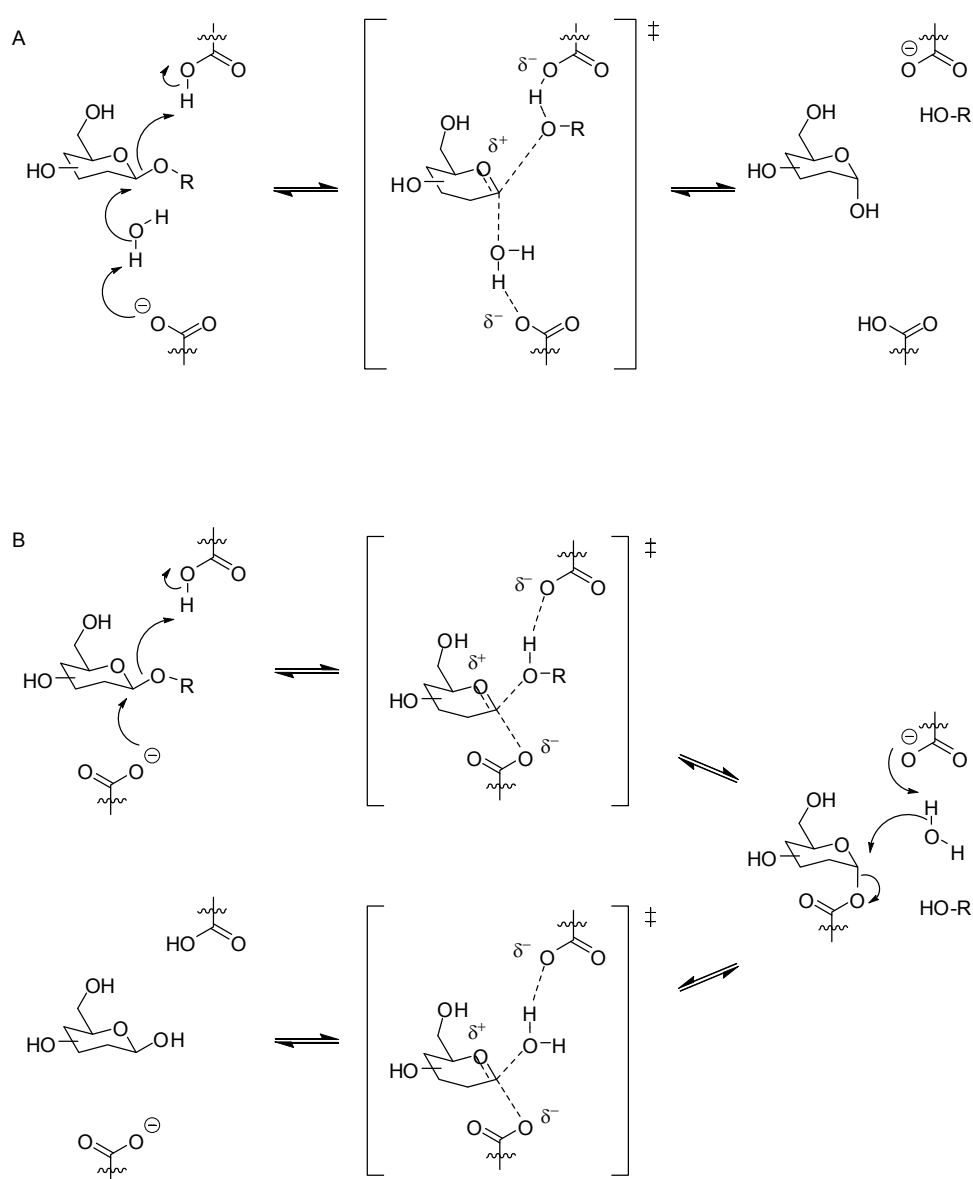


Figure 1: A inverting mechanism **B** retaining mechanism

In the first case, A in figure 1, a carboxylate side chain in the enzyme active site acts as a general base which attacks the hydrogen atom of a water molecule, which is also present in the active site. The water molecule attacks the anomeric centre of the sugar simultaneously to allow the rest of the chain originally attached to the terminal residue to leave and abstract a proton, from a second carboxylic acid group in the active site which acts as a general acid. The resulting product is a free monosaccharide with inverted stereochemistry at the anomeric centre compared to the substrate. A second mechanism, B, resulting in overall retention at the anomeric centre, occurs in other enzymes by virtue of the carboxylate residue attacking the anomeric centre of the terminal residue directly causing loss of the rest of the chain. This leaving group takes a proton from a carboxylic acid side chain as before. A water molecule now participates. The newly formed carboxylate residue which just lost its proton removes a proton from the water molecule whilst concurrently the electron pair from that breaking O-H bond attacks the anomeric centre, releasing the carboxylate side chain. Two inversions have therefore taken place, via S_N2 reactions, leading to overall retention. For both of these mechanisms it is proposed that the sugar goes through a transition state with oxocarbenium character where it adopts a half chair configuration. Interestingly, the distance between the two amino acid residues for inverting enzymes is greater at around $9.5\text{\AA}$ compared to $5.3\text{\AA}$ for retaining enzymes, presumably because the presence of water in the active site is required in the inverting case.¹

Iminosugars

Iminosugars are structurally the same as monosaccharides except the endo oxygen atom has been replaced by a nitrogen. This similarity allows iminosugars to behave as carbohydrates mimics *in vivo*, for example they interact with the same enzymes as their

natural counter parts. However, the presence of the nitrogen atom prevents their metabolism which means iminosugars are set up to behave as ideal enzyme inhibitors. Under physiological pH the nitrogen is protonated which allows the iminosugar to behave as a transition state mimic in the enzyme mechanism because it displays electro- and stereochemical features similar to the proposed oxocarbenium transition state.

There are a number of naturally occurring iminosugars. The first natural iminosugar to be found was the glucose mimic nojirimycin, whose structure was identified in 1966.^{2,3} This is related to the 1-deoxy equivalent deoxynojirimycin (DNJ) **1**, found in Mulberry tree roots. 1,4-Dideoxy-1,4-imino-D-arabinitol (DAB) **2** is isolable from *Anglyocalyx boutiqueanus*.⁴ Figure 2 shows the structure of some examples of the five different natural forms of iminosugars: pyrrolidines, piperidines, indolizidines, pyrrolizidines and nortropanes. DAB **2** will be described in more detail later.

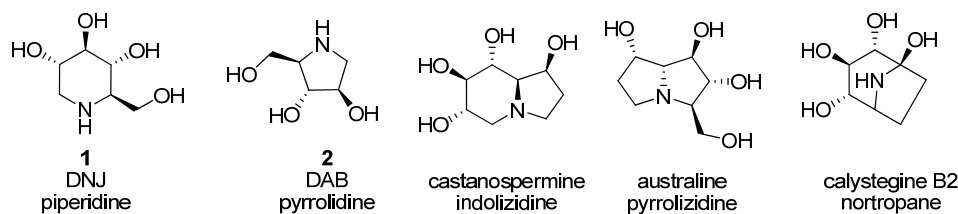
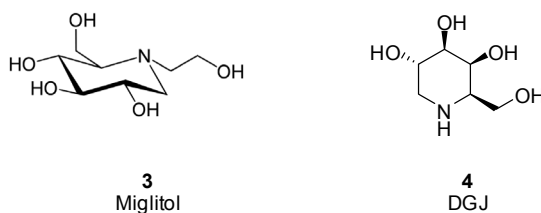
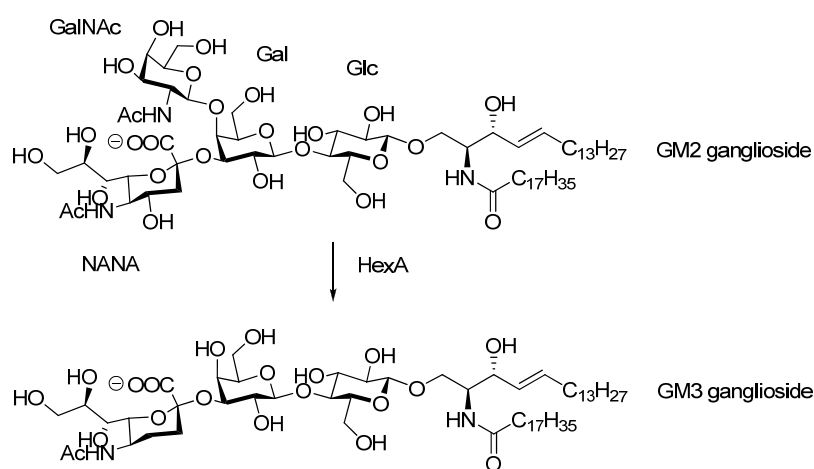


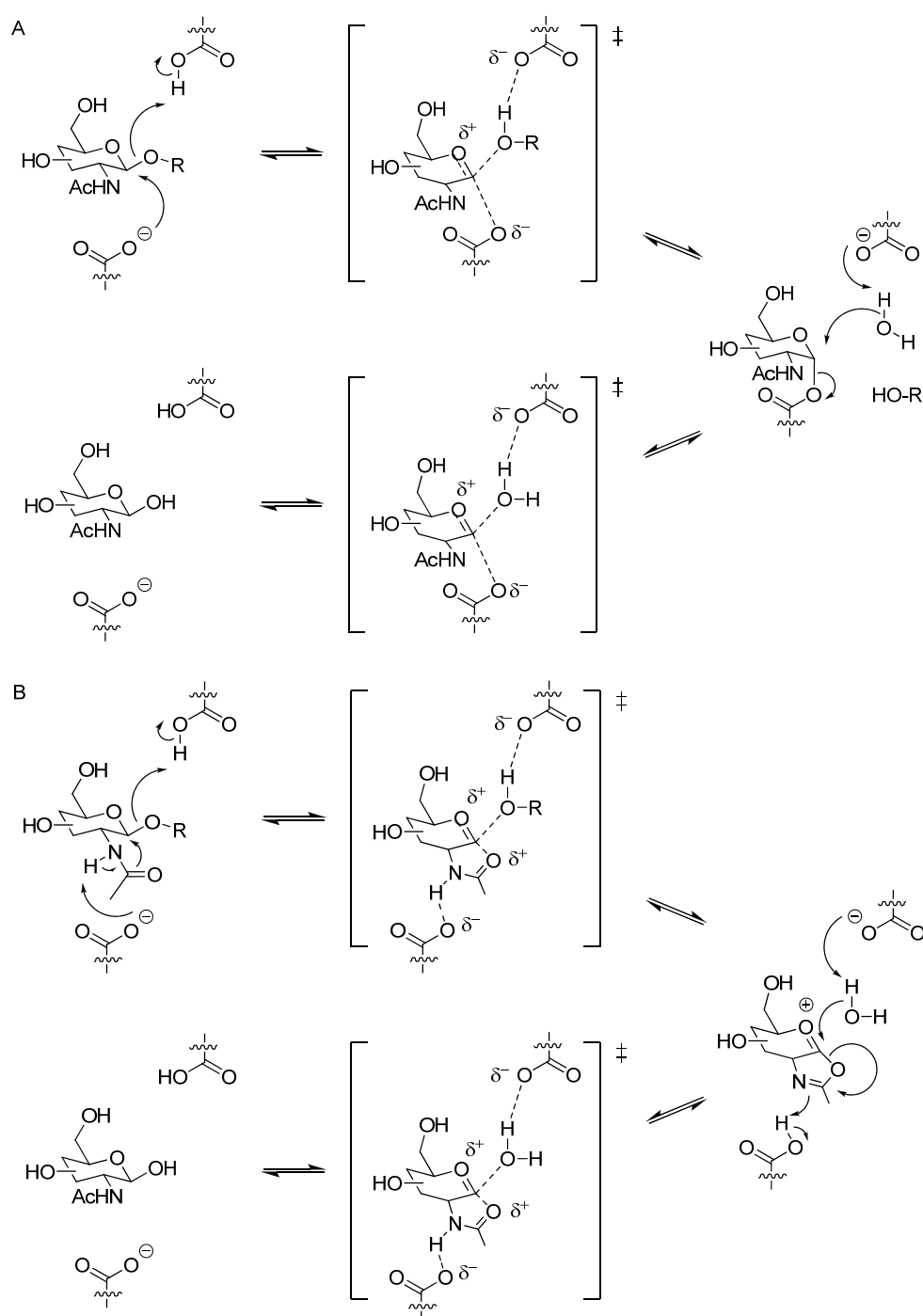
Figure 2

Iminosugars have found applications in treatment of a range of diseases including HIV,⁵ hepatitis B⁶ and C,⁷ diabetes,⁸ osteoarthritis and cancer. One synthetic iminosugar currently on the drug market is Miglitol **3** (Glyset®) (figure 3). It is an oral drug used to treat type II diabetes, which was introduced in 1999. It is an inhibitor of α -glucosidase so it is used to prevent digestion of carbohydrates to reduce glucose levels in the blood following ingestion. 1-Deoxygalactonojirimycin (DGJ) **4** (migalastat hydrochloride, Amigal™) is currently in phase III clinical trials for Fabry disease, which will be described in more detail later.

**Figure 3*****N*-Acetylhexosaminidases**

The *N*-acetylhexosaminidases are a subclass of the glycosidases and these enzymes are responsible for catalysing the cleavage of *N*-acetylhexosamine residues from glycoconjugates such as glycosphingolipids (GSLs) (for example GM2 gangliosides) and glycoproteins (for example proteoglycans) in the lysosome (figure 4).⁹ Perhaps surprisingly, the enzyme catalyses the cleavage of both *N*-acetylgalactosamine (GalNAc) and *N*-acetylglucosamine (GlcNAc) residues from glycolipids and glycoproteins. Pyranosides without an acetamido or equivalent substituent at C-2 are poorly recognised by the enzyme.¹⁰ Glycosidases are grouped into so called families based on the similarities of their sequences and there exist 113 of these families.¹¹ β -*N*-Acetylhexosaminidases are grouped into two classes and the hydrolysis mechanisms are given below (figure 5).^{12,13,14,15}

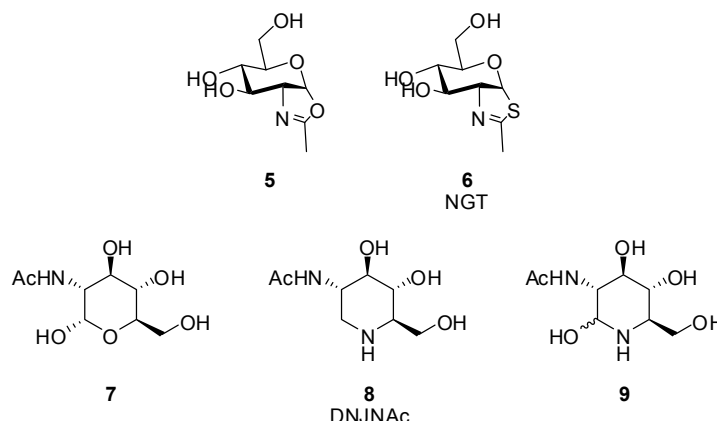
**Figure 4**

**Figure 5: A Family 3; B Family 20**

With reference to A in figure 5, the mechanism of family 3 (EC 3.2.1.52) is as for the retaining glycosidase mechanism given in figure 1. This class consists of enzymes derived from bacteria and the catalytic base is aspartic acid (Asp), whilst glutamic acid (Glu) is the

catalytic proton donor.¹¹ Family 20, mechanism B of figure 5, is the largest and most studied family and contains examples from prokaryotes and eukaryotes, including lysosomal β -*N*-acetylhexosaminidase. In the active site the Asp and Glu residues are still present, as for family 3, however the Asp is now not involved in the mechanism as the carbonyl oxygen of the C-2 acetamido group behaves as an intramolecular catalytic base in this family, via neighbouring group participation. However the Asp is used to stabilise the transition state (TS) and assist correct orientation of the substrate in the active site.^{11,16} A third class, family 84, is *gluco*- specific but has the same mechanism as for family 20. A member of family 84 is β -*N*-acetylglucosaminidase which is present in the cytoplasm where it cleaves glycoproteins in which serine and threonine residues have been posttranslationally modified with *O*-GlcNAc.¹⁷ The intramolecular reaction proceeds via a suspected oxazolinium ion intermediate. The neighbouring group participation leads to overall retention at the β -anomeric centre.

Some research groups have tried to exploit the oxazolinium intermediate by synthesising sugars with hetero-atom containing rings.^{9,10} *N*-Acetylglucosamine-oxazoline (NAG-oxazoline) **5** itself would not be suitable as an inhibitor because it would be too facile to hydrolyse but NAG-thiazoline (NGT) **6** is a stable equivalent (figure 6) and it was found to be a potent competitive inhibitor of *N*-acetylhexosaminidase from Jack bean (K_i 280nM).¹⁰ This compares favourably with the natural substrate *N*-acetyl- β ,D-glucosamine **7** (K_i 5mM) and known inhibitors 2-acetamido-1,5-imino-1,2,5-dideoxy-D-glucitol (DNJNAc) **8** (K_i 140-230nM) and 2-acetamido-2-deoxynojirimycin **9** (K_i 1.2nM). As for NAG-oxazoline, **5** is intrinsically unstable because of the presence of the hemiaminal.¹⁸

**Figure 6**

As with many biological systems, a fine balance in enzyme activity is required for processes to work in harmony with other processes and a breakdown of this leads to outcomes such as disease. Below some diseases are described involving *N*-acetylhexosaminidases including how iminosugar inhibitors could have applications as possible therapeutics.

Cancer

α -*N*-Acetylgalactosaminidase lysosomal enzymes cleave *O*-glycoside bonds between a terminal α -*N*-acetylgalactosamine residue and a threonine (Thr) or serine in a glycoprotein, in the lysosome. It has been reported that there are elevated levels of extracellular α -*N*-acetylgalactosaminidase in the serum in some cancer patients, especially in those where the disease is far progressed.^{19,20} This is a different enzyme to that found in the absence of cancer which is present in the lysosome. It has been proposed that the extracellular α -*N*-acetylgalactosaminidase derives from the tumor cells themselves.²¹ In fact the two α -*N*-acetylgalactosaminidase enzymes are active at different pHs; the normal lysosomal form ceases to be active at pH 7 but this is not so for the tumor derived form. There exists a glycoprotein in the serum called Vitamin D₃ binding protein (Gc protein) which is normally

deglycosylated and desialidated to form macrophage activating factor (MAF) which in turn activates macrophages, responsible for immunity in the body (figure 7). MAF displays a terminal α -N-acetylgalactosamine residue attached to a Thr side chain which is unfortunately cleaved by the tumor derived enzyme. This termination of the macrophage activation cascade has been observed in all types of cancers and leads to increased levels of immunosuppression in the patient. HIV patients also have elevated blood α -N-acetylgalactosaminidase levels, which leaves them at risk of developing malignant tumors because of immunosuppression.²² Some studies have shown the extracellular enzyme to be an exo-type glycosidase as presented in figure 7, however other papers have postulated it to be an endo-type.¹⁹ The end result is the same with formation of deglycosylated Gc protein. One therapeutic application of iminosugars here would be to find a molecule which is an inhibitor for the α -N-acetylgalactosaminidase to prevent the deglycosylation of Gc protein. However to prevent undesired side effects resulting from inhibition of the lysosomal form, inducing a Schindler/Kanzaki type phenotype which is described later, it would need to be specific for the extracellular enzyme.

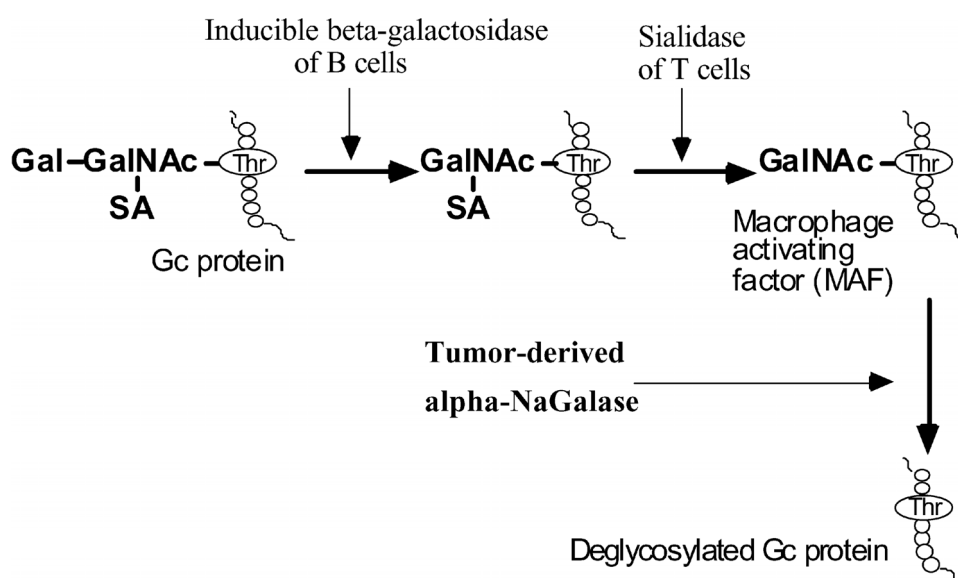


Figure 7 (taken from reference ²¹)

Osteoarthritis

Another example of the potential problems associated with unnatural extracellular enzyme activity, this time β -*N*-acetylhexosaminidase, is the case of osteoarthritis.²³ Cartilage matrix, produced and maintained by chondrocytes, contains proteoglycans and glycosaminoglycans (GAGs). However with osteoarthritis the presence of the proinflammatory cytokine interleukin-1 β stimulates the chondrocytes to secrete β -*N*-acetylhexosaminidase.²⁴ This causes breakdown of both the proteoglycans and GAGs in the cartilage matrix. This is unfavourable as GAGs are important for providing hydraulic resistance in the synovial cavity. The group that confirmed the enzyme involvement, Wong *et al.*, also showed that inhibition of this enzyme would slow the degradation process. A number of iminocyclitols were synthesised and incubated with human articular chondrocyte cells and human chondrosarcoma. Accumulation of GAGs was displayed in the cell fraction and decreased presence in the culture supernatant which is a result of deceleration of degradation. The best inhibitor, whose structure is given in figure 8, had a K_i of 24nM against human placenta β -*N*-acetylhexosaminidase.

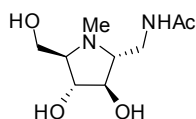


Figure 8

Lysosomal storage disorders

Lysosomal storage disorders (LSDs) are a relatively rare class of disease affecting around 1 in 7700-13000 births, combined.²⁵ There are around 50 different types, each stemming from a deficiency in a different enzyme in the relatively long glycosidase pathway (figure 9).²⁶ A complete lack or deficiency of an enzyme in the sequence causes a build up of

undegraded substrate in the lysosome, an organelle responsible for controlled macromolecular digestion in the cell. This can cause a range of phenotypes including neurological, motor and developmental problems. The diseases range in severity from mild in the adult onset forms, through juvenile onset to serious infant onset which often leads to death before the age of five. The disorders are genetic and usually autosomal and recessive. The most common LSD is Gaucher disease which is caused by a loss of lysosomal glucosylceramidase (glucocerebrosidase, acid β -glucosidase, GCase) activity. This leads to accumulation of glucosylceramide (GlcCer) in macrophage lysosomes, causing hepatosplenomegaly, anemia and neurological and skeletal dysfunctions.²⁵ However, the focus of this thesis is LSDs involving *N*-acetylhexosaminidases. These diseases are known as the GM2 gangliosidoses because of the inability by patients to degrade GM2 ganglioside in the lysosome. GM2 ganglioside therefore accumulates and because this molecule has a high presence in the neurones, neurological symptoms are common in patients.

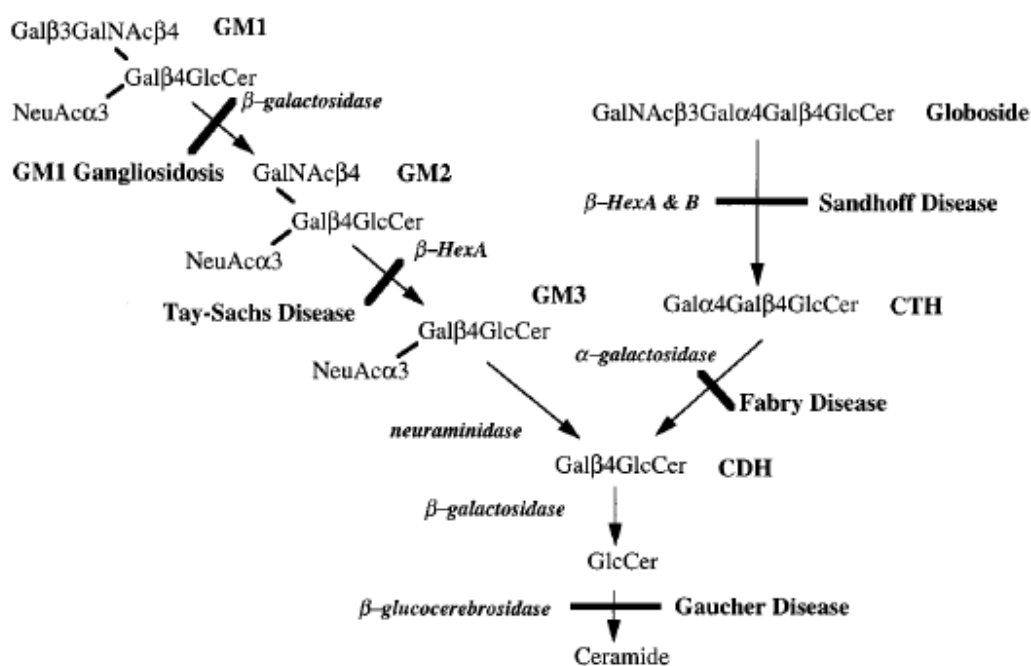


Figure 9: Examples of disorders in the glycosidase pathway (taken from reference ²⁷)

Hexosaminidases are made up of two subunits and both are required for the enzyme to be active. There are two types of subunit: α coded for by the gene *HEXA* and β coded for by the *HEXB* gene. This leads to three isoforms of the enzyme: HexA ($\alpha\beta$), HexB ($\beta\beta$) and HexS ($\alpha\alpha$) which is rarer. All cleave GalNAc and GlcNAc residues from glycoproteins, glycolipids and GAGs and are exo-type enzymes. Only the α subunit on HexA can cleave negatively charged substrates, therefore only HexA is necessary for life as it contains both types of subunit.^{27,28,29} Mutations in these genes lead either to no active enzyme being synthesised, on ribosomes associated with the rough endoplasmic reticulum, or only low levels with the rest being misfolded. Misfolded enzymes are degraded by the quality control mechanism of the endoplasmic reticulum and so are not transported, via the Golgi apparatus, to the lysosome where they are needed. GM2 gangliosidosis occurs if there is a mutation in either the *HEXA* or *HEXB* gene because HexA is needed, along with the GM2 activator protein, for hydrolysis to occur. HexA is required because GM2 ganglioside contains a negatively charged sialic acid group.^{9,30,31}

If there is a mutation, which could include a gene deletion, splicing, nonsense or missense mutation, in the *HEXA* gene it results in reduced availability of the α subunit. This is known as Tay Sachs disease. This affects HexA and HexS levels, but the latter is less prevalent anyway. However, HexB levels are unaffected so total Hexosaminidase activity is not too different from normal. In fact only low levels of HexA are actually needed to ameliorate the disease phenotype. Less than 10% of normal enzyme levels removes it completely and 1-5% gives a milder chronic phenotype. This observation has given rise to a so called 'critical threshold' of 5-10% enzyme.³² The highest incidence of Tay Sachs disease is present in the Ashkenazi Jewish population which has a carrier rate of 1 in 35, almost ten times higher than for the general population.³³ If the mutation is instead present in the *HEXB*

gene then HexA and HexB levels are affected. The unstable HexS isoform cannot make up for this loss. This disorder is known as Sandhoff disease. The residual level of enzyme activity is directly correlated to the severity of the disease. There is also a third rare form called AB variant which derives from GM2 activator mutations.

An example of a disorder involving the enzyme α -N-acetylglucosaminidase is Sanfilippo Syndrome B which is classed with the mucopolysaccharidoses; LSDs associated with inability to process GAGs, specifically heparan sulfate. Sanfilippo Syndrome B is also a rare autosomal recessive disease characterised by mental and motor degeneration.³⁴

Excessive levels of extracellular α -N-acetylgalactosaminidase were mentioned earlier in relation to cancer but there also exists a LSD where this enzyme is deficient in the lysosome, known as Schindler disease in which glycolipids and glycopeptides accumulate, leading to neurodegeneration.³⁵ The adult onset form is called Kanzaki disease with milder symptoms including skin lesions and angiokeratoma, a skin discolouration resulting from capillary lesions.

Fabry disease is an LSD resulting from mutations coding for α -galactosidase A. This X-linked recessive genetic disorder leads to globotriaosylceramide accumulation in vascular endothelium, causing progressive heart, kidney and central nervous system (CNS) damage.^{36,37}

Currently there are only limited therapies on the market for LSDs, partly because the neurological effects of most of the diseases are difficult to address because of the inaccessibility of the CNS by molecules not being able to cross the blood brain barrier (BBB). The other consideration is the rarity of the diseases, making drug research into this area financially less viable. For this reason Gaucher disease has received most attention because it is the most common LSD. Currently, an iminosugar called Miglustat (Zavesca®),

N-butyl-1-deoxynojirimycin (NB-DNJ) (figure 10), is licensed in the US, Europe and Israel for type 1 Gaucher, which does not have any CNS involvement. NB-DNJ is an inhibitor of glucosylceramide synthase (ceramide glucosyltransferase), the enzyme responsible for glucosylceramide synthesis.³⁸ This therapy is a substrate reduction therapy (SRT) whereby the GSL biosynthetic pathway is partially halted to reduce the burden on the low level of enzyme. The level of inhibition of substrate synthesis is adjusted to balance the rate of enzyme degradation, to reduce storage. It is not necessary to deplete to very low levels and in fact this may cause side effects.³⁹ This can be a generic therapy as inhibition at the start of the GM2 synthetic pathway can be used for disorders having a deficiency in any of the subsequent enzymes.⁴⁰ One advantage of SRT is that NB-DNJ is water soluble so the drug is orally available. However it is necessary for residual enzyme to be present for this therapy, to catabolise the GSLs synthesised, and the drugs ideally need to be small enough to cross the BBB.⁴¹

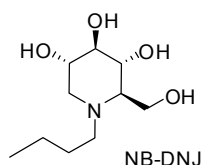


Figure 10

When it comes to therapeutics for LSDs there are two possible approaches. One is to decrease the level of substrate to allow the enzyme that is present to cope such as in the case of SRT; the other is to increase the level of enzyme available. Examples of the latter type are gene therapy and bone marrow transplantation. These two aim to raise enzyme levels by a few percent to alleviate the phenotype.²⁷ The idea of gene therapy is to introduce the wild type gene into the patient by use of a vector. However the safety of these viral vectors and some other technical issues still need to be resolved in this therapy.⁴¹ Also each disorder

would need an individual therapy. Bone marrow transplantation works by introducing normal cells which can synthesise the enzyme, however this requires suitable donors to be found and mortality rates are relatively high.

Another way of increasing enzyme levels is by enzyme replacement therapy (ERT). This involves regular intravenous enzyme infusions. Currently ERT has been approved for type 1 Gaucher disease in the US. Biweekly transfusions of purified enzyme are required, at a cost of \$150,000-\$200,000 per patient per annum. This treatment is only suitable for patients with no CNS involvement as the enzyme cannot cross the BBB. ERT may be combined with an SRT therapy to reduce the level of enzyme required in an attempt to reduce costs.⁴² Of the GM2 gangliosidoses, current treatment of Sanfilippo Syndrome B and Schindler/Kanzaki disease is supportive management. Tay Sachs disease has an SRT treatment in the pre-clinical stage, as does Sandhoff as well as pre-clinical chaperone mediated therapy (CMT), described below.^{43,44}

Another way to try to increase enzyme levels is by the use of pharmacological chaperones (PC) in a therapy called CMT. In normal cells, protein folding is a thermodynamic process with the native correct fold being in equilibrium with other possible yet inactive folds. Molecules present in the cell normally help the correct fold to be attained and these are called molecular chaperones; examples include calnexin and calreticulin.⁴² There exists only a small thermodynamic difference between the native and other folds so the theory behind CMT is that addition of a molecule which is known to interact with the enzyme would allow it to behave like one of the natural molecular chaperones to engender correct folding of a misfolded protein. A competitive inhibitor of the enzyme would be ideal here as it interacts with the residues of the active site of the enzyme. PCs prefer to bind to the active site of the enzyme so they help to shift the equilibrium back to the native active

form.^{25,45} This leads to a point to note about CMT, it is only viable if the mutation in the gene coding for the enzyme does not prevent the protein from folding and then being active.^{46,47} *N*-acetylglucosamine-thiazoline NGT **6** (figure 6 on page 7) is an example of a PC as it only interacts with the conserved residues.⁴⁵ It is a known active site specific chaperone of β -*N*-acetylhexosaminidase.²⁵

An ideal PC would be a better inhibitor at neutral pH compared to lower pH. This is so that in the endoplasmic reticulum (ER), where the enzyme is made, the inhibitor would bind tightly to the defective enzyme to cause correct folding. Then the enzyme would pass the ER quality control system to allow transportation to the lysosome where the substrate is. Here the lower pH and high substrate concentration would cause the inhibitor to be displaced. An advantage of CMT is that small molecules can cross the BBB and are more likely to be orally available. The concentrations of inhibitor used are at sub-inhibitory levels so as to act as a chaperone but not act as an inhibitor of the enzyme which would only exacerbate the disorder.

As dictated by the preference of the enzyme, when considering suitable inhibitors of *N*-acetylhexosaminidase it is important for an *N*-acetyl group or suitable substituent to be present. Some piperidine examples of existing competitive inhibitors are given in figure 11. PUGNAc **10** inhibits both β -hexosaminidase and *O*-GlcNAcase with a K_i value of 50nM.⁴⁸ Piperidine inhibitors NAc-nojirimycin **9** and NGT **6** have already been mentioned on page 6. Gluco-nagstatin **11** inhibits *O*-GlcNAcase with a K_i of 0.42 μ M and has a K_i of 0.01 μ M against β -hexosaminidase.⁴⁸ In order to increase the selectivity of the inhibitor for hexosaminidase over GlcNAcase a *galacto*- configuration is ideal as GlcNAcases cannot accept substrates of this type but hexosaminidase can, as well as accepting *gluco*- configured substrates. Gal-PUGNAc **12** and Gal-NAG-thiazoline **13** are indeed selective inhibitors. They

inhibit HexA with K_i s of 51nM and 820nM respectively and **12** inhibits HexB with a K_i of 18nM.^{49,50} Despite having the *galacto*- configuration, Nagstatin **14**, produced by *Streptomyces amakusaensis* is reported to inhibit β -N-acetylglucosaminidase with a K_i value of 12nM.⁵¹ It is reported to inhibit α -N-acetylgalactosaminidase with an IC_{50} value of 19 μ gml⁻¹.

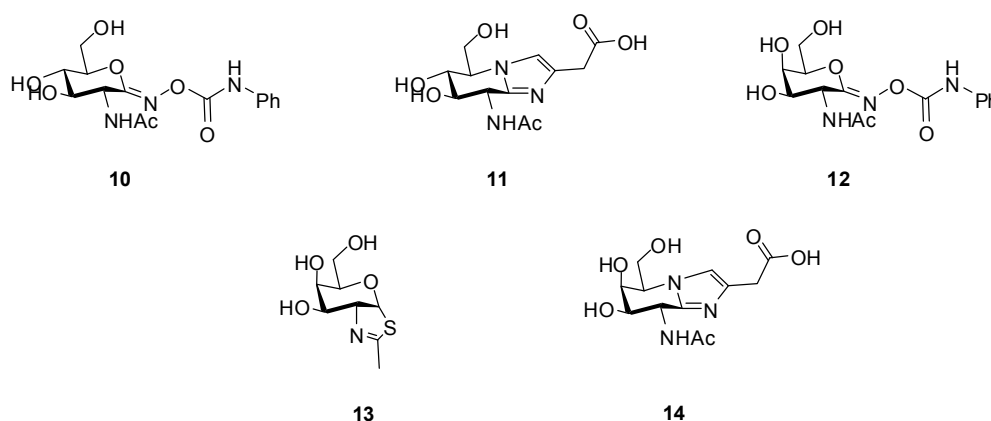
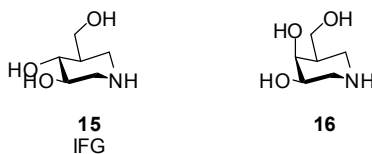


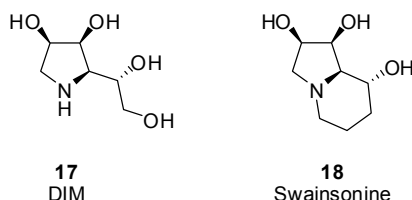
Figure 11

Other Iminosugars

Isofagomine (IFG) **15** (figure 12) was synthesised by Bols *et al.* as it was considered to mimic the resonance structure for the oxocarbenium transition state.^{52,53} The tartrate salt, Plicera, is an identified PC for type 1 Gaucher disease and has undergone phase II trials. It displays the desired pH profile as it inhibits GCcase at pH 5.2 with an IC_{50} of 30nM compared to an IC_{50} of 5nM at pH 7.2.^{54,55} IFG is of the class of iminosugar called 1-*N*- or isoiminosugar, where the ring N is shifted to C-1 compared to DNJ. The *galacto*- analogue of IFG **15**, 4-*epi*-isofagomine **16**, has displayed competitive inhibition against acid β -galactosidase which is deficient in some LSDs.^{25,56}

**Figure 12**

As the natural substrates of the glycosidase enzymes are pyranoses it makes sense that piperidine iminosugars would make good mimics. However pyrrolidine iminosugars also make suitable TS mimics as they still possess the ring nitrogen and a NAc at C-2. In fact the envelope structure of the 5-membered ring may be better suited to mimicking the half-chair conformation of the glycosyl cation TS than a 6-membered chair.⁵⁷ The first pyrrolidine found to inhibit an enzyme was 1,4-dideoxy-1,4-imino-D-mannitol (DIM) **17**, synthesised in 1984 by the Fleet group (figure 13). It displays competitive inhibition against Jack bean α -mannosidase (K_i 0.76 μ M). Structurally related Swainsonine **18** also inhibits the enzyme, though slightly less potently (K_i 9.5 μ M).⁵⁸

**Figure 13**

The first known natural polyhydroxylated pyrrolidine was 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) **19** (figure 14).^{59,60} It is widely occurring, being present in a range of plants for example *Hyacinthoides nonscripta* (bluebells). Its presence has also been identified in *Streptomyces* strains.⁶¹ Pyrrolidine **19** is an inhibitor of almond β -glucosidase (K_i 10 μ M) and yeast α -glucosidase (K_i 7 μ M). DAB **2** is related to DMDP **19** by removal of one of the hydroxymethyl groups.⁶² DAB can be found in *Morus alba* (white mulberry) as well as in bluebells. Pyrrolidine **2** is a competitive inhibitor of yeast α -D-glucosidase (K_i 0.16 μ M) and

rat intestinal isomaltase (K_i 2.4 μ M).^{63,64} The C-2 epimer of **2**, 1,4-dideoxy-1,4-imino-D-ribitol (DRB) **20**, also inhibits yeast α -glucosidase (K_i 23 μ M). *Lyxo*- derivative **21**, also related to Swainsonine **18**, displays potent competitive inhibition of green coffee bean α -galactosidase (K_i 0.1 μ M) and weak activity against Jack bean α -mannosidase (IC_{50} 14 μ M).^{64,65} The remaining pyranose mimic, *xylo*- derivative **22**, displays only low (mM) inhibition of almond β -glucosidase.⁶⁶

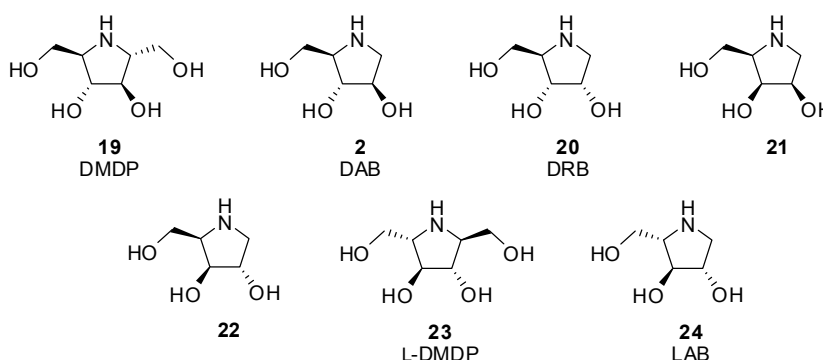


Figure 14

The enantiomers of some of these compounds have also been synthesised and biologically evaluated. L-DMDP **23** is actually a more potent and selective inhibitor of plant and mammalian derived α -glucosidases than DMDP **19**.⁵⁹ Iminosugar **23** inhibits rice α -glucosidase (K_i 0.1 μ M) and rat intestinal maltase (K_i 0.023 μ M).⁶³ Similarly 1,4-dideoxy-1,4-imino-L-arabinitol (LAB) **24**, the enantiomer of **2**, is also an inhibitor of yeast α -glucosidase (K_i 11.5 μ M) and rat intestinal maltase (K_i 0.07 μ M). Both **23** and **24** displayed non-competitive inhibition in contrast to the natural enantiomers.⁶³ This suggests that L-iminosugars bind at an alternative site on the enzyme compared to the D-iminosugars which bind at the active site.

There are not many known pyrrolidine inhibitors for the *N*-acetylhexosaminidase enzyme class. Prior to the research presented in this thesis, the most potent inhibitor of β -*N*-acetylhexosaminidase was 2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol (LABNAc) **25**,

the C-2 NAc analogue of LAB (figure 15), synthesised by Rountree of the Fleet group which will be discussed in the introduction to Chapter 2.⁶⁵ Iminosugars **26** and **27** also display some inhibition against β -*N*-acetylhexosaminidase.⁶⁷ Recently in the Fleet group some pyrrolidines with a methyl amide at C-1 have shown promising inhibition, including **28** which inhibits β -*N*-acetylhexosaminidase (HL60) with a K_i of 27nM.⁶⁸

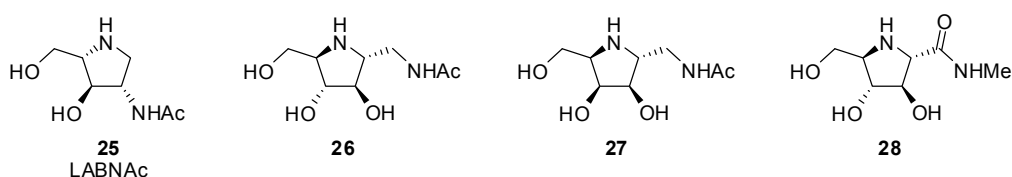


Figure 15

This thesis describes the efforts towards finding a potent and specific β -*N*-acetylhexosaminidase inhibitor through the synthesis of some pyrrolidine and piperidine iminosugars containing C-2 NAc groups to mimic the natural substrates.

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68. unpublished results

Chapter 2: 2-Acetamido Pyrrolidine Iminosugars

As highlighted in the general introduction, the majority of potential glycosidase inhibitors previously studied have displayed piperidine based structures. The five membered ring iminosugar pyrrolidine analogues have been less extensively studied. It was hoped that these would make interesting targets, especially considering the similarity between the envelope conformation of the pyrrolidines and the half-chair conformation envisaged for the glycosyl cation transition state during the proposed hydrolysis mechanism (figure 1 in general introduction). Therefore a previous Fleet group member, Rountree, set out to synthesise the four target stereochemistries displayed in figure 16; along with their enantiomers as the unnatural form had been observed to inhibit in other systems.^{1,2}

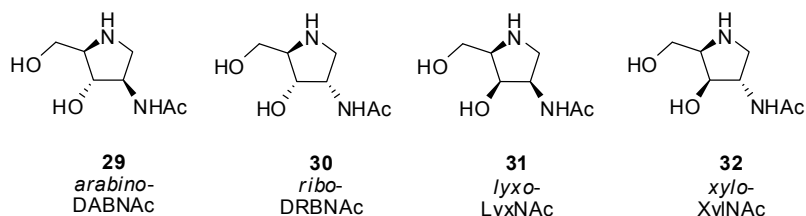


Figure 16

A common method for forming the ring of the iminosugar in the Fleet group has been to adopt a double displacement methodology and this was considered by Rountree to be a suitable strategy here using the generalised retrosynthetic route outlined in figure 17.

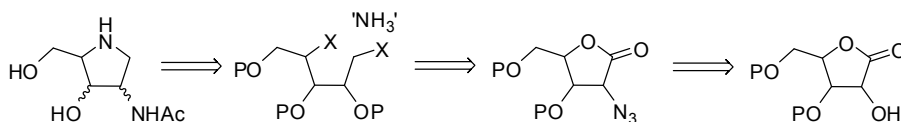


Figure 17: P protecting group, X leaving group

The first target to be synthesised by Rountree was LABNAc **25**, with *L-arabino*-stereochemistry, using D-lyxonolactone **33** as the starting material.³ The first step involved protection of the 3,5-diol using benzaldehyde to give benzylidene **34** (figure 18). Then the nitrogen, which eventually forms the *N*-acetyl (NAc) group, was introduced by a triflate/azide protocol using 0.97 eq azide to form the kinetic product **35**. The lactone was opened using lithium borohydride and the resulting diol activated as the di-mesylate. Benzylamine was used as the nitrogen source for imine ring formation. The first displacement of the primary mesylate group required the use of neat benzylamine at 95°C for 14h. The second displacement did not occur with the benzylidene group present but on removal of the protecting group the displacement proceeded *in situ* to give the desired product **36**.

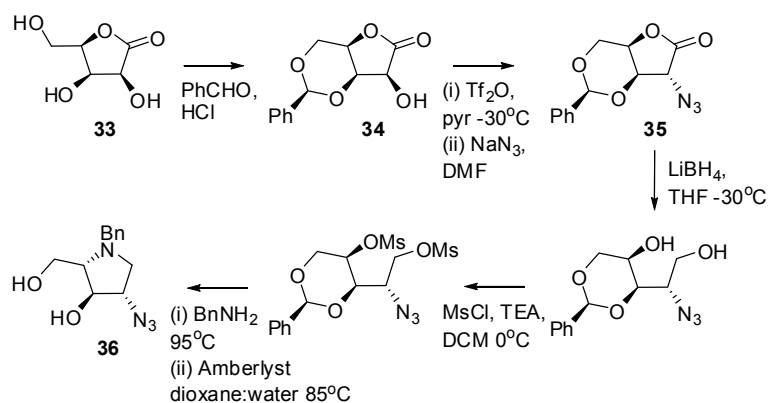


Figure 18

With the pyrrolidine ring installed the azide group was first reduced to form the amine and then acetylated to form the NAc group (figure 19). Selective acetylation of the amine was not possible so the esters formed were hydrolysed using sodium methoxide in methanol in a catalytic Zemplén reaction. Finally, reductive removal of the benzyl group yielded LABNAc **25**. The enantiomer of LABNAc, DABNAc **29**, was synthesised in a

parallel fashion using 2,3-*O*-isopropylidene-L-lyxonolactone which can be readily accessed from D-gulonolactone or D-ribose.^{4,5}

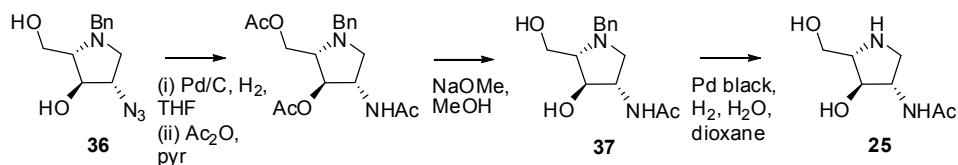


Figure 19

Rountree also synthesised the two pyrrolidines with *ribo*- stereochemistry, 2-acetamido-1,2,4-trideoxy-1,4-imino-D-ribitol (DRBNAc) **30** and 2-acetamido-1,2,4-trideoxy-1,4-imino-L-ribitol (LRBNAc).⁶ This was achieved using the same route as used for LABNac and DABNac but for the azide displacement of the triflate formed from **34** 2.5 eq azide were used to form the thermodynamic product **38**, with retained stereochemistry (figure 20). It is thought the excess azide acts as a base to epimerise the centre α - to the carbonyl. The rest of the synthesis, and that of the enantiomer, was as equivalent to the *arabino*- syntheses. Rountree also hoped to synthesise the other two compounds **31** and **32** along with their enantiomers but issues prevented the successful completion of this; these attempts will be expounded in due course.

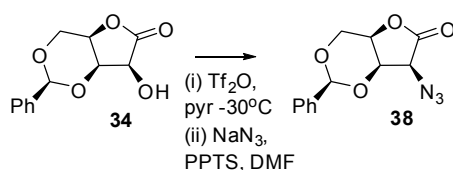


Figure 20

Rountree and collaborators performed enzyme assays on the target compounds *N*Bn-LABNac, LABNac, *N*Bn-DABNac, DABNac, *N*Bn-LRBNac and *N*Bn-DRBNac.¹ The *ribo*- compounds displayed no inhibition against a range of *N*-acetylhexosaminidases or glycosidases so the de-benzylated compounds were not tested.

The *arabino*- compounds displayed no significant inhibition against a range of glycosidases but there were interesting results against β -*N*-acetylhexosaminidases, which are summarised in table 1 and 2 below. No inhibition was observed against α -*N*-acetylgalactosaminidase (*Charonia Lampas*).

Enzyme	NBn-LABNAc 37	LABNAc 25	NBn-DABNAc	DABNAc 29
	IC ₅₀ /μM			
Jack bean	5.2	3.4	446	-
Bovine kidney	0.36	0.64	41.2	326
Human placenta	2.8	13	320	-

Table 1: IC₅₀ (μM) values against some β -*N*-acetylhexosaminidases; - no significant inhibition (at 1mM)

Enzyme	NBn-LABNAc 37	LABNAc 25	NBn-DABNAc	DABNAc 29
	K _i /μM			
Jack bean	3.4	1.9	-	-
Bovine kidney	0.28	0.095	16.9	104
Human placenta	3.7	ND	-	-

Table 2: K_i (μM) values against some β -*N*-acetylhexosaminidases; ND not determined; - no significant inhibition

Enzyme kinetic studies indicated that the mode of action of NBn-LABNAc was non-competitive but LABNAc displayed competitive inhibition. Molecular modelling studies of LABNAc and DABNAc were performed that predicted LABNAc would

overlay well with the natural substrate and therefore behave as a good competitive inhibitor whereas the enantiomer would not, suggesting it to be a poor inhibitor. The experimental data supported this theory. It was speculated that hydrophobic interactions involving the benzyl group of NBn-LABNAc could be changing its mode of action from the one which might have been expected. It is worth pointing out that a collaborator, Asano, noticed a trend that some L-iminosugars are more potent and non-competitive inhibitors of the enzyme against which the D- counterpart is competitive.^{2,7}

L-LyxNAc

Having made the *arabino*- and *ribo*- stereochemistries, Rountree hoped that similar strategies could be adopted to synthesise the remaining *lyxo*- and *xylo*- stereochemistries. Initially, attempts were made to synthesise the 2-acetamido-1,2,4-trideoxy-1,4-imino-L-lyxitol (L-LyxNAc) derivative; at the time the *lyxo*- compound was expected to be the more potent inhibitor by comparison with 1,4-imino-D-lyxitol which was biologically more interesting with respect to glycosidase inhibition compared to 1,4-imino-D-xylitol.^{8,9} The previous attempts towards L-LyxNAc will be described here followed by the completion of this work as part of this thesis. The synthesis of 2-acetamido-1,2,4-trideoxy-1,4-imino-D-xylitol (XylNAc) and its enantiomer will be described in the second half of this chapter.

The synthesis of L-LyxNAc was envisaged to proceed via a double displacement methodology starting from D-ribose **39** (figure 21). First a bromine oxidation was performed by Rountree and the resulting ribonolactone was protected without isolation using benzaldehyde to form lactone **40** in 49% on 100g.¹⁰ The product resides in a 6-

membered ring and is 3,4-protected, as shown crystallographically in 1985 by Bagget *et al.*¹¹ For almost 20 years preceding that it had been thought to reside as the 3,5-protected 1,4-lactone.¹² Azide **41** was formed by reacting alcohol **40** with triflic anhydride and displacing with sodium azide; the reaction proceeded with retention. The lactone is held in a boat conformation with the azide group in the bowsprit position. This is the thermodynamic product as the epimeric *arabino*-azide would have the azide group in the very sterically hindered flagpole position. It is thought that the kinetic *arabino* product is initially formed by the expected S_N2 mechanism, followed by very fast equilibration to the *ribo* product **41** via enolisation by the sufficiently basic azide ion. Finally deprotection of the benzylidene protecting group using aqueous TFA afforded azido lactone **42**.

In the original scheme by Rountree it was decided to use *tert*-butyldimethylsilyl (TBS) protection, as protection of the 3,5-diol by acetonide or benzylidene would be disfavoured because of their trans relationship. Reaction of **42** with 3 eq of TBSCl and 6 eq imidazole in DMF caused consumption of starting material in 2h. However two products **43** and **44** were formed which were deemed to be epimeric at the azide position as a result of the basic reaction conditions causing epimerisation by enolisation. The desired product **43** was separated from the side product by column chromatography using chloroform as eluent.

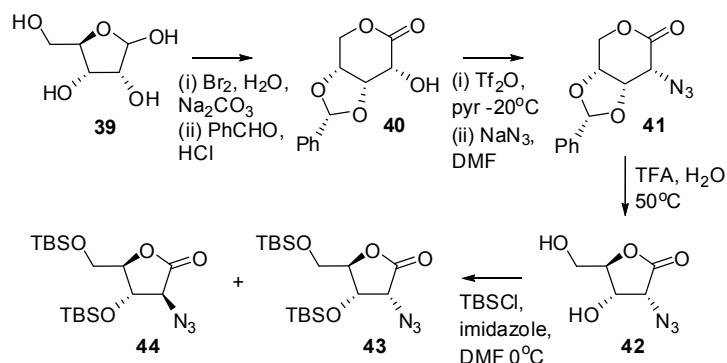


Figure 21

Taking pure **43** it was necessary to open the lactone to the diol using a reducing reagent. Rountree tried various reagents but in all cases a mixture of epimers **45** was observed (figure 22). A borohydride reduction in the presence of CeCl_3 was performed as this suppresses silyl migration,¹³ however a mixture was still recovered confirming that silyl migration was not the cause of the side product. Reduction using DIBAL gave a single major lactol product **46** however further reduction using sodium borohydride again produced the side product. Mixture **45** was di-mesylated using mesyl chloride and triethylamine (TEA) in DCM but the products **47** were not separable and silica purification lead to partial TBS deprotection.

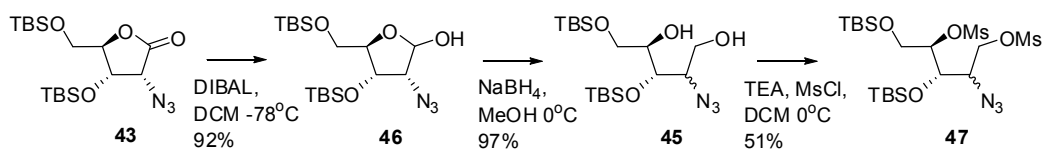


Figure 22

This prompted alternative protection of **42** with *tert*-butyldiphenylsilyl (TBDPS) and benzyl (Bn) but neither of these solved the problem. It was considered that converting the azide group to NAc might reduce the acidity of the proton α - to the carbonyl and so disfavour epimerisation processes so **43** was subjected to palladium

black under a hydrogen atmosphere, followed by acetylation using acetic anhydride and pyridine and isolated mainly as the desired product **48** in up to 85% over 2 steps (figure 23). Reduction of **48** using lithium borohydride formed the corresponding diol, however subsequent mesylation formed a complex mixture of products, possibly including intramolecular displacement by the NAc group. Time prevented Rountree from performing further studies.

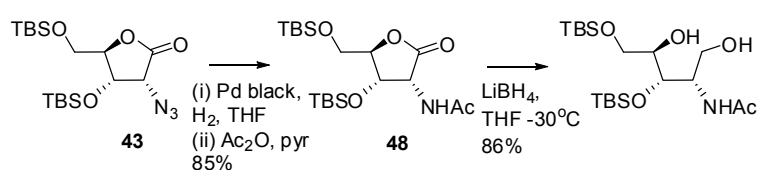


Figure 23

In summary of this previous work by Rountree, the epimerisation issues encountered proved to be the main obstacle in the synthesis meaning that either a new route or protecting group strategy would be required before a successful synthesis could be completed. It was envisaged that the use of the benzhydryl protecting group, subsequently developed in the Fleet group, could circumvent the problems of epimerisation experienced in the original synthesis as it crucially can be introduced under neutral conditions.¹⁴ A co-worker used the retrosynthetic analysis in figure 24 for the synthesis of D-LyxNAc **31** exploiting this reagent, using L-arabinose **49** as the starting material.¹⁵ Therefore, for the synthesis of L-LyxNAc as part of this thesis it was decided to use the benzhydryl reagent to protect intermediate **42**.

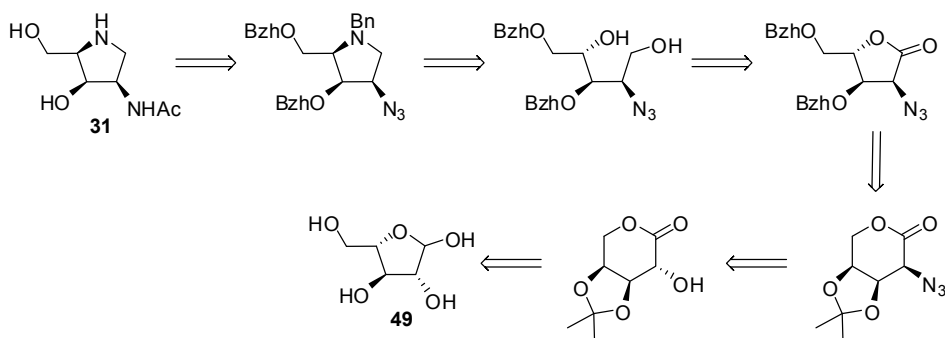


Figure 24

Using azido lactone **42**, the two hydroxyl groups were benzhydryl protected using 4 eq of diphenyldiazomethane in refluxing toluene at a high dilution of 7mgml^{-1} . One proposed mechanism for the protection is given in figure 25.^{14,16} During column chromatography it was necessary to start with a low polarity solvent mixture of cyclohexane:ethyl acetate 50:1 to allow elution of the azene which is a side product of the reaction.

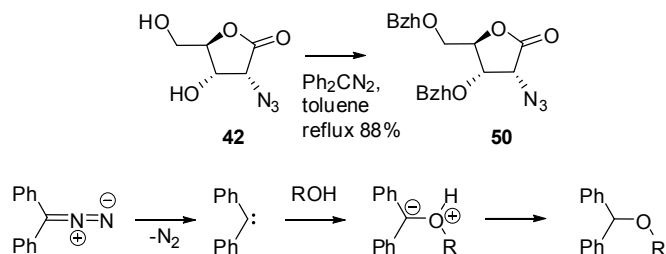


Figure 25

The next stage was a two step reduction of the lactone. As seen in the previous route, if sodium borohydride were used directly the basic conditions could promote undesired epimerisation at the azide carbon via the enolate. However use of the Lewis acidic reagent DIBAL followed by sodium borohydride minimised this problem. 1.5 Eq of DIBAL were used, as a 1.5M solution in toluene, at -78°C in DCM. The resulting lactol was reacted crude with sodium borohydride at 0°C in methanol for 3h. The desired

product **51** was isolated as a colourless oil in 62% yield over two steps (figure 26). The remaining 38% of material was found to be a mixture of epimers. It was envisaged that the ring could be formed by the Fleet double displacement methodology by di-mesylate formation. During di-mesylation of related systems formation of a tetrahydrofuran by attack of the as yet unreacted secondary alcohol on the primary mesylate was observed.¹ To prevent this, diol **51** was stirred at 0°C with 4 eq TEA in DCM for 20 min before 4 eq of mesyl chloride were added dropwise. The resulting di-mesylate was concentrated and purified by column chromatography to yield **52** in excellent 98% yield. Pyrrolidine formation was then facilitated by heating **52** at 80°C in toluene in the presence of benzylamine for 45h. Product **53** was isolated in 65% yield.

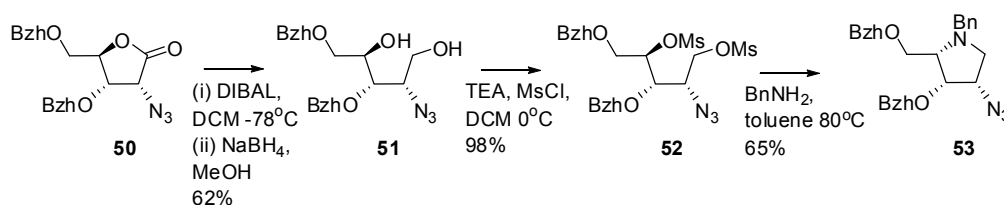


Figure 26

Initial work on the enantiomeric synthesis by a co-worker to introduce the NAc group by transformation of the azide group was successful employing a one-pot procedure using powdered zinc activated by saturated copper sulfate solution.¹⁷ However subsequent attempts to remove the benzhydryl protection using TFA in water and dioxane were unsuccessful. Therefore the strategy outlined in figure 27 was performed instead and subsequently adopted for the L- synthesis here. First the benzhydryl protecting groups were removed using boron trifluoride diethyletherate in acetic anhydride, which resulted in re-protection of the hydroxyl groups. The mixture was concentrated *in vacuo* and then partitioned between ethyl acetate and sodium bicarbonate

solution to neutralise the mixture. This was performed carefully with cooling to prevent excessive effervescence. Then the zinc and copper sulfate procedure was employed for installation of the NAc group. THF:acetic acid:acetic anhydride 3:2:1 was used as the solvent. Work up involved filtration followed by purification by column chromatography. The reaction was complete in 1h to give **55** in a 74% yield.

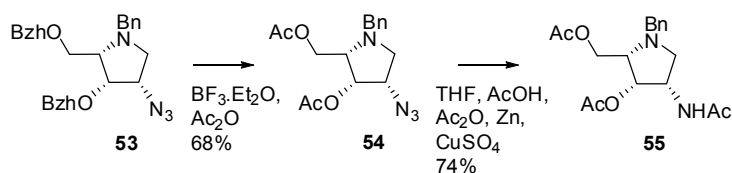


Figure 27

The final step was hydrolysis of the acetyl esters. This was achieved using 0.2 eq sodium methoxide in methanol in a catalytic Zemplén reaction.¹⁸ After 2h the reaction was complete and the product purified by column chromatography, eluting with acetone. This afforded the *N*-benzyl (NBn) derivative of L-LyxNAc **56** in a moderate yield of 60%. To obtain the free base **57**, the NBn derivative **56** was hydrogenated in the presence of palladium black in 1,4-dioxane and water. The palladium was removed by filtration through Celite® and the product received no further purification. L-LyxNAc **57** was isolated as a solid in quantitative yield (figure 28). The biological evaluation of **56** and **57** will be discussed at the end of the chapter. The overall yield of **57** was 11% over nine steps from **42**.

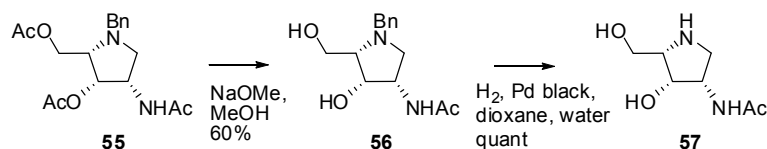


Figure 28

Molecular modelling study

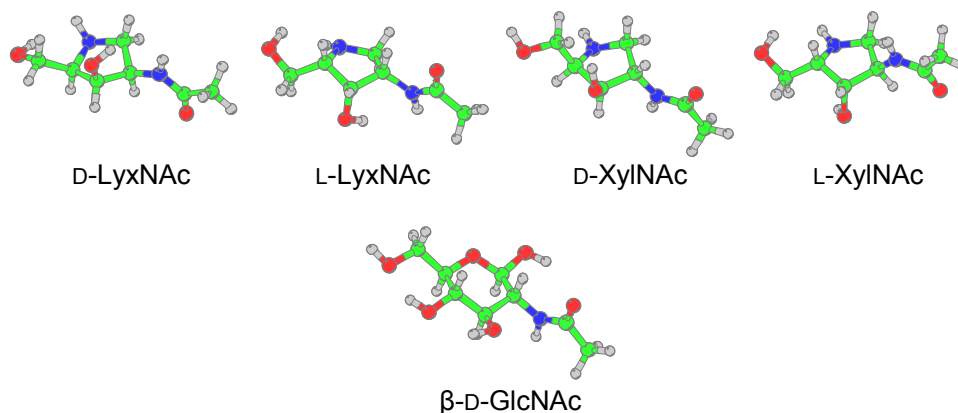


Figure 29

In the Rountree thesis,¹ molecular modelling studies on DABNAc and LABNAc were presented. Overlay studies with the natural substrate β-D-GlcNAc were performed and by prioritising NAc and ring N overlap, LABNAc was indicated to be a potential inhibitor of β-N-acetylhexosaminidase whereas DABNAc was predicted to be a not very good inhibitor as a result of this modelling. Similarly, in an attempt to rationalise the biochemical results obtained for the compounds synthesised in this thesis, both enantiomers of XylNAc and LyxNAc were modelled and overlayed with β-D-GlcNAc by Mark Wormald of the Oxford Glycobiology Institute (figure 29). If the stereochemistry at C-2 is the critical interaction then D-XylNAc and L-LyxNAc were predicted to display the best inhibition.

D- and L-XylNAc

This half of the chapter describes the syntheses of the final compounds to be made in the pyrrolidine series, with *xyl*o stereochemistry. Initially Rountree planned to synthesise the enantiomers with this stereochemistry by adapting the parallel route for the

LyxNAc synthesis using the azide **44** in figure 21, which would be accessible by base promoted enolisation. However as these efforts proved unsuccessful an alternative method was adopted which is the work performed as part of this thesis. It is worth noting that a collaborator, Atsushi Kato, independently predicted that D-XylNAc **32** would be a good inhibitor by comparing it to the structures of known inhibitors synthesised by members of the Fleet group (figure 30). The values given in the figure are K_i values against β -N-acetylglucosaminidase. XylNAc **32** overlays the natural substrate of the enzyme, β -D-GlcNAc **7**, in a similar fashion to known inhibitor LABNAc **25**.

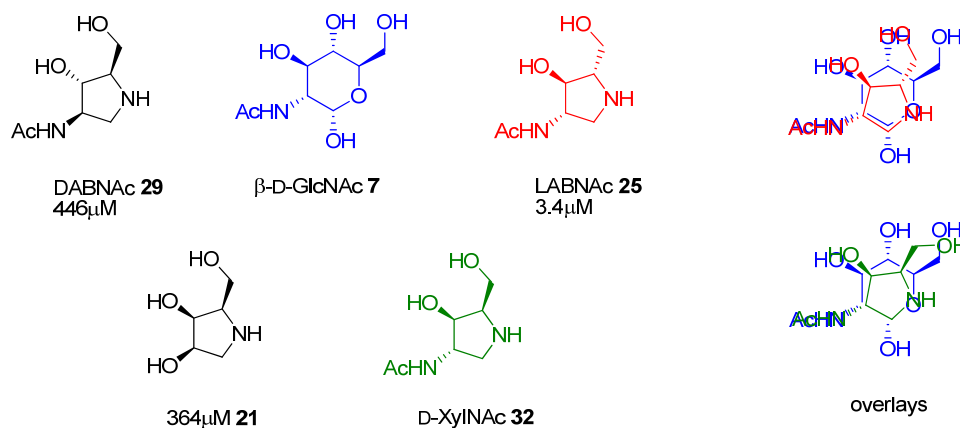


Figure 30: Overlays of LABNAc and XylNAc with β -D-GlcNAc

Using intermediate **58**, which can be synthesised from glucuronolactone,¹⁶ it was envisaged that XylNAc **32** could be synthesised as outlined in the retrosynthetic analysis in figure 31; the key pyrrolidine ring being formed by an intramolecular reductive amination reaction using an azide group selectively introduced on the primary position.

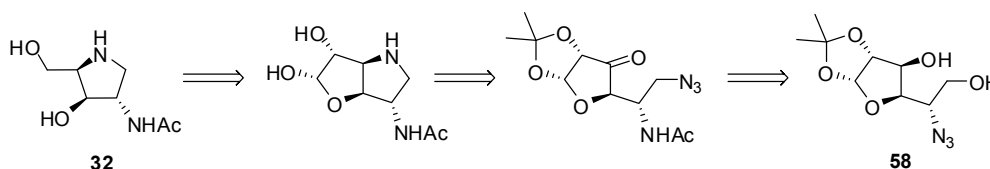


Figure 31

For the synthesis of **58**, isopropylidene protected glucuronolactone **59** was reacted with triflic anhydride in the presence of anhydrous pyridine and DCM at -30°C (figure 32). After 1h, the triflate was washed with 1M HCl and used without any further purification as it decomposed readily. The displacement reaction used 1 eq of sodium azide and the reaction temperature was maintained at -20°C . These measures were to minimise epimerisation at the carbon α - to the carbonyl which is possible as sodium azide is basic; however epimerisation was difficult to suppress completely. The azide was purified by flash column chromatography, however silica appeared to accelerate decomposition of the compound. Azide **60** was isolated as a white crystalline solid in excellent 89% yield.

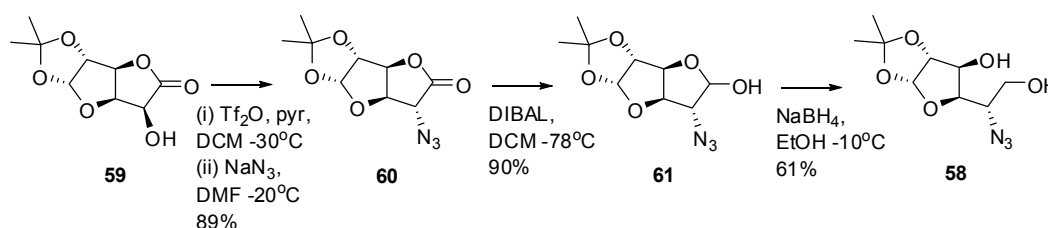


Figure 32

The next stage required opening of lactone **60** to diol **58**. Based on previous work in the group a DIBAL/borohydride protocol was employed to avoid epimerisation of **60** at the azide centre under basic conditions. The first step involved reduction using DIBAL at -78°C in DCM. On work up with saturated potassium sodium tartrate solution it was necessary to extract the product at least five times with DCM using relatively large volumes because of the highly viscous gel-like nature of the aqueous layer. Crude lactol **61** was then further reduced using 0.25 eq sodium borohydride. The temperature of the reaction was monitored carefully and kept below -10°C . This step allowed isolation of diol **58** after purification in a yield of 61%.

In order to eventually form the pyrrolidine ring it would be necessary to introduce a nitrogen derivative at C-6 such as an azide. However reduction of this would cause complications considering the azide just installed at C-5. Hence it was decided to introduce the NAc group at C-5 at this stage. First the azide was reduced in the presence of palladium black under a hydrogen atmosphere. The mixture was filtered through Celite® and concentrated. Acetylation using acetic anhydride in pyridine as the solvent produced per-acetylated product **62** in excellent 85% yield. It was not possible to selectively acetylate the amine using these reagents, however it was facile to deprotect the hydroxyl groups by employing Zemplén conditions of catalytic sodium methoxide in methanol. Amide **63** was isolated in 91% yield from **62** (figure 33).

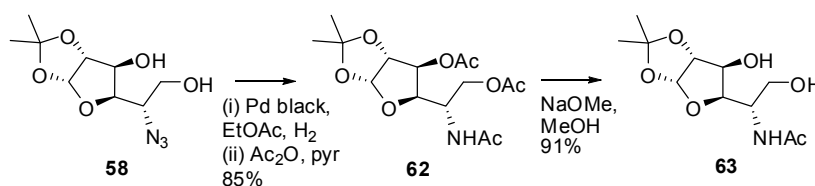


Figure 33

To allow the introduction of an azide group at C-6 a selective mesylation on **63** was required. It was thought that the epimeric compound **64** (figure 34) could also undergo the same transformations. Diol **64** had been synthesised in an attempt to obtain LyxNAc but this synthesis had been aborted in favour of the route presented in the previous half of this chapter. However diol **64** could be used to test the reaction on material that was no longer required so initial attempts to introduce azide were carried out on this molecule. Addition of 1.1 eq mesyl chloride with 0.2 eq DMAP in pyridine at 0°C followed by concentration and purification by flash column chromatography afforded 46% of the desired mono-mesylate **65** along with 19% of recovered starting material. The

mesylate was taken with 10 eq sodium azide in DMF (100 mgml^{-1}) at 80°C for 4h. The reaction yielded 15% of an azide which may have been the desired compound **66** along with 76% of THF **67**, arising from intramolecular attack of OH-3 on the primary mesylate (figure 34).

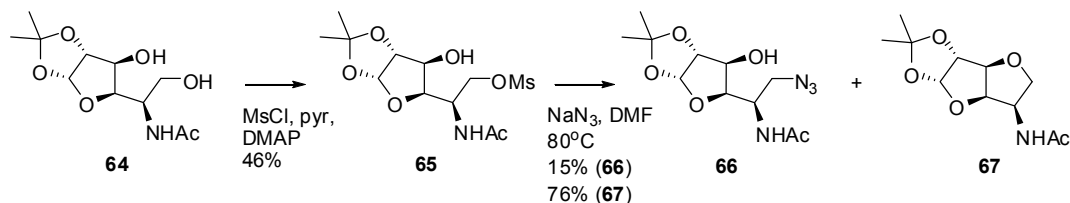


Figure 34

Following this result it was decided to employ a different strategy. An alternative way to introduce nitrogen at C-6 would be reductive amination of lactol **61** (figure 35). Suitable reducing reagents for reductive amination are sodium triacetoxyborohydride and sodium cyanoborohydride because the electron withdrawing groups on the boron attenuate the nucleophilicity of the reagent. The most reliable conditions for this system were found to be 1.3 eq sodium cyanoborohydride with 1.1 eq benzylamine in THF, adjusted to pH 6-7 with acetic acid to allow protonation of the imine but not the aldehyde. The reaction was quenched after 23h with sodium bicarbonate solution and the product extracted with ethyl acetate. At this stage it was necessary to protect the free amine to ease purification. Groups such as Fmoc and acetyl were tried but the best yields were achieved using 1.2 eq Boc anhydride with 2 eq TEA as base in DMF. Product **68** was isolated in a yield of 63% from **61**. It was decided to leave the C-5 substituent as azide at this stage as NAc groups make compounds much more polar and therefore more difficult to purify.

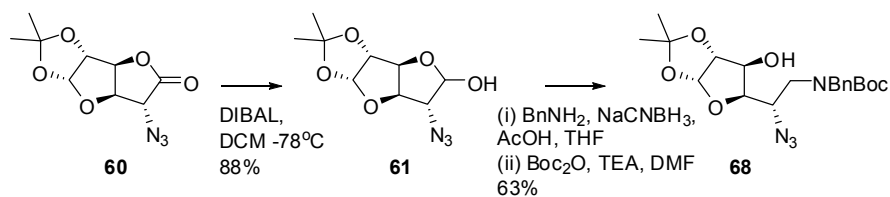


Figure 35

In order to form the pyrrolidine ring by activation of OH-3 followed by attack by the deprotected amine on C-6, it was first necessary to invert the stereochemistry of the alcohol to get the correct configuration for the desired product. The inversion was envisaged to proceed by an oxidation-reduction protocol. The first step was effected by pyridinium chlorochromate (PCC). A number of oxidants were trialled prior to this including Dess Martin periodinane (DMP), Moffatt, Swern and TPAP, however none were successful which prompted the use of PCC despite its toxicity and difficulty in handling. For the PCC oxidation it was vital that the reaction was carried out in anhydrous conditions for the reaction to proceed in an acceptable yield. Anhydrous DCM was used and the reaction performed in the presence of powdered molecular sieves. After reaction overnight the mixture was part filtered part purified by passing down a silica column topped with Celite®. Under these conditions it was possible to obtain the crude ketone in excess of 80% yield. The ketone was then reduced to form **69** using sodium borohydride leading to overall inversion relative to the original alcohol as the hydride attacks from the least hindered top face (figure 36).

The molecule was now set up to form the pyrrolidine ring. OH-3 was activated by converting it into a triflate, using triflic anhydride in pyridine at 0°C, which was purified by flash column chromatography to give **70** in 90% yield. The Boc protecting group was removed by stirring in neat TFA for 7 min. It was not left under acidic conditions for long

to prevent removal of the isopropylidene group. The TFA was removed by concentration *in vacuo* and the residue stirred with 5 eq sodium acetate in a mixture of 1,4-dioxane and water. After 2h a single product had formed and was extracted from the aqueous layer using DCM. Column chromatography yielded pyrrolidine **71** as an oil in a good yield of 84% from **70**.

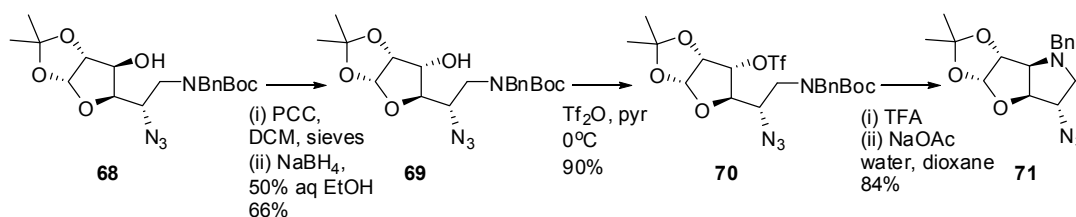


Figure 36

Owing to the relatively low yielding reductive amination step it was envisaged that an alternative route to pyrrolidine **71** could be achieved by adopting inversion of OH-3 prior to introduction of the N functionality. THF formation would not be a problem in this case if both hydroxyls OH-3 and OH-6 were activated. A stable di-triflate had been successfully ring closed on a similar system.¹⁹ TBS protected **72** was easily synthesised from diol **58**, whose synthesis was mentioned earlier, using 2 eq TBSCl in pyridine in excellent 91% yield (figure 37).¹⁹ This allowed selective oxidation of C-3 using the PCC method in the previous route. 3 Eq of oxidant were used under dry conditions. It transpired later during a test reaction that **72** could be oxidised in good yield using freshly made DMP, however it appeared crucial that the work up was performed by stirring with an aqueous mixture of saturated sodium bicarbonate and sodium thiosulfate (8g per 100ml). The partially purified ketone was reduced further with sodium borohydride to give *talo*-alcohol **73** in a quantitative yield. Subjecting **73** to 1.1 eq TBAF in THF gave 81% of *talo*-diol **74**.

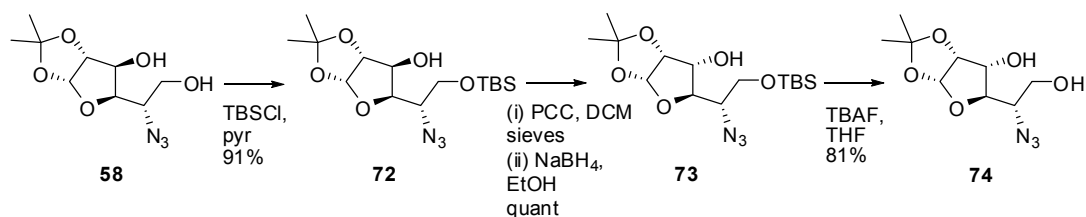


Figure 37

The di-triflate of the enantiomer of **74** had previously been found to form in good yield and be relatively stable as an intermediate.²⁰ Its formation was achieved by pre-mixing 3 eq of triflic anhydride with 6 eq of anhydrous pyridine in DCM at -40°C before adding a relatively dilute solution of starting material dropwise. The temperature was then maintained at -25°C and the reaction was complete within an hour. The mixture was washed with 2M HCl to remove the pyridine and excess triflic anhydride. The crude di-triflate was stirred with 6 eq benzylamine in THF for 3h whereupon LRMS showed complete loss of starting material peak at 509 and a peak at 316 corresponding to product. It was difficult to follow the reaction by t.l.c as pyrrolidine **71** co-spotted with the di-triflate. The reaction of **74** proceeded smoothly to form **71** in an 87% yield over two steps (figure 38). This was the same intermediate as attained by the reductive amination route described previously and the two routes are summarised in figure 38 for comparison. It was decided to use the di-triflate approach for scale up and synthesis of the L-enantiomer as the yield of **71** from **58** on the initial scale was 69% overall compared to 31% when using the reductive amination route, both over 7 steps. Upon scale up the yield for the route fell to 39%.

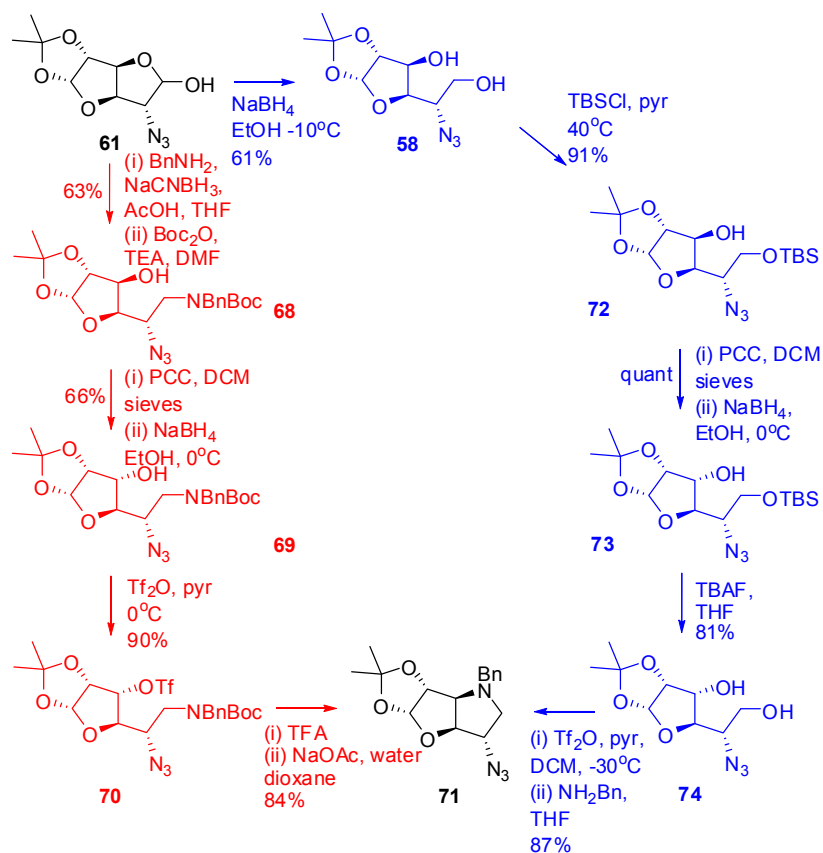


Figure 38

With pyrrolidine **71** in hand the synthesis now required the cleavage of one carbon unit and introduction of the NAc group. Firstly the isopropylidene protecting group on **71** was removed using 80% TFA in water, in 5h. The lactol **75** was purified by column chromatography in quantitative yield. At first it was hoped that the synthesis of **76** could be accessed by the route proposed in figure 39, however during initial attempts identification of the product was complicated by the presence of side products. Therefore a stepwise approach was taken. Lactol **75** was reacted with 1 eq sodium borohydride to form triol **77** (figure 40). Because of the polarity of this molecule it was necessary when purifying by column chromatography to elute using up to 20% methanol in ethyl acetate and the product eluted slowly on occasion. Therefore it was preferable to per-acetylate **77**

using the DIPEA, DMAP, acetic anhydride in DCM protocol mentioned before. This allowed purification by chromatography using cyclohexane:ethyl acetate 4:1.

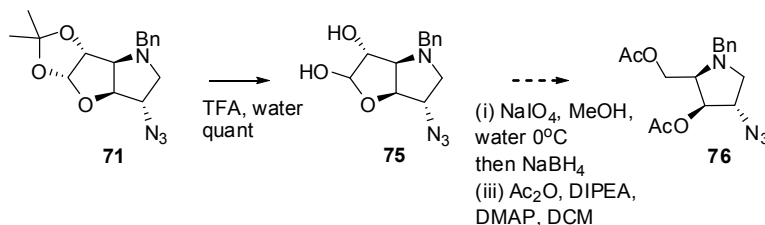


Figure 39

On one occasion the reduction of **75** did not react to completion so following acetylation compound **78** was isolated instead of the expected product **79**. However the acetylated lactol **78** could itself be taken with sodium borohydride, nearly 4 eq to force the reaction, and following acetylation and purification **79** was isolated in 66% yield from **78**. The increased yield could be a symptom simply of using elevated amounts of borohydride in the latter reaction. In any case it was decided to acetylate lactol **75** during scale up reactions.

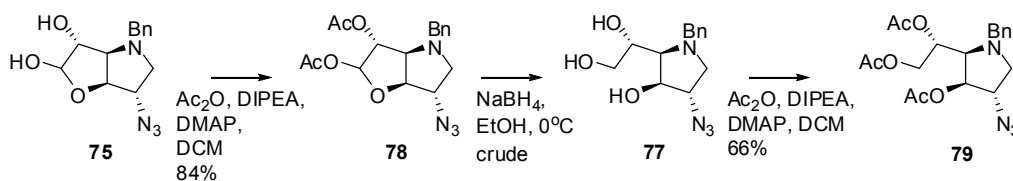


Figure 40

At this stage compound **79** had one extra carbon in the chain compared to the product which needed to be cleaved. To facilitate this transformation it was necessary to remove the acetyl protection using 0.3 eq sodium methoxide in methanol (figure 41). After a couple of hours the reaction had proceeded to completion and sodium periodate and water were added to the mixture directly. 1.6 Eq were used and it was necessary to stir the mixture vigorously as a thick white precipitate formed; the reaction was complete

in 2h. 2 Eq sodium borohydride were then added to the mixture. Acetic acid was used to quench the reaction after 4h and the product was washed. To ease purification the molecule was again per-acetylated to allow isolation of **76** in 62% over four steps from **79**. During the subsequent synthesis of the L-enantiomer, the equivalent of molecule **76** was successfully obtained using the route in figure 39 in a yield of 44% over 2 steps from the corresponding lactol; it is possible these steps could be optimised.

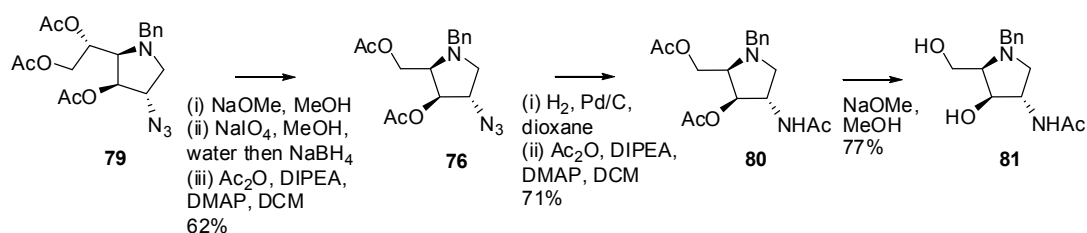


Figure 41

In order to introduce the NAc group, azide **76** was first put under a hydrogen atmosphere with 10% palladised carbon in 1,4-dioxane. It was necessary to carefully monitor the reaction as removal of the benzyl group was possible. De-benzylation, however, was seen to be slower and after 1h the reaction was complete and the amine was acetylated to afford **80** in 71% over 2 steps. With this molecule in hand *N*Bn-XylNAc **81** could be accessed by a simple Zemplén deprotection in good yield of 77% (figure 41). Chromatography was used to purify the compound using acetone as eluent. To obtain XylNAc **32** itself required de-benzylation using palladium black in the presence of hydrogen (figure 42). The catalyst was removed by filtration through Celite® and the molecule required no further purification, being obtained in an excellent 93% yield. The overall yield of **32** was 6% over 21 steps.

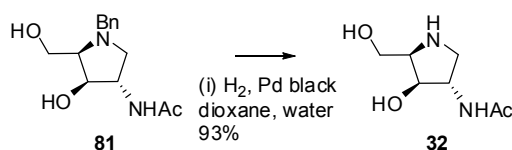


Figure 42

Synthesis of L-XylNAc

In order to complete the full set of pyrrolidine 2-*N*-acetylated iminosugars, L-XylNAc **82** needed to be synthesised. Despite the length of the final route used to synthesise the D-enantiomer **32**, it was nonetheless employed here as it had allowed the synthesis of the final compound in a reasonable overall yield (figure 43). Enantiomers have the same chemical properties as each other and so should behave in the same fashion with non-chiral molecules such as reagents and solvents. Acetonide protected L-glucuronolactone **83** was used as the starting material which was either obtained from a collaborator or synthesised from heptogluconolactone.²¹

The synthesis proceeded as for the enantiomeric route as expected. The experimental procedures used were parallel to those used for the D-series and as reported in the experimental for this chapter. The yields obtained are given in the scheme below and are not noticeably disparate from the enantiomeric synthesis. L-XylNAc **82** was synthesised in a yield of 8% over 21 steps.

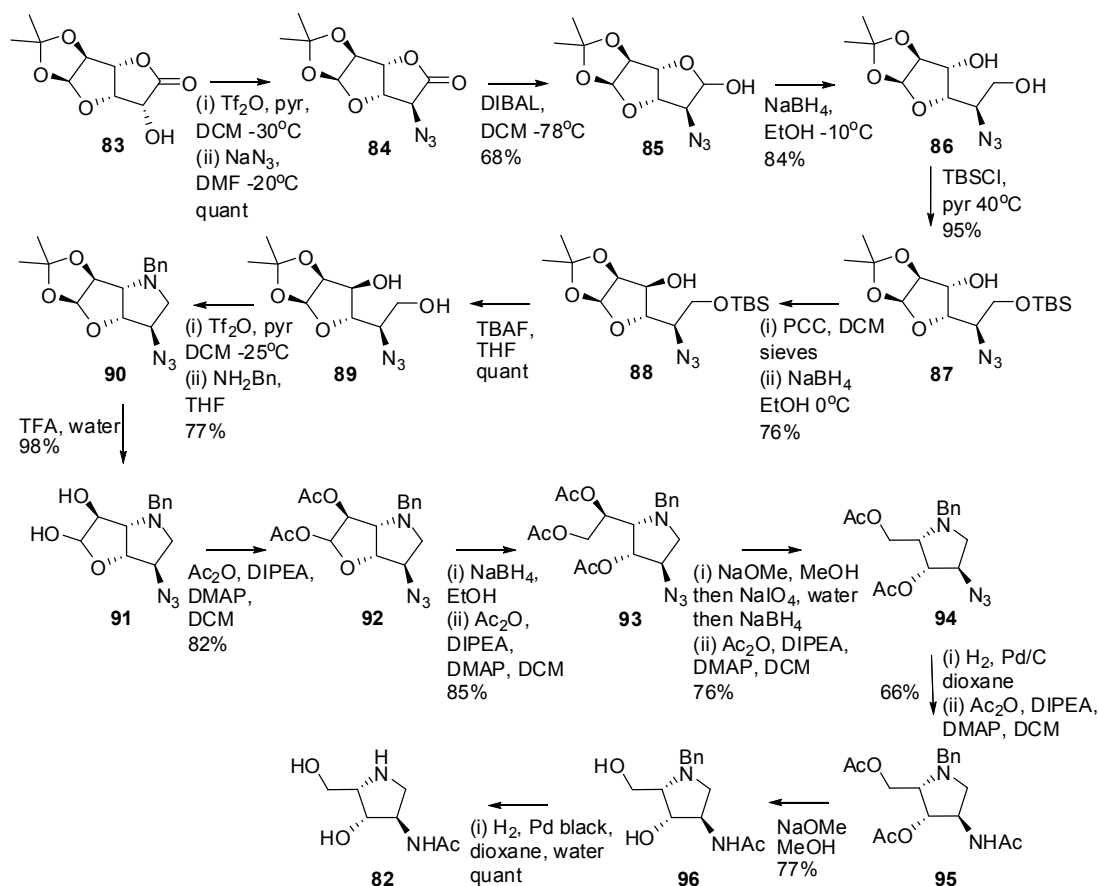


Figure 43

Crystal structure of NBn-L-XylNAc

Crystals suitable for X-ray crystallography were grown from a concentrated solution of NBn-L-XylNAc **96** in acetonitrile. After a week crystals had formed. A co-worker performed the data collection to elucidate the molecular structure (figure 44). The structure confirmed that the expected stereochemistry had been synthesised. The absolute configuration was assigned with reference to use of L-glucuronolactone as the starting material. The pyrrolidine ring is in an envelope configuration with the nitrogen out of the plane and the molecule is involved in six H-bonds (figure 45). The crystal structure has been published and a copy is given in the appendix of this thesis.²²

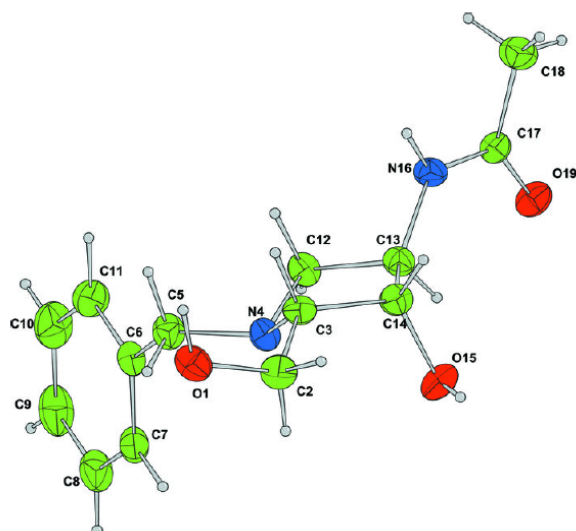


Figure 44: Single molecule of NBn-L-XylNAc, with displacement ellipsoids drawn at 50% probability level

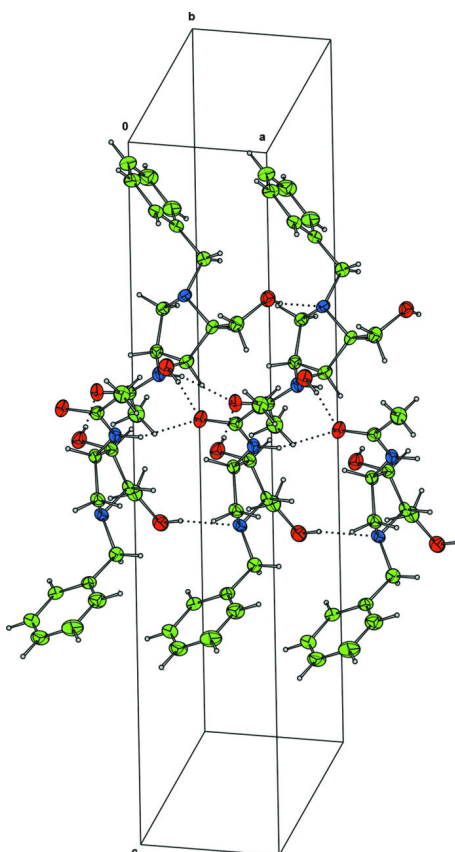


Figure 45: View of H-bonding

Biological evaluation

Initial enzyme screening on the *xylo*- compounds **32** and **82**, along with the C-2 epimer **57**, and its enantiomer **31** synthesised by a co-worker, and their respective NBn compounds, was performed in the Oxford Glycobiology department in the Butters group. The IC₅₀ data against β -N-acetylhexosaminidase (HL60) is summarised in Table 3. The data highlighted the D-*xylo* derivatives as particularly interesting. L-LyxNAc **57** did not display any significant inhibition and none of the compounds tested displayed any significant inhibition against α -N-acetylgalactosaminidase (*Charonia Lampas*).

Compound	IC ₅₀ /μM
NBn-XylNAc 81	1.1
XylNAc 32	2.6
NBn-L-XylNAc 96	132
L-XylNAc 82	416
NBn-LyxNAc	77.6
LyxNAc 31	180

Table 3: IC₅₀ data against β -N-acetylhexosaminidase (HL60)

The compounds were also assayed against a number of other glycosidases by a collaborator in Japan, Atsushi Kato, including α -glucosidase (yeast), β -glucosidase (almond), α -galactosidase (coffee beans), β -galactosidase (bovine liver) and α -N-acetylgalactosaminidase (chicken liver). No significant inhibition was observed except against β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase and these results, given in Tables 4 and 5, confirmed those collected in Oxford.²³ LABNAc **25** is given for comparison.

Enzyme		XylNAc 32	L-XylNAc 82	LyxNAc 31	LABNAc 25
		IC ₅₀ /μM			
β-N-acetyl galactos aminidase	<i>Aspergillus oryzae</i>	426	-(12.3%)	ND	ND
	HL60	50	-(14.4%)	ND	ND
β-N-acetyl glucosaminidase	Jack bean	5.6	ND	108	3.4
	Bovine kidney	6.5	-(25.3%)	125	0.64
	Human placenta	4.5	-(40.7%)	205	13
	HL60	6.6	ND	ND	ND
	<i>Aspergillus oryzae</i>	816	-(5.6%)	ND	-

Table 4: - no significant inhibition () inhibition at 1000μM; ND not determined

Enzyme		NBn-XylNAc 81	NBn-L-XylNAc 96	NBn-LyxNAc	NBn-LABNAc 37
		IC ₅₀ /μM			
β-N-acetyl galactosaminidase	<i>Aspergillus oryzae</i>	1.1	463	ND	ND
	HL60	22	-(30.0%)	ND	ND
β-N-acetyl glucosaminidase	Jack bean	2.5	ND	20	5.2
	Bovine kidney	4.0	807	69	0.36
	Human placenta	1.9	247	76	2.8
	HL60	2.2	429	ND	ND
	<i>Aspergillus oryzae</i>	2.6	-(42.5%)	ND	ND

Table 5: - no significant inhibition () inhibition at 1000μM; ND not determined

From the data given in tables 3, 4 and 5 it is possible to deduce some information on structure activity relationships with respect to inhibition of the β-N-acetylhexosaminidase enzyme. For LyxNAc and both enantiomers of XylNAc the introduction of the Bn substituent on the ring nitrogen increased the potency of the inhibition of the pyrrolidines. The two best inhibitors were NBn-XylNAc **81** and LABNAc **25** which have the same C-2 and C-3 configuration (figure 46). These two were predicted by the molecular modelling studies to be the best inhibitors. Both of the enantiomers lost their inhibitory effects. The C-2 epimer of XylNAc **32**, LyxNAc **31**, displayed a weak inhibition profile against the β-N-acetylglucosaminidases tested. L-

LyxNAc **57**, the enantiomer, displayed no significant inhibition, as for DRBNAc **30** which displays the same C-2 and C-3 configuration.⁶ Interestingly though, LRBNAc, which would be comparable to LyxNAc **31** in this respect, did not show even weak inhibition. Molecular modelling had predicted L-LyxNAc to be a potential inhibitor based on NAc group and ring N overlap which could indicate C-3 configuration is more important than was considered. These results would suggest that variations in C-4 configuration are better tolerated by the enzyme than at either C-2 or C-3.

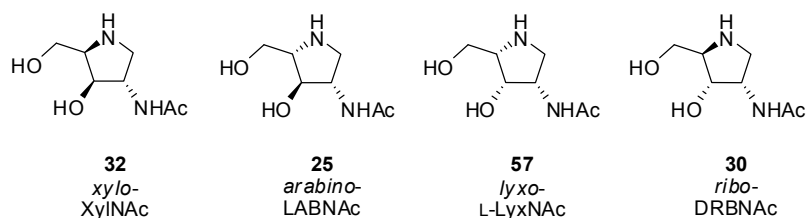


Figure 46

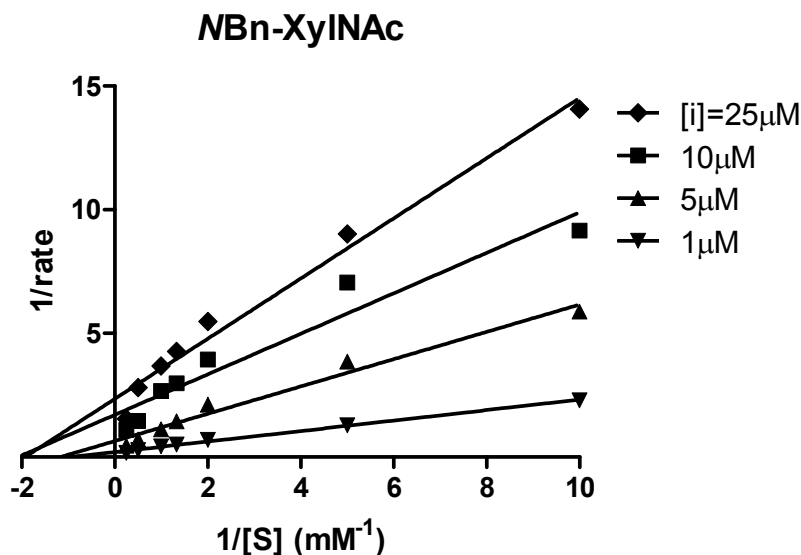
Table 6 shows the K_i values measured in the Oxford Glycobiology Institute for the active compounds against HL60 derived β -*N*-acetylhexosaminidase. The overall trend is comparable to the IC_{50} data. It appears that as predicted both the NBn derivative and XylNAc itself are very good inhibitors.

Compound	$K_i/\mu\text{M}$
NBn-XylNAc 81	0.54
XylNAc 32	0.29
NBn-L-XylNAc 96	39.6
L-XylNAc 82	171
NBn-LyxNAc	18.6
LyxNAc 31	20.9

Table 6: K_i data against β -*N*-acetylhexosaminidase (HL60)

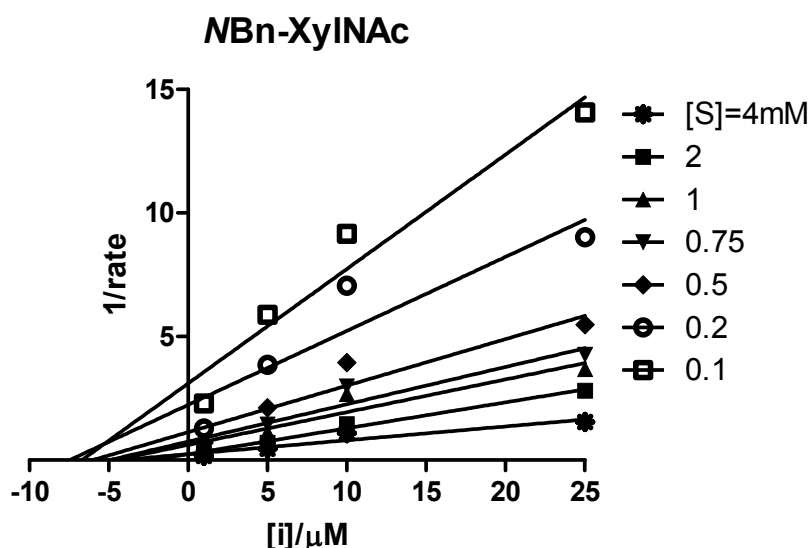
Kinetic analysis of β -N-acetylhexosaminidase inhibitors

In order to extract information regarding the mode of action of the six active inhibitors a Lineweaver-Burk graph was plotted for each. This type of graph is a double reciprocal plot with the inverse of the substrate concentration ($[S]$) on the x -axis and the inverse of the catalytic rate on the y -axis, which in this case is the relative fluorescence reading from the assay. The gradient intersects the y -axis at $V_{\max}$, the maximum velocity, and the x -axis at the inverse of $-K_m$, the Michaelis Menten constant. For a competitive inhibitor $V_{\max}$ remains unchanged with varying inhibitor concentration ($[i]$). However, in the case of a non-competitive inhibitor $V_{\max}$ decreases with increasing inhibitor concentration but K_m is unchanged, hence the gradients have the same x -intercept. In the case of NBn-XylNAc (graph 1) the gradients, within experimental error, appear to meet on the x -axis indicating a non-competitive mode of inhibition.



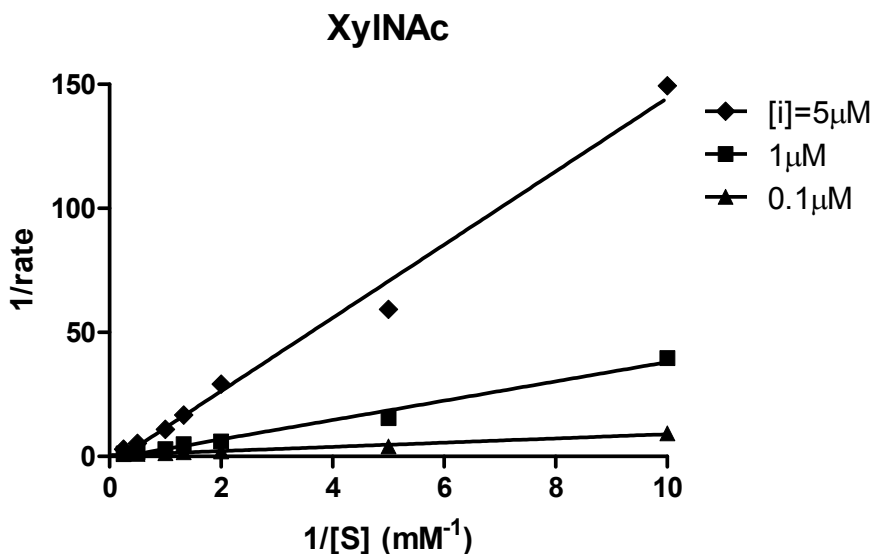
Graph 1: Lineweaver-Burk plot of NBn-XylNAc **81** against HL60 β -N-acetylhexosaminidase

However the Lineweaver-Burk plot, being a double reciprocal plot, has a propensity to attach increased significance to low concentrations or readings which are prone to greater error. Another method for inferring the mode of action of an enzyme inhibitor plots the inhibitor concentration against the inverse of the rate at constant substrate concentration; known as a Dixon plot. The gradients again intersect at $y=0$ for non-competitive inhibition but for linear competitive inhibition they intersect at neither $x=0$ or $y=0$. From graph 2 it appears that NBn-XylNAc displays non-competitive inhibition which agrees with the Lineweaver-Burk plot (graph 1).

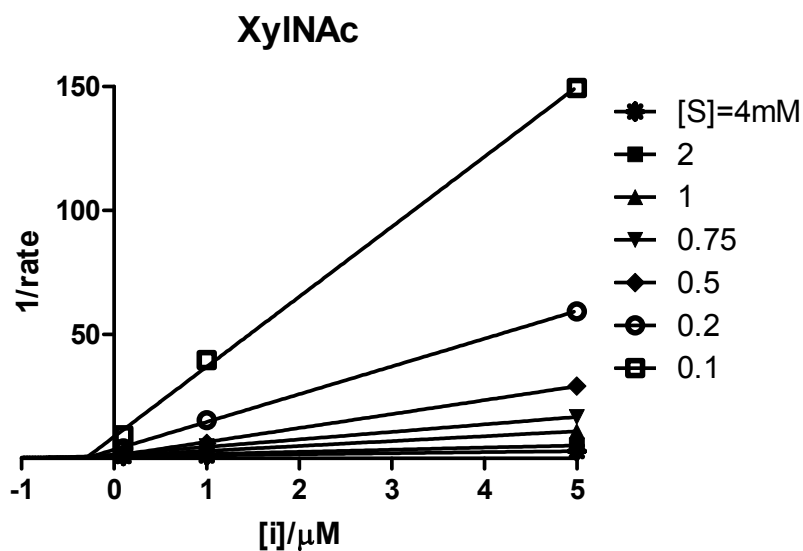


Graph 2: Dixon plot of NBn-XylNAc **81** against HL60 β -N-acetylhexosaminidase

For the Lineweaver-Burk plot of XylNAc (graph 3) the gradients intersect very close to the origin making deduction of the mode difficult. It is possible that the inhibitor is displaying mixed inhibition, which graphically is represented by the gradients intersecting at a point other than on one of the axes. With reference to the Dixon plot (graph 4) it appears that the mode could be non-competitive.



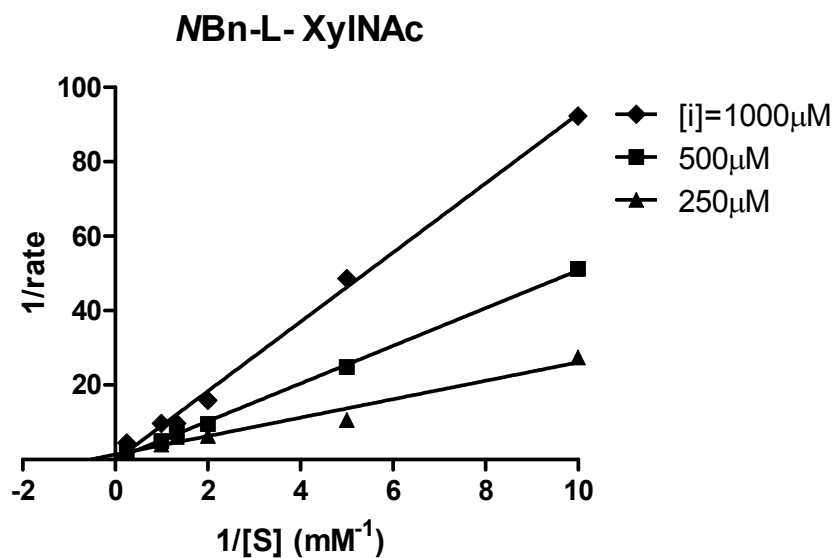
Graph 3: Lineweaver-Burk plot of XylINAc **32** against HL60 β -N-acetylhexosaminidase



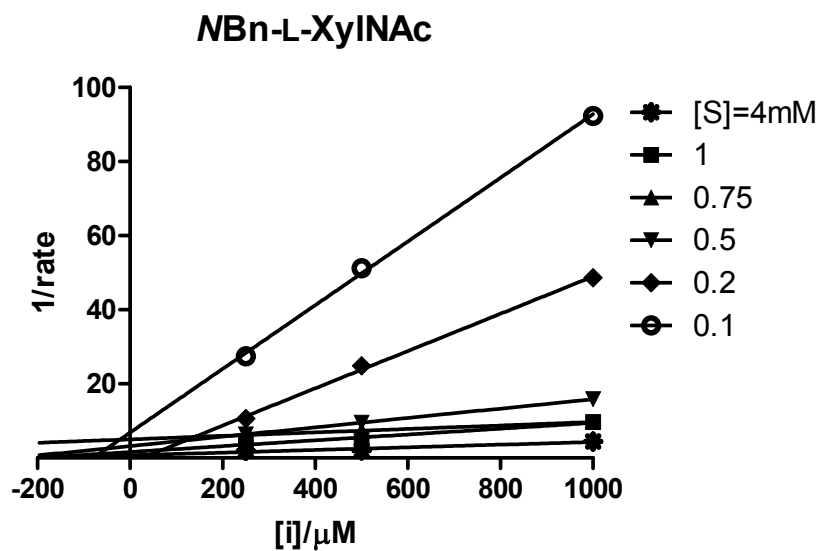
Graph 4: Dixon plot of XylINAc **32** against HL60 β -N-acetylhexosaminidase

As for the case above, the mode of inhibition of NBn-L-XylINAc is a little unclear from graph 5 with competitive or mixed inhibition being possibilities. The Dixon plot in Figure 6 does not provide any clear information either. In this case it is difficult to say with any degree of certainty and the mode may be mixed. Fortunately the graphs for L-

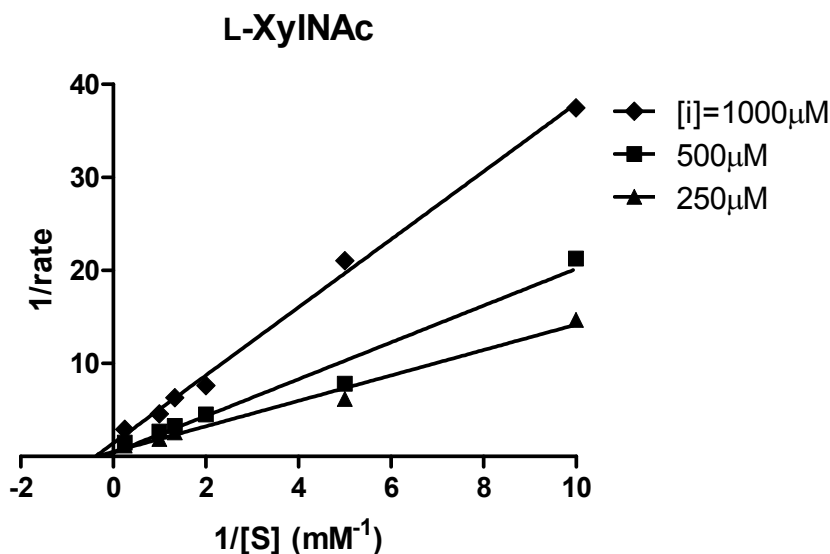
XylINAc (graphs 7 and 8) are a little more informative with a non-competitive mode seeming most likely as both converge on the x -axis.



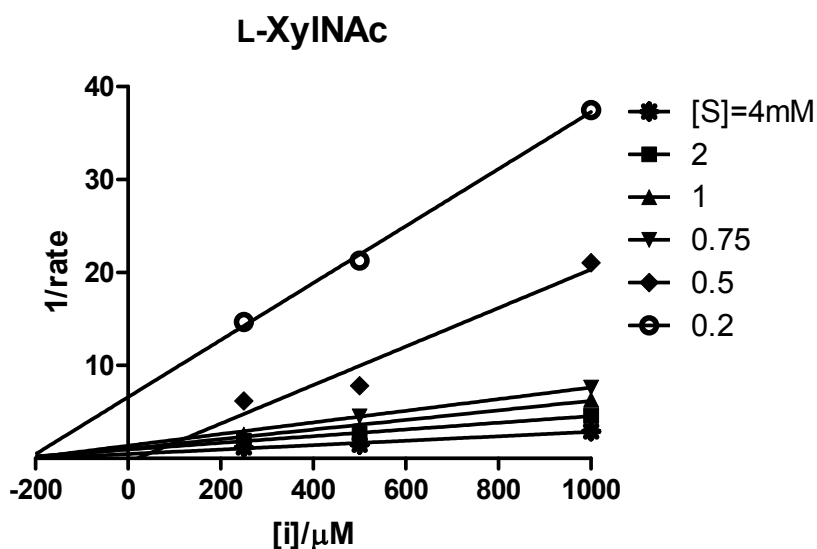
Graph 5: Lineweaver-Burk plot of NBn-L-XylINAc **96** against HL60 β -N-acetylhexosaminidase



Graph 6: Dixon plot of NBn-L-XylINAc **96** against HL60 β -N-acetylhexosaminidase



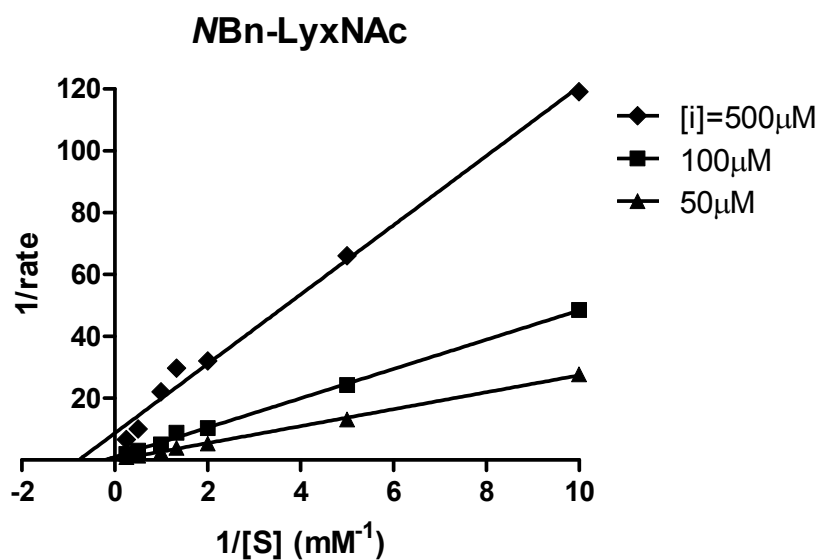
Graph 7: Lineweaver-Burk plot of L-XylNAc **82** against HL60 β -N-acetylhexosaminidase



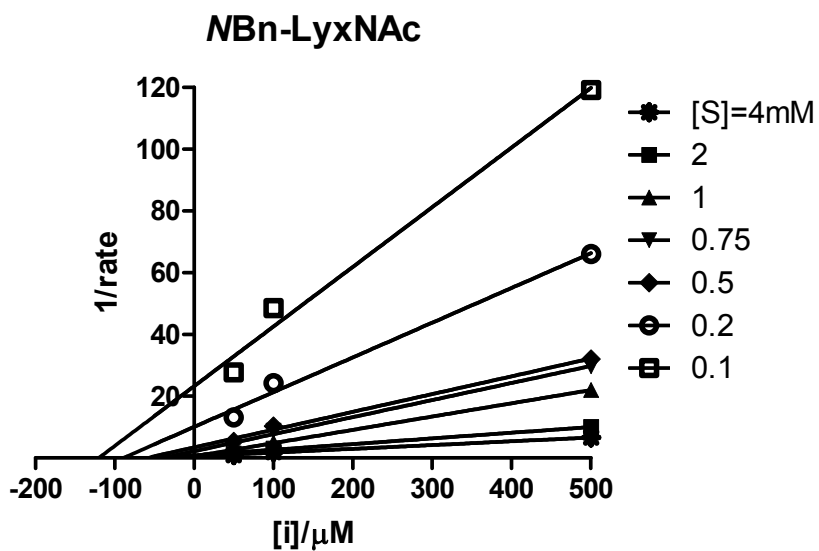
Graph 8: Dixon plot of L-XylNAc **82** against HL60 β -N-acetylhexosaminidase

From the graphs below, 9 to 12, both the NBn and un-substituted LyxNAc compounds appear to be displaying non-competitive inhibition. The fact most of these inhibitors are potentially displaying non-competitive inhibition is promising from a chaperone mediated therapy point of view. In the substrate burdened environment of a

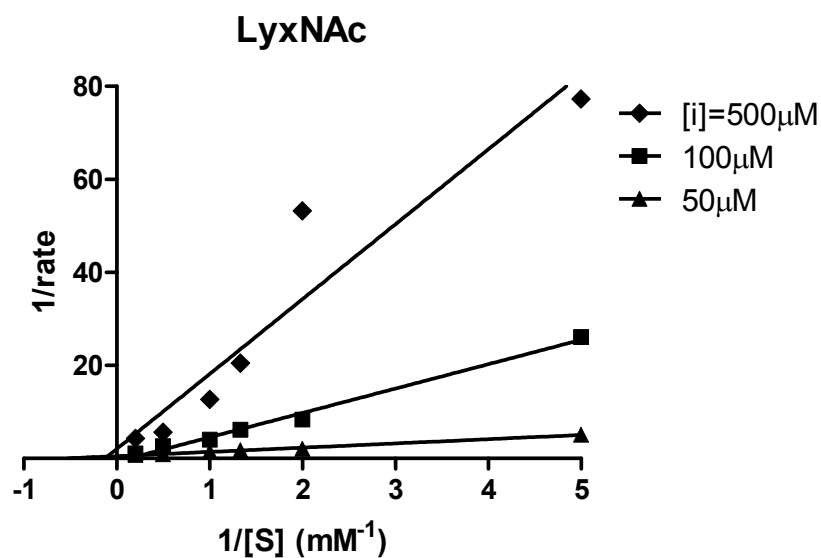
storage disorder inflicted lysosome a competitive inhibitor competing for the enzyme active site with the substrate may be disadvantageous.



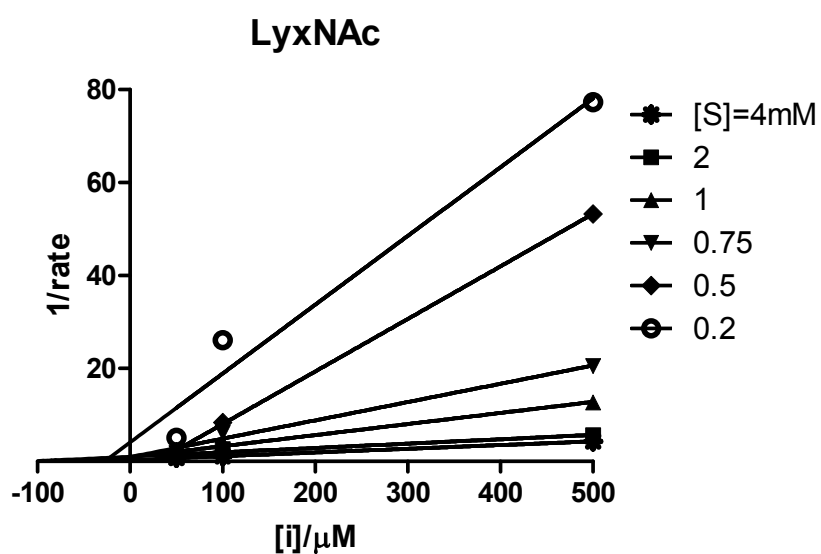
Graph 9: Lineweaver-Burk plot of NBn-LyxNAc against HL60 β -N-acetylhexosaminidase



Graph 10: Dixon plot of NBn-LyxNAc against HL60 β -N-acetylhexosaminidase



Graph 11: Lineweaver-Burk plot of LyxNAc **31** against HL60 β -N-acetylhexosaminidase



Graph 12: Dixon plot of LyxNAc **31** against HL60 β -N-acetylhexosaminidase

Summary

The syntheses of the target compounds L-LyxNAc **57** and XylNAc **32** were achieved, from D-ribose and D-glucuronolactone respectively, in good yield overall considering the length of the routes used. The synthesis of L-LyxNAc **57** exploiting the neutrally introduced benzhydryl protecting group successfully circumvented the problems with epimerisation at the azide centre encountered in the previous attempts utilising alternative protecting groups. Both the NBn derivative and the free amine were accessed. Biological evaluation of D-LyxNAc against *N*-acetylhexosaminidases revealed moderate inhibition. Kinetic analysis suggested a non-competitive mechanism to be probable.

With reference to the epimerisation issues encountered in the original synthesis of LyxNAc, an alternative strategy was pursued in the synthesis of XylNAc **32** using glucuronolactone as the starting material. Two routes to the key pyrrolidine intermediate **71** were achieved. An approach involving sodium periodate was employed to cleave the excess carbon unit. Both the NBn derivative and free amine were synthesised in both D- and L- enantiomeric forms. A crystal structure confirmed the structure of L-XylNAc **82**. In terms of chronology, the synthesis reported here was started before the benzhydryl group had been successfully employed in the L-LyxNAc **57** synthesis, so it may be possible to synthesise XylNAc **32** by a more efficient route exploiting this reagent. Biological analysis found that the D-enantiomer was a potent inhibitor against β -*N*-acetylhexosaminidase. Kinetic analysis indicated a non-competitive mode of inhibition which is interesting considering molecular modelling predicted potential potent competitive inhibition. No α -*N*-acetylgalactosaminidase inhibition was observed. The L-enantiomer displayed only weak inhibition.

General Experimental

Solvents and reagents

DMF and pyridine were purchased dry from the Aldrich chemical company in Sure-SealTM bottles. Water was deionised. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Reactions performed under an atmosphere of nitrogen, argon or hydrogen gas were maintained by an inflated balloon.

Chromatography

Thin layer chromatography (t.l.c.) was performed on aluminium backed sheets coated with 60 F₂₅₄ silica. Sheets were developed using a dip of 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid or a dip of 1% KMnO₄ and 6.3% K₂CO₃ in 0.015M NaOH. Flash column chromatography was performed using Sorbsil C60 40/60 silica.

NMR spectroscopy

NMR spectra were recorded on a Bruker DPX 400 (¹H: 400.1 MHz) spectrometer, a Bruker DQX 400 (¹H: 400.2 MHz and ¹³C: 100.6 MHz) spectrometer, or a Bruker AV 500 (¹H: 500 MHz) spectrometer in the deuterated solvent stated. All chemical shifts (δ) are quoted in ppm. Coupling constants (*J*) are quoted in Hz. Residual signals from the solvents were used as an internal reference. For samples in D₂O, MeCN was added as an internal reference. The ¹³C resonances were assigned using DEPT sequences.

Melting points

Melting points were recorded on a Kofler hot block and are uncorrected.

Infrared spectroscopy

Infrared spectra were recorded at room temperature on a Perkin-Elmer 1750 IR Fourier Transform Spectrometer, using thin films on NaCl or Ge plates as stated and were recorded over 32 scans at a resolution of 4cm^{-1} . Only the characteristic peaks are quoted.

Mass spectrometry

Low resolution mass spectra (m/z) were recorded on a Micromass BioQ II-ZS LCT mass spectrometer using the technique of electrospray ionisation (ESI) or field ionisation (FI). High resolution mass spectra (HRMS) were recorded on a Micromass Autospec 500 OAT mass spectrometer using the technique of electrospray ionisation (ESI).

Polarimetry

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1dm. Concentrations are quoted in g/100mL. The wavelength at which the rotations were measured corresponds to the sodium D line.

Enzyme inhibition assays

For the tests performed in the Oxford Glycobiology Institute β -*N*-acetylhexosaminidase was used as an enzyme homogenate from HL60 cells and tested using 4-methylumbelliferyl *N*-acetyl- β -D-glucosaminide as the substrate. α -*N*-

Acetylgalactosaminidase, partially purified from *Charonia lampas*, was used with PNP-*N*-acetyl- α -D-galactosaminide as substrate.

Reagents

- Enzyme solutions were made up using distilled water with 0.02% sodium azide. These solutions were stored on ice whilst in use and at -20°C otherwise.
- Stock solutions of the inhibitors (50mM) were made using distilled water.
- Enzyme substrate solutions were made from a stock solution (4mM) in 10ml batches by dissolving the appropriate mass of substrate in citrate/citric acid buffer (pH5 buffer made using citric acid and sodium citrate solutions). These were kept at 4°C when not in use.

Method

Assays were carried out in duplicate, using water as a blank in place of inhibitor. Linearity over the time course of the reaction was checked using a series of incubation times.

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

5 μ l enzyme solution

5 μ l inhibitor solution

40 μ l substrate solution

The reaction mix was incubated at 37°C for 15 minutes and was quenched by the addition of 200 μ l of 0.5M Na₂CO₃. The plates were then immediately read using either a

Molecular Devices SpectraMax M5 or Molecular Devices UVmax kinetic microplate reader and SOFTmax 2.35 software at 405nm.

Determination of IC_{50} values

Percentage inhibition was plotted against the log of the inhibitor concentration and a trend line was plotted using GraphPad Prism 5. The IC_{50} value for each compound was calculated from the value of the log of the inhibitor concentration at 50% inhibition of enzyme activity.

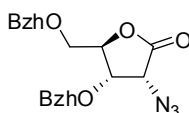
Determination of K_i values

The inhibition concentrations were determined for compounds that displayed significant inhibition in the initial screening. K_i values were calculated from Lineweaver-Burk plots ($1/v$ against $1/[S]$) using a suitable range of substrate solutions and inhibitor concentrations. Values were plotted and subjected to linear regression using GraphPad Prism 5.

For known compounds the data was in agreement with the literature and therefore only $[\alpha]_D$, m.p. and proton NMR data are quoted. For the enantiomers of compounds given in this thesis only $[\alpha]_D$ and m.p. are quoted as the proton NMR data matched.

Chapter 2: Experimental

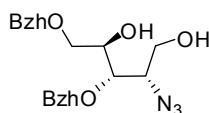
L-LyxNAC

2-Azido-3,5-di-*O*-benzhydryl-2-deoxy-D-ribo-1,4-lactone **50**

A suspension of 2-azido-2-deoxy-ribo-1,4-lactone **42** (638mg, 3.69mmol) in toluene (89ml) was heated to reflux. Diphenyldiazomethane (2.86g, 14.7mmol) was added. After 4.5h, t.l.c. analysis (cyclohexane:ethyl acetate 3:1) revealed product formation (R_f 0.44) and no remaining starting material (R_f 0.02). The mixture was concentrated *in vacuo* and purified by flash column chromatography (cyclohexane:ethyl acetate 50:1→10:1). This afforded product **50** (1.64g, 88%) as a yellow oil.

Data: $[\alpha]_D^{25}$: +32.6 (*c*, 0.56 in CHCl_3) [Lit.¹⁵ enantiomer $[\alpha]_D^{26}$: -42 (*c*, 1.14 in CHCl_3)].

δ_H (400MHz, CDCl_3): 3.39 (1H, dd, $J_{5a,5b}$ 11.0, $J_{5a,4}$ 2.4, H-5a), 3.65 (1H, dd, $J_{5b,5a}$ 11.0, $J_{5b,4}$ 3.1, H-5b), 4.29 (1H, d, $J_{2,3}$ 6.0, H-2), 4.34 (1H, d, $J_{3,2}$ 6.2, H-3), 4.59 (1H, a-t, $J_{4,5a+5b}$ 2.7, H-4), 5.27, 5.59 (2x1H, 2xs, 2xCHPh₂), 7.14-7.37 (4x5H, m, 20xArCH).

2-Azido-3,5-di-*O*-benzhydryl-2-deoxy-D-ribitol **51**

Protected lactol **50** (1.64g, 3.24mmol) was suspended in DCM (16ml) and cooled to -78°C. DIBAL in toluene (1.5M, 3.24ml, 4.87mmol) was added and stirred at -78°C. After 4h, t.l.c. analysis (cyclohexane:ethyl acetate 3:1) indicated consumption of starting material (R_f 0.44) and formation of product (R_f 0.40). The reaction was diluted with

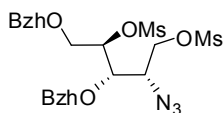
DCM (50ml) and saturated sodium potassium tartrate solution (70ml) was added. The mixture was stirred vigorously at room temperature for 20h. The aqueous and organic layers were then separated and the product re-extracted from the aqueous layer with DCM (4x70ml). The organic layers were dried (MgSO_4), filtered and concentrated to give crude lactol (1.64g, assumed quant).

The crude lactol (1.64g, 3.24mmol) was dissolved in methanol (20ml) and initially cooled to 0°C . Sodium borohydride (123mg, 3.24mmol) was added and stirred at room temperature. After 3h, t.l.c. analysis (cyclohexane:ethyl acetate 3:1) indicated product formation (R_f 0.20) and no residual starting material (R_f 0.40). The reaction was quenched with glacial acetic acid and concentrated, co-evaporating with methanol twice. The residue was purified by flash column chromatography (cyclohexane:ethyl acetate 3:1) to afford clean diol **51** (1.02g, 62%) as a colourless oil, plus some product mixed with what was probably the C-2 epimer (640mg, 38%).

Data: $[\alpha]_D^{25}$: -32.5 (c , 0.73 in CHCl_3) [Lit.¹⁵ enantiomer $[\alpha]_D^{26}$: +32 (c , 0.49 in CHCl_3)].

δ_H (400MHz, CD_3CN): 3.26 (1H, br-s, OH-1), 3.36 (1H, dd, $J_{5a,5b}$ 9.9, $J_{5a,4}$ 6.1, H-5a), 3.50 (1H, d, $J_{\text{OH},4}$ 4.4, OH-4), 3.56 (1H, dd, $J_{5b,5a}$ 9.9, $J_{5b,4}$ 3.8, H-5b), 3.61 (1H, br-a-s, H-1a), 3.65 (1H, dd, J 6.3, J 3.3, H-3), 3.78-3.85 (2H, m, H-2, H-1b), 3.97-4.02 (1H, m, H-4), 5.35, 5.72 (2x1H, 2xs, 2x CHPh_2), 7.26-7.37 (4x5H, m, 20xArCH).

2-Azido-3,5-di-*O*-benzhydryl-2-deoxy-1,4-di-*O*-methanesulfonyl-D-ribitol **52**

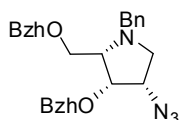


Diol **51** (1.02g, 2.00mmol) was dissolved in DCM (10ml) at 0°C and TEA (1.11ml, 8.01mmol) was added. After 20 min methanesulfonyl chloride (0.62ml, 8.01mmol) was added dropwise. After 3h, t.l.c. analysis (cyclohexane:ethyl acetate 2:1) indicated formation of product (R_f 0.43) from starting material (R_f 0.35). The mixture was concentrated and purified by flash column chromatography (cyclohexane:ethyl acetate 3:1) to afford di-mesylate **52** (1.30g, 98%) as a colourless oil.

Data: $[\alpha]_D^{25}$: -22.6 (c , 0.83 in CHCl_3) [Lit.¹⁵ enantiomer $[\alpha]_D^{26}$: +21 (c , 0.99 in CHCl_3)].

δ_H (400MHz, CDCl_3): 2.93, 2.97 (2x3H, 2xs, CH_3), 3.64-3.69 (1H, m, H-5a), 3.77 (1H, dd, $J_{5b,5a}$ 11.1, $J_{5b,4}$ 3.9, H-5b), 3.86 (1H, dd, $J_{3,2}$ 5.6, $J_{3,4}$ 3.9, H-3), 3.95 (1H, ddd, $J_{2,1a}$ 8.1, $J_{2,3}$ 5.6, $J_{2,1b}$ 3.1, H-2), 4.08 (1H, dd, $J_{1a,1b}$ 10.8, $J_{1a,2}$ 8.2, H-1a), 4.45 (1H, dd, $J_{1b,1a}$ 10.8, $J_{1b,2}$ 3.1, H-1b), 5.09-5.13 (1H, m, H-4), 5.34, 5.71 (2x1H, 2xs, 2x CHPh_2), 7.19-7.36 (4x5H, m, 20xArCH).

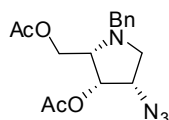
2-Azido-3,5-di-*O*-benzhydryl-*N*-benzyl-1,4-imino-1,2,4-trideoxy-L-lyxitol **53**



Di-mesylate **52** (1.30g, 1.96mmol) was dissolved in toluene (15ml) and stirred with benzylamine (3.23ml) at 80°C for 45h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) then indicated product (R_f 0.83), some side products (R_f 0.73, 0.39) and no remaining starting material (R_f 0.68). The mixture was concentrated and purified by flash column chromatography (cyclohexane:ethyl acetate 7:1). Pyrrolidine **53** (743mg, 65%) was obtained as a yellow oil.

Data: $[\alpha]_{\text{D}}^{25}$: +1.6 (*c*, 0.95 in CHCl₃) [Lit.¹⁵ enantiomer $[\alpha]_{\text{D}}^{26}$: -4 (*c*, 1.02 in CHCl₃)]. δ_{H} (400MHz, CDCl₃): 2.37 (1H, dd, $J_{1\text{a},1\text{b}}$ 10.7, $J_{1\text{a},2}$ 4.7, H-1a), 2.96 (1H, a-d, $J_{1\text{b},1\text{a}}$ 10.7, H-1b), 3.13 (1H, a-dt, $J_{4,3}$ 7.5, $J_{4,5\text{a}+5\text{b}}$ 5.8, H-4), 3.43 (1H, d, J_{gem} 13.4, CH₂Ph), 3.61 (1H, a-t, $J_{2,1+3}$ 4.2, H-2), 3.66 (1H, dd, $J_{5\text{a},5\text{b}}$ 9.8, $J_{5\text{a},4}$ 5.3, H-5a), 3.97 (1H, dd, $J_{5\text{b},5\text{a}}$ 9.7, $J_{5\text{b},4}$ 6.1, H-5b), 4.17 (1H, d, J_{gem} 14.1, CH₂Ph), 4.23 (1H, dd, $J_{3,4}$ 7.9, $J_{3,2}$ 5.9, H-3), 5.33, 5.59 (2x1H, 2xs, 2xCHPh₂), 7.24-7.35 (4x5H, m, 20xArCH).

3,5-Di-*O*-acetyl-2-azido-*N*-benzyl-1,4-imino-1,2,4-trideoxy-*L*-lyxitol **54**

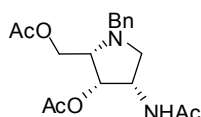


The di-benzhydrylated compound **53** (110mg, 0.19mmol) was dissolved in acetic anhydride (1ml) and cooled to 0°C. Boron trifluoride diethyl etherate (0.15ml, 1.23mmol) was added. After 22h, t.l.c. (cyclohexane:ethyl acetate 4:1) indicated a major baseline product and no remaining starting material (*R_f* 0.68). The mixture was concentrated *in vacuo* and the residue partitioned carefully between ethyl acetate (15ml) and saturated sodium bicarbonate solution (10ml). The aqueous layer was re-washed with more ethyl acetate (2x10ml). The organic layers were combined and dried (MgSO₄), filtered and concentrated. The crude was purified by flash column chromatography (cyclohexane:ethyl acetate 4:1→2:1) to afford di-acetate **54** (43mg, 68%) as a brown oil.

Data: $[\alpha]_{\text{D}}^{25}$: +63.2 (*c*, 1.31 in CHCl₃) [Lit.¹⁵ enantiomer $[\alpha]_{\text{D}}^{20}$: -69 (*c*, 1.06 in CHCl₃)]. δ_{H} (400MHz, CDCl₃): 2.04, 2.17 (2x3H, 2xs, 2xCO₂CH₃), 2.62 (1H, dd, $J_{1\text{a},1\text{b}}$ 11.0, $J_{1\text{a},2}$ 5.4, H-1a), 3.02 (1H, dd, $J_{1\text{b},1\text{a}}$ 11.0, $J_{1\text{b},2}$ 2.9, H-1b), 3.20 (1H, a-q, $J_{4,3+5}$ 6.7, H-4), 3.53 (1H, d, J_{gem} 13.5, CH₂Ph), 3.97 (1H, a-dt, $J_{2,1+3}$ 5.4, $J_{2,1\text{b}}$ 3.0, H-2), 4.10 (1H, d, J_{gem}

13.5, CH_2Ph), 4.25-4.27 (2H, m, H-5a, H-5b), 5.43 (1H, dd, $J_{3,4}$ 7.4, $J_{3,2}$ 5.7, H-3), 7.27-7.34 (5H, m, 5ArCH).

2-Acetamido-3,5-di-O-acetyl-N-benzyl-1,2,4-trideoxy-1,4-imino-L-lyxitol 55

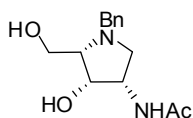


Azide **54** (211mg, 0.64mmol) was dissolved in THF:AcOH:Ac₂O (3:2:1, 7.2ml). Powdered zinc (831mg, 12.7mmol) was added, followed lastly by saturated aqueous copper sulfate solution (1.6ml). After 1h, t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated baseline product and no remaining starting material (R_f 0.58). The mixture was filtered through Celite®, eluting with THF. After concentration, the residue was purified by flash column chromatography (cyclohexane:ethyl acetate 1:1→0:1) to yield **55** (164mg, 74%) as a brown oil.

Data: $[\alpha]_D^{25}$: +16.7 (c , 1.15 in CHCl_3) [Lit.¹⁵ enantiomer $[\alpha]_D^{20}$: -14 (c , 0.97 in CHCl_3)].

δ_H (400MHz, CDCl_3): 1.98 (3H, s, NHCOCH_3), 2.07, 2.08 (2x3H, 2xs, 2x CO_2CH_3), 2.65 (1H, dd, $J_{1a,1b}$ 10.6, $J_{1a,2}$ 6.1, H-1a), 2.81 (1H, dd, $J_{1b,1a}$ 10.6, $J_{1b,2}$ 3.2, H-1b), 3.14 (1H, a-dt, $J_{4,3}$ 6.8, $J_{4,5}$ 5.8, H-4), 3.50 (1H, d, J_{gem} 13.2, CH_2Ph), 4.02 (1H, d, J_{gem} 13.2, CH_2Ph), 4.16-4.17 (2H, m, H-5a, H-5b), 4.61-4.67 (1H, m, H-2), 5.45 (1H, a-t, $J_{3,2+4}$ 6.6, H-3), 6.22 (1H, d, $J_{\text{NH},2}$ 8.0, NH), 7.26-7.36 (5H, m, 5ArCH).

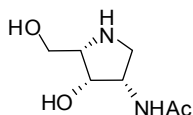
2-Acetamido-N-benzyl-1,2,4-trideoxy-1,4-imino-L-lyxitol 56



Di-ester **55** (156mg, 0.45mmol) was stirred with sodium methoxide (5mg, 0.2mmol) in methanol (1.5ml). After 2h, t.l.c. analysis (20% methanol in ethyl acetate) indicated product (R_f 0.21) and no remaining starting material (R_f 0.63). The mixture was concentrated *in vacuo* and purified by flash column chromatography (acetone). This afforded diol **56** (71mg, 60%) as a yellow oil which solidified on scratching.

Data: m.p.: 58-61 °C [Lit.¹⁵ enantiomer m.p.: 68-70 °C]. $[\alpha]_D^{25}$: +60.5 (*c*, 0.91 in MeOH) [Lit.¹⁵ enantiomer $[\alpha]_D^{20}$: -62 (*c*, 1.45 in MeOH)]. δ_H (400MHz, MeOD): 1.96 (3H, s, NHCOCH₃), 2.70 (1H, dd, $J_{1a,1b}$ 10.4, $J_{1a,2}$ 7.4, H-1a), 2.81 (1H, dd, $J_{1b,1a}$ 10.4, $J_{1b,2}$ 5.8, H-1b), 2.86 (1H, a-dt, $J_{4,3+5b}$ 5.7, $J_{4,5a}$ 4.1, H-4), 3.51 (1H, d, J_{gem} 13.2, CH₂Ph), 3.69 (1H, dd, $J_{5a,5b}$ 11.2, $J_{5a,4}$ 4.0, H-5a), 3.80 (1H, dd, $J_{5b,5a}$ 11.2, $J_{5b,4}$ 6.1, H-5b), 4.00 (1H, d, J_{gem} 13.2, CH₂Ph), 4.27 (1H, a-dt, $J_{2,1a}$ 7.0, $J_{2,1b+3}$ 5.7, H-2), 4.33 (1H, a-t, $J_{3,2+4}$ 5.4, H-3), 7.24-7.37 (5H, m, 5ArCH).

2-Acetamido-1,4-imino-1,2,4-trideoxy-L-lyxitol (L-LyxNAc) **57**



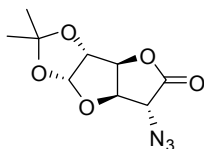
Compound **56** (44mg, 0.17mmol) was dissolved in 1,4-dioxane:water (1:1, 0.6ml), palladium black (9mg) was added and the flask was degassed. After flushing the flask with hydrogen gas the mixture was stirred for 18h. T.l.c. analysis (20% methanol in ethyl acetate) indicated a baseline product and no remaining starting material (R_f 0.21). The palladium was removed by filtration through Celite®, eluting with water. Concentration *in vacuo* afforded amine **57** (30mg, quant) as a yellow oil.

Data: $[\alpha]_D^{25}$: -25.8 (*c*, 0.81 in MeOH) [Lit.¹⁵ enantiomer $[\alpha]_D^{25}$: +32 (*c*, 1.06 in MeOH)].

δ_H (400MHz, MeOD): 1.99 (3H, s, NHCOCH₃), 2.85 (1H, dd, $J_{1a,1b}$ 10.6, $J_{1a,2}$ 9.4, H-1a), 3.19 (1H, dd, $J_{1b,1a}$ 10.7, $J_{1b,2}$ 8.4, H-1b), 3.26 (1H, a-dt, $J_{4,5a+5b}$ 6.0, $J_{4,3}$ 3.8, H-4), 3.70 (1H, dd, $J_{5a,5b}$ 11.1, $J_{5a,4}$ 6.2, H-5a), 3.82 (1H, dd, $J_{5b,5a}$ 11.1, $J_{5b,4}$ 6.2, H-5b), 4.20 (1H, a-t, $J_{3,2+4}$ 4.0, H-3), 4.32 (1H, a-dt, $J_{2,1a+1b}$ 8.6, $J_{2,3}$ 4.4, H-2).

D-XylNAc

5-Azido-5-deoxy-1,2-*O*-isopropylidene- β -L-idurono-3,6-lactone **60**



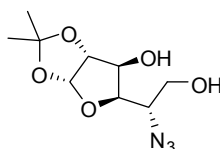
Alcohol **59** (6.21g, 28.7mmol) was dissolved in DCM (60ml) and pyridine (6.65ml, 82.2mmol) was added. The reaction mixture was cooled to -30°C and trifluoromethanesulfonyl anhydride (6.96ml, 86.1mmol) was added dropwise. After 1h, t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated formation of a product (R_f 0.55) and consumption of the starting material (R_f 0.27). The product was washed with HCl (1M, 2x100ml) and extracted using DCM (3x200ml). The organic fractions were combined, dried (MgSO₄), filtered and concentrated *in vacuo*.

The crude triflate (quant) was dissolved in DMF (25ml) and stirred with sodium azide (1.87g, 28.7mmol) at -20°C. After 2.5h t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated formation of one major product (R_f 0.74). The mixture was washed with 5% aqueous brine (150ml) and the product extracted from the aqueous layer using DCM (3x150ml). The combined organic fractions were dried (MgSO₄), filtered and

concentrated. The residue was purified using flash column chromatography (cyclohexane:ethyl acetate 6:1). Azide **60** (6.16g, 89%) was isolated as a white solid.

Data: m.p.: 100-102°C [Lit.²⁴ m.p.: 114-116°C]. $[\alpha]_D^{25}$: +203.4 (*c*, 1.11 in CHCl₃) [Lit.²⁴ $[\alpha]_D^{25}$: +243 (*c*, 1.1 in CHCl₃)]. δ_H (400MHz, CDCl₃): 1.35, 1.52 (2x3H, 2xs, C(CH₃)₂), 4.24 (1H, s, H-5), 4.66 (1H, d, *J*_{3,4} 3.2, H-3), 4.85 (1H, d, *J*_{2,1} 3.1, H-2), 4.96 (1H, d, *J*_{4,3} 3.2, H-4), 5.95 (1H, d, *J*_{1,2} 3.7, H-1).

5-Azido-5-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose **58**



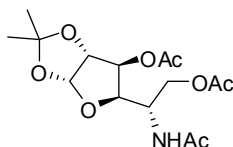
A solution of **60** (5.67g, 23.5mmol) in DCM (25ml) was cooled to -78°C. 1.5M DIBAL in toluene (25.8ml, 38.9mmol) was added dropwise and the mixture was stirred for 1h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated conversion of starting material (*R*_f 0.79) to a major product (*R*_f 0.54). The reaction mixture was diluted with DCM (150ml) and stirred with saturated aqueous potassium sodium tartrate solution (190ml) for 15h. The organic layer was then collected and the aqueous layer was extracted with DCM (5x150ml). The organic fractions were combined, dried (MgSO₄), filtered and concentrated to afford hemi-acetal **61** (5.17g, 90%).

Crude hemi-acetal **61** (5.17g, 21.3mmol) was dissolved in methanol (40ml) and cooled to -20°C. Sodium borohydride (201mg, 5.33mmol) was added portionwise, keeping the internal temperature below -10°C. The mixture was stirred at -10°C for 1h before being cooled to -20°C and a further portion of sodium borohydride (242mg, 6.39mmol) added. After stirring at -10°C for a further 1h t.l.c. analysis

(cyclohexane:ethyl acetate 1:1) indicated formation of a major (R_f 0.17) and a minor (R_f 0.24) product from starting material (R_f 0.52). Glacial acetic acid was used to neutralise the reaction mixture which was subsequently concentrated *in vacuo*. The residue was purified using flash column chromatography (cyclohexane:ethyl acetate 3:1→2:5) to afford the major product **58** (3.17g, 61%) as a white solid.

Data: m.p.: 119°C [Lit.¹⁶ m.p.: 120-122°C]. $[\alpha]_D^{25}$: -77.4 (*c*, 0.37 in MeOH) [Lit.²⁵ $[\alpha]_D^{21}$: -77 (*c*, 1.2 in MeOH)]. δ_H (400MHz, MeOD): 1.31, 1.46 (2x3H, 2xs, C(CH₃)₂), 3.60 (1H, dd, $J_{6a,6b}$ 11.3, $J_{6a,5}$ 6.0, H-6a), 3.67-3.72 (1H, m, H-5), 3.76 (1H, dd, $J_{6b,6a}$ 11.3, $J_{6b,5}$ 3.1, H-6b), 4.09 (1H, d, $J_{3,4}$ 2.8, H-3), 4.14 (1H, dd, $J_{4,5}$ 9.1, $J_{4,3}$ 2.8, H-4), 4.52 (1H, d, $J_{2,1}$ 3.7, H-2), 5.93 (1H, d, $J_{1,2}$ 3.7, H-1).

5-Acetamido-3,6-di-O-acetyl-5-deoxy-1,2-O-isopropylidene-β-L-idofuranose **62**



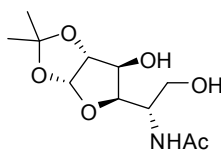
Azide **58** (422mg, 1.72mmol) was suspended in 1,4-dioxane (8ml) and palladium black (63mg) was added. The flask was degassed and flushed first with argon and then hydrogen. The mixture was stirred under a hydrogen atmosphere for 8h. T.l.c. analysis (ethyl acetate:cyclohexane 5:1) indicated complete conversion of starting material (R_f 0.56) to product (R_f 0.00). The mixture was filtered through Celite®, eluting with methanol, and concentrated *in vacuo*. This afforded the amine (374mg, 99%) as a white powder.

The crude amine (374mg, 1.71mmol) was dissolved in anhydrous pyridine (4ml) and stirred with acetic anhydride (0.98ml, 10.3mmol). After 13h t.l.c. analysis (ethyl

acetate:IPA 10:1) indicated product formation (R_f 0.60) and no remaining starting material (R_f 0.00). The mixture was concentrated and the residue purified using flash column chromatography (ethyl acetate:cyclohexane 2:1→5:1). This afforded **62** (506mg, 85%) as a colourless oil.

Data: HRMS: $C_{15}H_{23}NO_8Na$ ($M+Na^+$) calculated 368.1316 found 368.1315. ν_{max} (NaCl)/ cm^{-1} : 1741 (s, ester C=O), 1657 (m, C=O amides I), 1543 (w, C=O amides II). $[\alpha]_D^{22}$: -10.1 (c , 0.37 in $CHCl_3$). δ_H (400MHz, $CDCl_3$): 1.30, 1.50 (2x3H, 2xs, $C(CH_3)_2$), 1.93, 2.07, 2.11 (3x3H, 3xs, 3x CO_2CH_3), 4.04-4.14 (2H, m, 2xH-6), 4.32 (1H, dd, $J_{4,5}$ 5.5, $J_{4,3}$ 3.1, H-4), 4.50 (1H, d, $J_{2,1}$ 3.7, H-2), 4.47-4.54 (1H, m, H-5), 5.22 (1H, d, $J_{3,4}$ 3.0, H-3), 5.92 (1H, d, $J_{1,2}$ 3.7, H-1), 5.93 (1H, br-s, NH). δ_C (100.6MHz, $CDCl_3$): 20.7, 20.8 (CO_2CH_3), 23.4 ($NHCOCH_3$), 26.1, 26.6 ($C(CH_3)_2$), 46.6 (C-5), 64.0 (C-6), 76.5 (C-3), 76.9 (C-4), 83.6 (C-2), 104.3 (C-1), 112.3 ($C(CH_3)_2$), 169.6, 169.8 (CO_2CH_3), 170.7 ($NHCOCH_3$). m/z (ES+): 368.2 ($M+Na^+$, 89%), 713.3 ($2M+Na^+$, 100%).

5-Acetamido-5-deoxy-1,2-*O*-isopropylidene- β -L-idofuranose **63**

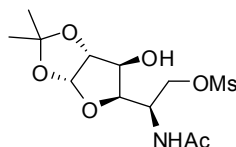


Sodium methoxide (16mg, 0.29mmol) was stirred with ester **62** (506mg, 1.47mmol) in methanol (10ml). After 4h, t.l.c. analysis (ethyl acetate:IPA 10:1) indicated complete conversion of starting material (R_f 0.60) to product (R_f 0.27). The mixture was concentrated and the resulting residue purified by flash column chromatography (ethyl acetate:IPA 1:0→10:1). Alcohol **63** (349mg, 91%) was isolated as a white amorphous solid.

Data: HRMS: $C_{11}H_{19}NO_6Na$ ($M+Na^+$) calculated 284.1105 found 284.1101. **m.p.:** 79-82°C. **ν_{max} (NaCl)/ cm^{-1} :** 3289 (m, br, OH), 1634 (m, C=O amides I), 1556 (w, C=O amides II). **$[\alpha]_D^{26}$:** -33.4 (c, 1.35 in $CHCl_3$). **δ_H (400MHz, $CDCl_3$):** 1.32, 1.50 (2x3H, 2xs, $C(CH_3)_2$), 2.02 (3H, s, $NHCOCH_3$), 3.62-3.67 (1H, m, H-6a), 3.79-3.82 (1H, m, H-6b), 4.14 (1H, br-s, OH-6), 4.18-4.22 (1H, m, H-4), 4.22-4.25 (2H, m, H-3, H-5), 4.41 (1H, d, $J_{OH,3}$ 4.6, OH-3), 4.55 (1H, d, $J_{2,1}$ 3.7, H-2), 5.93 (1H, d, $J_{1,2}$ 3.7, H-1), 6.44 (1H, d, $J_{NH,5}$ 7.1, NH). **δ_C (100.6MHz, $CDCl_3$):** 23.4 ($NHCOCH_3$), 26.0, 26.7 ($C(CH_3)_2$), 51.0 (C-5), 62.9 (C-6), 74.9 (C-3), 80.2 (C-4), 85.2 (C-2), 104.5 (C-1), 111.7 ($C(CH_3)_2$), 172.0 ($NHCOCH_3$). **m/z (ES+):** 284.1 ($M+Na^+$, 71%), 545.3 ($2M+Na^+$, 100%).

5-Acetamido-5-deoxy-1,2-O-isopropylidene-6-O-methanesulfonyl- α -D-glucofuranose

65

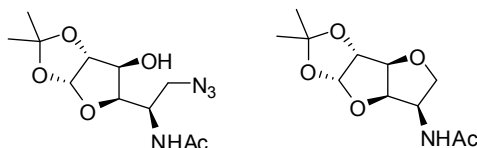


Diol **64** (257mg, 0.98mmol) was suspended in anhydrous pyridine (2ml) at 0°C. DMAP (24mg, 0.20mmol) and methanesulfonyl chloride (84 μ l, 1.08mmol) were added and the mixture was stirred for 30 min. The reaction was allowed to warm to room temperature and after 5h t.l.c. analysis (ethyl acetate:IPA 10:1) indicated a major product (R_f 0.62) along with starting material (R_f 0.44). The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (ethyl acetate:cyclohexane 2:1→ ethyl acetate:IPA 10:1) to afford **65** (153mg, 46%) as a colourless oil plus **64** (50mg, 19%).

Data: HRMS: $C_{12}H_{22}NO_8S$ ($M+H^+$) calculated 340.1061 found 340.1061. $[\alpha]_D^{25}$: +19.5 (c , 0.79 in $CHCl_3$). ν_{max} (NaCl)/ cm^{-1} : 3418 (m, br, OH), 1640 (m, C=O amide I), 1547 (w, C=O amide II). δ_H (400MHz, $CDCl_3$): 1.31, 1.47 (2x3H, 2xs, $C(CH_3)_2$), 2.08 (3H, s, CO_2CH_3), 3.11 (3H, s, SO_2CH_3), 3.99 (1H, dd, J 10.2, J 2.1, H-4), 4.03 (1H, a-s, H-3), 4.24-4.30 (1H, m, H-5), 4.46 (1H, dd, $J_{6a,6b}$ 10.9, $J_{6a,5}$ 2.4, H-6a), 4.59 (1H, d, $J_{2,1}$ 3.4, H-2), 4.61 (1H, dd, $J_{6b,6a}$ 10.8, $J_{6b,5}$ 3.6, H-6b), 5.07 (1H, a-d, $J_{OH,3}$ 2.0, OH-3), 5.92 (1H, d, $J_{1,2}$ 3.6, H-1), 6.45 (1H, d, $J_{NH,5}$ 8.4, NH). δ_C (100.6MHz, $CDCl_3$): 23.0 (CO_2CH_3), 26.1, 26.8 ($C(CH_3)_2$), 37.4 (SO_2CH_3), 47.1 (C-5), 69.9 (C-6), 73.6 (C-3), 79.1 (C-4), 84.5 (C-2), 105.5 (C-1), 111.8 ($C(CH_3)_2$), 172.4 (CO_2CH_3). m/z (ES⁺): 340.1 ($M+H^+$, 95%), 362.1 ($M+Na^+$, 89%), 701.2 ($2M+Na^+$, 100%).

5-Acetamido-6-azido-5,6-dideoxy-1,2-*O*-isopropylidene- α -D-glucofuranose **66**

5-Acetamido-3,6-anhydro-5-deoxy-1,2-*O*-isopropylidene- α -D-glucofuranose **67**

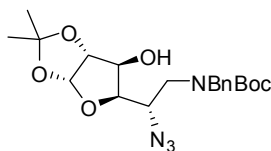


Mesylate **65** (24mg, 0.07mmol) was suspended in DMF (0.2ml) and sodium azide (46mg, 0.71mmol) was added. After stirring for 30 min the temperature was raised to 80°C. After 4h t.l.c. analysis (ethyl acetate:IPA 10:1) indicated consumption of starting material (R_f 0.61) and the formation of a major (R_f 0.51) and a minor (R_f 0.73) product. The mixture was concentrated *in vacuo* and the residue purified by flash column chromatography (ethyl acetate:cyclohexane 2:1 → ethyl acetate:IPA 10:1). This afforded **67** (13mg, 76%) and a product which could be **66** (3mg, 15%), as colourless oils.

Data for 67: HRMS: $C_{11}H_{18}NO_5$ ($M+H^+$) calculated 244.1180 found 244.1178. $[\alpha]_D^{25}$: -41.5 (c , 0.51 in $CHCl_3$). ν_{max} (NaCl)/ cm^{-1} : 1669 (s, C=O amide I), 1547 (m, C=O amide II). δ_H (400MHz, $CDCl_3$): 1.33, 1.50 (2x3H, 2xs, $C(CH_3)_2$), 2.01 (3H, s, $NHCOCH_3$), 3.95 (1H, a-dd, J 8.0, J 2.5, H-5), 4.30-4.43 (4H, m, H-3, H-4, 2xH-6), 4.55 (1H, d, $J_{2,1}$ 3.6, H-2), 5.94 (1H, d, $J_{1,2}$ 3.7, H-1). δ_C (100.6MHz, $CDCl_3$): 13.8 ($NHCOCH_3$), 26.2, 26.9 ($C(CH_3)_2$), 64.3 (C-5), 71.6 (C-6), 74.3, 83.6 (C-3, C-4), 85.4 (C-2), 105.1 (C-1), 111.6 ($C(CH_3)_2$), 186.0 ($NHCOCH_3$). m/z (ES+): 244.2 ($M+H^+$, 100%), 284.1 ($M+MeCN$, 62%), 307.1 ($M+MeCN+Na^+$, 56%), 509.2 ($2M+Na^+$, 54%), 545.2 ($2M+MeCN+NH_4^+$, 60%).

Selected data for 66: ν_{max} (NaCl)/ cm^{-1} : 3318 (m, br, OH, NH), 2105 (s, N_3), 1651 (m, C=O amides I), 1545 (m, C=O amides II). δ_H (400MHz, $CDCl_3$): 1.33, 1.51 (2x3H, 2xs, $C(CH_3)_2$), 2.08 (3H, s, $NHCOCH_3$), 3.53 (1H, dd, $J_{6a,6b}$ 12.5, $J_{6a,5}$ 3.3, H-6a), 3.96-4.02 (3H, m, H-3, H-4, H-6b), 4.12-4.19 (1H, m, H-5), 4.58 (1H, d, $J_{2,1}$ 3.5, H-2), 5.06 (1H, d, $J_{OH,3}$ 2.3, OH-3), 5.93 (1H, d, $J_{1,2}$ 3.5, H-1), 5.97 (1H, d, $J_{NH,5}$ 11.7, NH). m/z (ES-): 285.1 ($[M-H]^-$, 100%), 321.1 ($M+Cl^-$, 60%), 571.2 ($[2M-H]^-$, 26%).

5-Azido-6-*N*-benzyl-6-*N*-tert-butoxycarbonyl-5,6-dideoxy-1,2-*O*-isopropylidene- β -L-idofuranose 68



A solution of **60** (6.16g, 25.5mmol) in DCM (25ml) was cooled to $-78^\circ C$. DIBAL in toluene (1.5M, 20.0ml, 30.6mmol) was added dropwise and the mixture was stirred for 1h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated conversion of starting

material (R_f 0.79) to a major product (R_f 0.54). The reaction mixture was diluted with DCM (180ml) and stirred with saturated aqueous potassium sodium tartrate solution (260ml) for 15h. The organic layer was then collected and the aqueous layer extracted with DCM (5x100ml). The organic fractions were combined, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography (cyclohexane:ethyl acetate 3:1) to afford lactol **61** (5.48g, 88%).

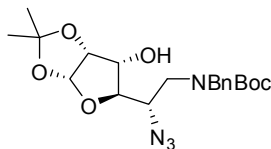
Lactol **61** (3.40g, 14.0mmol) was suspended in anhydrous THF (66ml) and benzylamine was added (1.68ml, 15.4mmol). Acetic acid was added until the pH was 6-7 (6ml) and this was followed by sodium cyanoborohydride (1.14g, 18.1mmol). After stirring at room temperature for 23h t.l.c. analysis (ethyl acetate) indicated complete conversion of starting material (R_f 0.77) to product (R_f 0.42). The reaction was quenched with saturated aqueous sodium bicarbonate solution and the aqueous layer extracted using ethyl acetate (4x20ml). The combined organic fraction was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford crude amine.

The amine (assumed quant) was dissolved in anhydrous DMF (30ml) and stirred with TEA (3.90ml, 28.0mmol). Di-*tert*-butyl dicarbonate (3.67g, 16.8mmol) was added. After 15h t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated conversion of starting material (R_f 0.40) to product (R_f 0.68). The mixture was concentrated, co-evaporating with toluene. The residue was purified using flash column chromatography (cyclohexane:ethyl acetate 3:1→2:1) to afford **68** (3.83g, 63%) as a colourless oil.

Data: HRMS: $C_{21}H_{30}N_4O_6Na$ ($M+Na^+$) calculated 457.2058, found 457.2037. $[\alpha]_D^{23}$: -61.9 (*c*, 1.15 in $CHCl_3$). ν_{max} (**NaCl**)/ cm^{-1} : 3408 (m, br, OH), 2124 (s, N_3), 1667 (s, C=O). δ_H (**400MHz**, $CDCl_3$): 1.32, 1.43 (2x3H, 2xs, $C(CH_3)_2$), 1.47 (3x3H, s, $C(CH_3)_3$),

2.71 (1H, dd, $J_{6a,6b}$ 14.7, $J_{6a,5}$ 8.1, H-6a), 3.49 (1H, d, $J_{6b,6a}$ 14.8, H-6b), 3.91 (1H, a-t, $J_{5,6a+4}$ 9.0, H-5), 4.01 (1H, dd, $J_{4,5}$ 9.8, $J_{4,3}$ 2.4, H-4), 4.29-4.33 (1H, m, H-3), 4.31 (1H, d, J_{gem} 16.8, CH_2Ph), 4.55 (1H, d, $J_{2,1}$ 3.5, H-2), 4.86 (2H, d, J_{gem} 16.8, CH_2Ph , OH-3), 5.98 (1H, d, $J_{1,2}$ 3.6, H-1), 7.20-7.36 (5H, m, 5xArCH). δ_C (100.6MHz, $CDCl_3$): 26.2, 26.9 ($C(CH_3)_2$), 28.3 ($C(CH_3)_3$), 48.6 (C-6), 52.6 (CH_2Ph), 63.2 (C-5), 75.2 (C-3), 81.9 ($C(CH_3)_3$), 82.1 (C-4), 85.1 (C-2), 105.0 (C-1), 111.7 ($C(CH_3)_2$), 127.3, 127.5, 128.7 (ArCH), 137.5 (Ar_{quat}), 156.8 ($CO_2C(CH_3)_3$). m/z (ES⁺): 435.2 (M+H⁺, 37%), 886.4 (M+NH₄⁺, 53%), 891.3 (2M+Na⁺, 100%).

5-Azido-6-*N*-benzyl-6-*N*-tert-butoxycarbonyl-5,6-dideoxy-1,2-*O*-isopropylidene- β -L-talofuranose 69



Alcohol **68** (3.83g, 8.83mmol) was suspended in DCM (38ml) with 3Å powdered molecular sieves. PCC (5.71g, 26.5mmol) was added and the mixture was stirred under Argon for 15h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated consumption of starting material (R_f 0.75) to form product (R_f 0.77). The mixture was purified by passing through a silica plug topped with Celite® (cyclohexane:ethyl acetate 4:1) to afford ketone (3.17g, 83%).

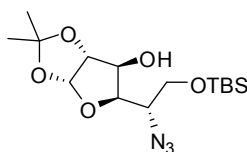
The ketone (3.17g, 7.34mmol) was dissolved in ethanol (63ml) and cooled to 0°C. Sodium borohydride (278mg, 7.34mmol) suspended in 50% aqueous ethanol (7ml) was added dropwise. After 2h t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated formation of a product (R_f 0.50) and consumption of starting material (R_f 0.68). The

reaction was quenched using saturated ammonium chloride solution and the product extracted using DCM (3x20ml). The combined organic fraction was dried (MgSO₄), filtered and concentrated. The residue was purified using flash column chromatography (cyclohexane:ethyl acetate 4:1→2:1) to afford **69** (2.53g, 79%) as a colourless oil.

Data: HRMS: C₂₁H₃₀N₄O₆Na (M+Na⁺) calculated 457.2058, found 457.2055. **[α]_D²⁵:** -7.5 (*c*, 0.85 in CHCl₃). **ν_{max} (NaCl)/cm⁻¹:** 3453 (m, br, OH), 2121 (s, N₃), 1696 (s, C=O). **δ_H (400MHz, CDCl₃):** 1.37, 1.43 (2x3H, 2xs, C(CH₃)₂), 1.46 (3x3H, s, C(CH₃)₃), 3.30 (1H, dd, *J*_{6a,6b} 14.1, *J*_{6a,5} 9.0, H-6a), 3.63-3.67 (1H, m, H-6b), 4.03 (2H, br-a-s, H-4, H-5), 4.38 (1H, d, *J*_{gem} 15.8, CH₂Ph), 4.57-4.60 (2H, m, H-2, H-3), 4.74 (1H, d, *J*_{gem} 15.9, CH₂Ph), 5.81 (1H, br-a-s, H-1), 7.23-7.35 (5H, m, 5xArCH). **δ_C (100.6MHz, CDCl₃):** 26.5, 26.9 (C(CH₃)₂), 28.3 (C(CH₃)₃), 48.7 (C-6), 50.8 (C-3), 52.4 (CH₂Ph), 60.6 (C-5), 72.5 (C-4), 78.6 (C-2), 80.7 (C(CH₃)₃), 103.9 (C-1), 112.9 (C(CH₃)₂), 127.3, 127.8, 128.6 (ArCH), 137.7 (Ar_{quat}), 155.8 (CO₂C(CH₃)₃). ***m/z* (ES⁺):** 435.3 (M+H⁺, 7%), 457.2 (M+Na⁺), 891.4 (2M+Na⁺, 100%).

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

72



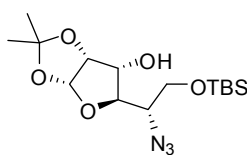
Diol **58** (1.04g, 4.25mmol) was suspended in anhydrous pyridine (10ml) and TBSCl (1.28g, 8.51mmol) was added. The mixture was heated to 40°C and stirred for 2h. T.l.c. analysis (cyclohexane:ethyl acetate 2:1) indicated complete conversion of starting material (*R_f* 0.07) to product (*R_f* 0.50). The mixture was quenched with methanol (1.7ml)

and concentrated *in vacuo*. The residue was purified by flash column chromatography (cyclohexane:ethyl acetate 3:1) to afford **72** (1.40g, 91%) as a colourless oil.

Data: $[\alpha]_D^{26}$: -7.4 (*c*, 1.35 in CHCl_3) [Lit.¹⁶ $[\alpha]_D^{25}$: -12.7 (*c*, 1.07 in CHCl_3)]. δ_{H} (400MHz, CDCl_3): 0.13 (2x3H, s, $\text{Si}(\text{CH}_3)_2$), 0.92 (3x3H, s, $\text{C}(\text{CH}_3)_3$), 1.33, 1.51 (2x3H, 2xs, $\text{C}(\text{CH}_3)_2$), 3.24 (1H, br-s, OH-3), 3.65-3.70 (1H, m, H-5), 3.77-3.83 (2H, m, 2xH-6), 4.13 (1H, dd, *J* 7.7, *J* 2.4, H-4), 4.21 (1H, br-a-s, H-3), 4.56 (1H, a-d, *J*_{2,1} 3.6, H-2), 5.97 (1H, d, *J*_{1,2} 3.6, H-1).

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

73



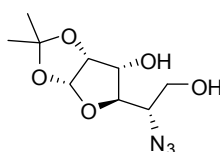
Alcohol **72** (1.40g, 3.90mmol) was dissolved in DCM (14ml) in the presence of 3Å molecular sieves and PCC (2.52g, 11.7mmol) was added. After stirring for 15h under argon, t.l.c. analysis (cyclohexane:ethyl acetate 2:1) indicated conversion of starting material (R_f 0.50) to product (R_f 0.53). The mixture was passed through Celite® topped silica, eluting with cyclohexane:ethyl acetate 2:1. This afforded ketone (1.39g, quant).

Ketone (1.39g, 3.90mmol) was dissolved in ethanol (10ml) and cooled to 0°C. Sodium borohydride (147mg, 3.90mmol) was added as a suspension in 50% aqueous ethanol (3ml). After 2h, analysis by t.l.c. (cyclohexane:ethyl acetate 2:1) revealed formation of product (R_f 0.57) and no remaining starting material (R_f 0.48). The reaction was quenched with glacial acetic acid and concentrated. The residue was then partitioned between DCM (20ml) and aqueous sodium bicarbonate solution (15ml) and the aqueous

layer was extracted with DCM (2x15ml). The washed organic layer was dried (MgSO₄), filtered and concentrated. Following purification by flash column chromatography (cyclohexane:ethyl acetate 3:1), **73** (1.40g, quant) was isolated as a colourless oil.

Data: $[\alpha]_D^{26}$: +45.7 (*c*, 0.91 in CHCl₃) [Lit.¹⁶ $[\alpha]_D^{25}$: +74.9 (*c*, 0.94 in CHCl₃)]. δ_H (400MHz, CDCl₃): 0.11 (2x3H, s, Si(CH₃)₂), 0.92 (3x3H, s, C(CH₃)₃), 1.38, 1.56 (2x3H, 2xs, C(CH₃)₂), 2.57 (1H, br-s, OH-3), 3.64 (1H, ddd, *J* 7.9, *J* 4.9, *J*_{5,4} 3.2, H-5), 3.81 (1H, dd, *J*_{4,3} 8.6, *J*_{4,5} 3.4, H-4), 3.87-3.92 (2H, m, 2xH-6), 4.05 (1H, dd, *J*_{3,4} 8.2, *J*_{3,2} 5.2, H-3), 4.59 (1H, dd, *J*_{2,3} 5.0, *J*_{2,1} 3.9, H-2), 5.82 (1H, d, *J*_{1,2} 3.7, H-1).

5-Azido-5-deoxy-1,2-*O*-isopropylidene- β -L-talofuranose **74**

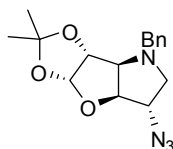


Silylated product **73** (1.38g, 3.84mmol) was dissolved in THF (14ml) and TBAF (4.2ml, 4.23mmol) was added. After stirring under argon for 2h, t.l.c. (cyclohexane:ethyl acetate 1:1) indicated complete conversion of starting material (*R*_f 0.63) to product (*R*_f 0.16). The mixture was preadsorbed onto silica and purification by flash column chromatography (ethyl acetate:cyclohexane 2:1) yielded **74** (760mg, 81%) as a white solid.

Data: HRMS: C₉H₁₅N₃O₅Na (M+Na⁺) calculated 268.0904, found 268.0902. **m.p.:** 91-93°C. $[\alpha]_D^{26}$: +28.4 (*c*, 1.11 in CHCl₃). $\nu_{\max}$ (NaCl)/cm⁻¹: 2115 (s, N₃), 3386 (m, br, OH). δ_H (400MHz, CDCl₃): 1.38, 1.57 (2x3H, 2xs, C(CH₃)₂), 2.81 (2H, br-s, OH-3, OH-6), 3.58 (1H, a-q, *J*_{5,4+6} 5.5, H-5), 3.86-3.89 (3H, m, H-4, 2xH-6), 3.95-4.01 (1H, m, H-3), 4.61 (1H, a-t, *J*_{2,1+3} 4.5, H-2), 5.84 (1H, d, *J*_{1,2} 3.7, H-1). δ_C (100.6MHz, CDCl₃): 26.4,

26.5 (C(CH₃)₂), 62.6 (C-6), 63.1 (C-5), 72.6 (C-3), 78.3 (C-2), 80.6 (C-4), 104.0 (C-1), 113.1 (C(CH₃)₂). *m/z* (ES⁺): 268.1 (M+Na⁺, 100%).

5-Azido-*N*-benzyl-3,5,6-trideoxy-3,6-imino-1,2-*O*-isopropylidene-β-L-idofuranose **71**



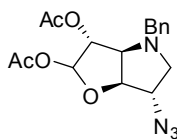
Method 1: Alcohol **69** (2.50g, 5.75mmol) was dissolved in anhydrous pyridine (25ml) and cooled to 0°C. Trifluoromethanesulfonyl anhydride (1.45ml, 8.63mmol) was added and the mixture was stirred for 1h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated total conversion of starting material (*R_f* 0.48) to product (*R_f* 0.66). The reaction was quenched by addition of water (100ml) and the product extracted using DCM (3x100ml). The organic fraction was dried (MgSO₄), filtered and concentrated *in vacuo* and the residue purified using flash column chromatography (cyclohexane:ethyl acetate 6:1), loading in DCM. This afforded **70** (2.97g, 90%) as a colourless oil.

Triflate **70** (2.97g, 5.24mmol) was suspended in TFA (15ml) and stirred for 7 min. After this time t.l.c. analysis (cyclohexane:ethyl acetate 2:1) indicated product formation (*R_f* 0.12) and no residual starting material (*R_f* 0.67). The mixture was concentrated *in vacuo*, co-evaporating with toluene. The crude residue was then dissolved in 1,4-dioxane (15ml) and sodium acetate (2.15g, 26.2mmol) was added in water (15ml). After 2h, t.l.c. (cyclohexane:ethyl acetate 1:1) indicated product (*R_f* 0.75). Water (50ml) was added and the product was extracted using DCM (3x50ml). The combined organic fraction was dried (MgSO₄), filtered and concentrated. Flash column chromatography

(cyclohexane:ethyl acetate 5:1) was used to afford pure **71** (1.40g, 84%) as a pale yellow oil.

Method 2: At -40°C DCM (5ml), anhydrous pyridine (1.12ml, 13.8mmol) and trifluoromethanesulfonyl anhydride (1.16ml, 6.91mmol) were pre-mixed. Diol **74** (564mg, 2.30mmol) was then added dropwise in DCM (15ml). The temperature was then maintained at -25°C. After 1h, t.l.c. analysis (ethyl acetate:cyclohexane 2:1) indicated complete conversion of starting material (R_f 0.24) to product (R_f 0.84). The mixture was washed with HCl (2M, 10ml). The aqueous layer was extracted using DCM (2x20ml). The combined organic layers were dried (MgSO₄), filtered and concentrated *in vacuo*. The residue was suspended in THF (15ml) and benzylamine (0.75ml, 6.91mmol) was added. After 3h, LRMS indicated product formation by the presence of a peak corresponding to a mass of 316 and no starting material peak of mass 509. The mixture was concentrated and purified by flash column chromatography (cyclohexane:ethyl acetate 3:1) to afford **71** (635mg, 87%) as a yellow oil.

Data: HRMS (FI+): C₁₆H₂₀N₄O₃ (M⁺) calculated 316.1535, found 316.1532. $[\alpha]_D^{26}$: -11.8 (*c*, 1.62 in CHCl₃). $\nu_{\max}$ (NaCl)/cm⁻¹: 2103 (s, N₃). δ_H (400MHz, CDCl₃): 1.33, 1.51 (2x3H, 2xs, C(CH₃)₂), 2.34 (1H, dd, $J_{6a,6b}$ 10.4, $J_{6a,5}$ 6.6, H-6a), 3.30 (1H, dd, $J_{6b,6a}$ 10.4, $J_{6b,5}$ 6.7, H-6b), 3.39 (1H, a-d, $J_{3,4}$ 5.1, H-3), 3.53 (1H, d, J_{gem} 13.3, CH₂Ph), 3.96 (1H, dt, $J_{5,6a+6b}$ 6.6, $J_{5,4}$ 1.4, H-5), 4.15 (1H, d, J_{gem} 13.3, CH₂Ph), 4.49 (1H, a-d, $J_{2,1}$ 3.6, H-2), 4.71 (1H, dd, $J_{4,3}$ 5.1, $J_{4,5}$ 1.6, H-4), 5.94 (1H, d, $J_{1,2}$ 3.6, H-1), 7.27-7.36 (5H, m, 5xArCH). δ_C (100.6MHz, CDCl₃): 26.7, 27.4 (C(CH₃)₂), 58.0 (C-6), 58.2 (CH₂Ph), 64.7 (C-5), 72.7 (C-3), 83.3 (C-2), 88.0 (C-4), 107.0 (C-1), 112.2 (C(CH₃)₂), 127.5, 128.5, 128.6 (ArCH), 137.7 (Ar_{quat}).

1,2-Di-O-acetyl-5-azido-N-benzyl-3,5,6-trideoxy-3,6-imino-L-idofuranose 78

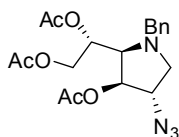
Pyrrolidine **71** (1.40g, 4.43mmol) was suspended in 80% TFA in water (14ml) and stirred at room temperature. After 5h t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated complete consumption of starting material (R_f 0.79) and formation of product (R_f 0.10). The mixture was concentrated, co-evaporating with toluene, and suspended in DCM (30ml). The product was washed with a saturated aqueous solution of sodium bicarbonate (30ml) and extracted with DCM (3x30ml). Flash column chromatography of the residue (cyclohexane:ethyl acetate 1:1→1:3) afforded **75** (1.22g, quant) as a yellow oil.

Diol **75** (805mg, 2.92mmol) was suspended in DCM (8ml) and mixed with DIPEA (2.03ml, 11.68mmol), DMAP (71mg, 0.58mmol) and acetic anhydride (1.65ml, 17.5mmol). After 16h, t.l.c. (cyclohexane:ethyl acetate 1:1) indicated complete conversion of starting material (R_f 0.10) to product (R_f 0.79). The mixture was concentrated *in vacuo* and purified by flash column chromatography (cyclohexane:ethyl acetate 4:1) to afford **78** (977mg, 84%) as a colourless oil in an anomeric ratio of 1:3.

Data: HRMS: $C_{17}H_{20}N_4O_5Na$ ($M+Na^+$) calculated 383.1326, found 383.1326. $[\alpha]_D^{26}$: +75.3 (*c*, 0.91 in $CHCl_3$). ν_{max} (NaCl)/ cm^{-1} : 2106 (s, N_3), 1755 (s, C=O). δ_H (400MHz, CD_3CN) **major anomer:** 1.94, 2.02 (2x3H, 2xs, 2x CO_2CH_3), 2.78 (1H, dd, $J_{6a,6b}$ 10.5, $J_{6a,5}$ 4.2, H-6a), 3.12 (1H, dd, $J_{6b,6a}$ 10.5, $J_{6b,5}$ 5.5, H-6b), 3.75 (1H, d, J_{gem} 13.5, CH_2Ph), 3.79 (1H, a-t, $J_{3,2+4}$ 5.5, H-3), 3.83 (1H, d, J_{gem} 13.8, CH_2Ph), 3.85-3.87 (1H, m, H-5), 4.71 (1H, a-d, J 6.9, H-4), 5.06 (1H, a-t, $J_{2,1+3}$ 4.4, H-2), 6.34 (1H, d, $J_{1,2}$ 4.2, H-1), 7.28-

7.37 (5H, m, 5xArCH). **minor anomer:** 2.04, 2.11 (2x3H, 2xs, 2xCO₂CH₃), 2.39 (1H, a-t, $J_{6a,6b+5}$ 9.0, H-6a), 3.22 (1H, dd, $J_{6b,6a}$ 9.1, $J_{6b,5}$ 6.9, H-6b), 3.49 (1H, a-t, $J_{3,4}$ 7.4, H-3), 3.64 (1H, d, J_{gem} 13.6, CH₂Ph), 4.07 (1H, d, J_{gem} 13.6, CH₂Ph), 4.07-4.11 (1H, m, H-5), 4.77 (1H, dd, $J_{4,3}$ 7.1, $J_{4,5}$ 3.9, H-4), 4.97 (1H, a-s, H-2), 6.17 (1H, a-s, H-1), 7.28-7.37 (5H, m, 5xArCH). δ_C (100.6MHz, CDCl₃) **major anomer:** 20.1, 20.5 (2xCO₂CH₃), 55.7, 56.0 (C-6, CH₂Ph), 64.04 (C-5), 68.1 (C-3), 73.5 (C-2), 86.4 (C-4), 96.3 (C-1), 127.6, 128.7, 129.1 (ArCH), 138.6 (Ar_{quat}), 169.9, 170.1 (2xCO₂CH₃). **minor anomer:** 20.4, 20.9 (2xCO₂CH₃), 56.4 (C-6), 57.4 (CH₂Ph), 65.9 (C-5), 71.6 (C-3), 80.2 (C-2), 90.6 (C-4), 102.0 (C-1), 127.6, 128.7, 129.0 (ArCH), 138.8 (Ar_{quat}), 169.7, 170.2 (2xCO₂CH₃). **m/z (ES⁺):** 361.2 (M+H⁺, 62%), 383.1 (M+Na⁺, 53%), 743.2 (2M+Na⁺, 100%).

3,5,6-Tri-*O*-acetyl-2-azido-*N*-benzyl-1,2,3-trideoxy-1,4-imino-L-idofuranose **79**

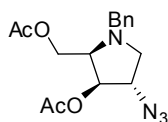


Protected lactol **78** (977mg, 2.17mmol) was suspended in ethanol (20ml) and cooled to 0°C. Sodium borohydride (308mg, 8.14mmol) was added and stirred for 17h, at which point LRMS analysis indicated product formation by the presence of a peak corresponding to a mass of 278. The reaction was quenched with AcOH and concentrated, co-evaporating with methanol five times. The crude was then suspended in DCM (10ml) and stirred with DIPEA (1.89ml, 10.8mmol), DMAP (66mg, 0.54mmol) and acetic anhydride (1.54ml, 16.3mmol) for 23h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated formation of product (R_f 0.60). The reaction mixture was

concentrated and purified by flash column chromatography (cyclohexane:ethyl acetate 4:1) resulting in **79** (726mg, 66% over 2 steps) as a yellow oil.

Data: HRMS: $C_{19}H_{24}N_4O_6Na$ ($M+Na^+$) calculated 427.1588, found 427.1586. $[\alpha]_D^{25}$: -8.3 (*c*, 0.42 in $CHCl_3$). ν_{max} ($NaCl$)/ cm^{-1} : 2107 (s, N_3), 1745 (s, $C=O$). δ_H (400MHz, CD_3CN): 1.99, 2.01, 2.09 (3x3H, 3xs, 3x CO_2CH_3), 2.34 (1H, dd, $J_{1a,1b}$ 10.1, $J_{1a,2}$ 7.6, H-1a), 3.18 (1H, dd, $J_{1b,1a}$ 10.2, $J_{1b,2}$ 6.6, H-1b), 3.42 (1H, dd, J 7.6, J 5.2, H-4), 3.62 (1H, d, J_{gem} 13.4, CH_2Ph), 4.03 (1H, d, J_{gem} 13.4, CH_2Ph), 4.13 (1H, a-q, J 6.7, H-2), 4.23 (1H, dd, $J_{6a,6b}$ 11.7, $J_{6a,5}$ 8.2, H-6a), 4.46 (1H, dd, $J_{6b,6a}$ 11.8, $J_{6b,5}$ 3.4, H-6b), 5.15-5.20 (2H, m, H-3, H-5), 7.28-7.36 (5H, m, 5xArCH). δ_C (100.6MHz, $CDCl_3$): 20.4, 20.6, 20.6 (3x CO_2CH_3), 54.7 (C-1), 60.5 (CH_2Ph), 63.1 (C-4), 63.3 (C-6), 63.8 (C-2), 71.2, 77.0 (C-3, C-5), 127.6, 128.8, 129.0 (ArCH), 139.1 (Ar_{quat}), 170.5, 170.6, 170.9 (3x CO_2CH_3). m/z (ES⁺): 405.2 ($M+H^+$, 83%), 427.1 ($M+Na^+$, 98%), 831.3 ($2M+Na^+$, 100%).

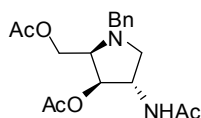
3,5-Di-*O*-acetyl-2-azido-*N*-benzyl-1,2,4-trideoxy-1,4-imino-D-xylitol **76**



Triester **79** (199mg, 0.49mmol) was suspended in methanol (2ml) and stirred with sodium methoxide (8mg, 0.15mmol). After 2h, t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated complete conversion of starting material (R_f 0.64) to product (R_f 0.08). The mixture was concentrated *in vacuo* and re-suspended in methanol:water 3:1 (1ml). Sodium periodate (126mg, 0.59mmol) was added and the mixture was stirred vigorously. After 1h a further portion of sodium periodate (42mg, 0.20mmol) was added (as t.l.c. analysis had shown remaining starting material). After a further 1h t.l.c. (20% methanol

in ethyl acetate) indicated conversion of starting material (R_f 0.79) to product (R_f 0.68). Sodium borohydride (46mg, 1.23mmol) was added to the reaction along with water (0.25ml). After 4h LRMS indicated formation of product and no remaining starting material which have masses of 248 and 246, respectively. The reaction was quenched with acetic acid and concentrated, co-evaporating with methanol five times. The residue was suspended in DCM (1ml) and DIPEA (0.34ml, 1.96mmol) was added followed by DMAP (12mg, 0.10mmol) and acetic anhydride (0.28ml, 2.94mmol). After stirring for 18h, t.l.c. (cyclohexane:ethyl acetate 1:1) indicated formation of product (R_f 0.55) and the mixture was preadsorbed onto silica and purified by flash column chromatography (cyclohexane:ethyl acetate 5:1) to afford **76** (101mg, 62%) as a colourless oil.

Data: HRMS: $C_{16}H_{20}N_4O_4Na$ ($M+Na^+$) calculated 355.1377, found 355.1370. $[\alpha]_D^{25}$: -39.6 (c , 0.82 in $CHCl_3$). ν_{max} ($NaCl$)/ cm^{-1} : 2106 (s, N_3), 1743 (s, $C=O$). δ_H (400MHz, $CDCl_3$): 2.05, 2.12 (2x CH_3 , 2xs, 2x CO_2CH_3), 2.29 (1H, dd, $J_{1a,1b}$ 9.8, $J_{1a,2}$ 7.9, H-1a), 3.19-3.26 (2H, m, H-1b, H-4), 3.51 (1H, d, J_{gem} 13.2, CH_2Ph), 3.96-4.01 (1H, m, H-2), 4.03 (1H, d, J_{gem} 13.4, CH_2Ph), 4.11 (1H, dd, $J_{5a,5b}$ 11.4, $J_{5a,4}$ 7.0, H-5a), 4.19 (1H, dd, $J_{5b,5a}$ 11.4, $J_{5b,4}$ 4.6, H-5b), 5.19 (1H, dd, J 6.9, J 5.5, H-3), 7.27-7.35 (5H, m, 5xArCH). δ_C (100.6MHz, $CDCl_3$): 20.8, 20.9 (2x CO_2CH_3), 55.2 (C-1), 58.8 (CH_2Ph), 62.0 (C-5), 62.2 (C-4), 63.4 (C-2), 77.3 (C-3), 127.4, 128.4, 128.7 (ArCH), 137.7 (Ar_{quat}), 170.3, 170.6 (2x CO_2CH_3). m/z (ES⁺): 333.1 ($M+H^+$, 96%), 355.1 ($M+Na^+$, 97%), 687.2 ($2M+Na^+$, 100%).

2-Acetamido-3,5-di-O-acetyl-N-benzyl-1,2,4-trideoxy-1,4-imino-D-xylitol 80

Azide **76** (89mg, 0.27mmol) was dissolved in 1,4-dioxane and palladised carbon (10%, 29mg, 0.03mmol) was added. The flask was degassed and flushed with hydrogen and stirred under a hydrogen atmosphere for 1h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated a baseline product and no remaining starting material (R_f 0.69). The mixture was filtered through Celite® eluting with 1,4-dioxane and concentrated *in vacuo*.

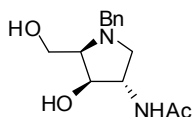
The crude amine (83mg, quant) was suspended in DCM (0.8ml) and stirred with DIPEA (0.09ml, 0.54mmol), DMAP (7mg, 0.05mmol) and acetic anhydride (0.08ml, 0.81mmol). After 18h, t.l.c. (ethyl acetate) indicated complete conversion of baseline starting material to product (R_f 0.15). The mixture was concentrated and purified by flash column chromatography (ethyl acetate:cyclohexane 12:1) to afford **80** (67mg, 71%) as a yellow solid.

Data: HRMS: $C_{18}H_{25}N_2O_5$ ($M+H^+$) calculated 349.1758, found 349.1751. **m.p:** 72-75°C.

$[\alpha]_D^{25}$: -35.4 (c , 0.70 in MeOH). **ν_{max} (NaCl)/ cm^{-1} :** 3280 (m, br, NH), 1741 (s, ester C=O), 1656 (m, C=O amide I), 1550 (m, C=O amide II). **δ_H (400MHz, MeOD):** 1.91 (3H, s, $NHCOCH_3$), 2.03, 2.07 (2x3H, 2xs, 2x CO_2CH_3), 2.19 (1H, a-t, $J_{1a,1b+2}$ 9.3, H-1a), 3.14 (1H, dd, $J_{1b,1a}$ 9.2, $J_{1b,2}$ 7.2, H-1b), 3.20 (1H, a-dt, $J_{4,3}$ 7.2, $J_{4,5}$ 4.7, H-4), 3.51 (1H, d, J_{gem} 13.1, CH_2Ph), 4.03 (1H, d, J_{gem} 13.1, CH_2Ph), 4.10-4.20 (2H, m, 2xH-5), 4.29 (1H, a-dt, $J_{2,1a}$ 9.2, $J_{2,3}$ 6.8, H-2), 5.23 (1H, dd, $J_{3,4}$ 7.7, $J_{3,2}$ 6.5, H-3), 7.24-7.33 (5H, m, 5xArCH). **δ_C (100.6MHz, MeOD):** 19.8, 19.8 (CO_2CH_3), 21.5 ($NHCOCH_3$), 53.4 (C-2), 55.4 (C-1), 58.6 (CH_2Ph), 62.1 (C-5), 62.5 (C-4), 77.0 (C-3), 127.4, 128.4, 129.1 (ArCH),

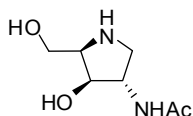
138.1 (Ar_{quat}), 171.1, 171.4, 172.3 ($2\times\text{CO}_2\text{CH}_3$, NHCOCH_3). m/z (ES^+): 349.2 ($\text{M}+\text{H}^+$, 100%), 407.2 ($\text{M}+\text{MeCN}+\text{NH}_4^+$, 24%).

2-Acetamido-*N*-benzyl-1,2,4-trideoxy-1,4-imino-D-xylitol **81**



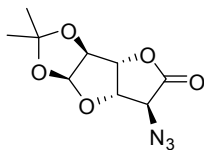
Ester **80** (69mg, 0.20mmol) was stirred with sodium methoxide (2mg, 0.04mmol) in methanol (1ml) for 2h. T.l.c. analysis (20% methanol in ethyl acetate) indicated product (R_f 0.31) and no starting material (R_f 0.60). The mixture was concentrated and purified by flash column chromatography (acetone) to afford **81** (41mg, 77%) as a pale yellow solid.

Data: HRMS: $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_3$ ($\text{M}+\text{H}^+$) calculated 265.1547, found 265.1545. **m.p.:** 114-117°C. $[\alpha]_D^{25}$: -30.3 (c , 0.85 in MeOH). ν_{max} (NaCl)/ cm^{-1} : 3282 (m, br, OH), 1651 (m, C=O amide I), 1557 (m, C=O amide II). δ_{H} (400MHz, MeOD): 1.93 (3H, s, NHCOCH_3), 2.12 (1H, a-t, J 9.0, H-1a), 2.82-2.86 (1H, m, H-4), 3.17 (1H, dd, $J_{1b,1a}$ 9.4, $J_{1b,2}$ 7.0, H-1b), 3.46 (1H, d, J_{gem} 13.0, CH_2Ph), 3.71 (1H, dd, $J_{5a,5b}$ 11.4, $J_{5a,4}$ 4.2, H-5a), 3.82 (1H, dd, $J_{5b,5a}$ 11.3, $J_{5b,4}$ 5.9, H-5b), 4.06 (1H, d, J_{gem} 12.9, CH_2Ph), 4.09-4.15 (2H, m, H-2, H-3), 7.23-7.36 (5H, m, $5\times\text{ArCH}$). δ_{C} (100.6MHz, MeOD): 21.5 (NHCOCH_3), 55.9 (C-1), 56.6 (C-2), 59.0 (CH_2Ph), 60.4 (C-5), 66.9 (C-4), 76.8 (C-3), 127.3, 128.3, 129.3 (ArCH), 138.4 (Ar_{quat}), 172.6 (NHCOCH_3). m/z (ES^+): 265.2 ($\text{M}+\text{H}^+$, 84%), 551.3 ($2\text{M}+\text{Na}^+$, 100%).

2-Acetamido-1,2,4-trideoxy-1,4-imino-D-xylitol (XylNAc) 32

Benzylated pyrrolidine **81** (41mg, 0.17mmol) was dissolved in 1,4-dioxane:water 1:1 (1ml) and palladium black (8mg) was added. The flask was degassed and flushed with hydrogen. After stirring for 4.5h under a hydrogen atmosphere, analysis by t.l.c. (20% methanol in ethyl acetate) indicated complete transformation of starting material (R_f 0.22) to baseline product. The mixture was filtered through Celite®, eluting with 1,4-dioxane and water. Concentration *in vacuo* afforded **32** (25mg, 93%) as a pale yellow solid.

Data: HRMS: $C_7H_{15}N_2O_3$ ($M+H^+$) calculated 175.1077, found 175.1077. **m.p.:** 162-170°C, decomposed. $[\alpha]_D^{25}$: -6.9 (c , 1.33 in H_2O). ν_{max} (**Ge**)/ cm^{-1} : 3274 (m, br, OH, NH), 1645 (m, C=O amide I), 1542 (m, C=O amide II). δ_H (**400MHz, D₂O**): 1.87 (3H, s, $NHCOCH_3$), 2.57 (1H, dd, $J_{1a,1b}$ 12.3, $J_{1a,2}$ 4.7, H-1a), 3.09 (1H, a-dd, J 11.2, J 6.3, H-4), 3.27 (1H, dd, $J_{1b,1a}$ 12.3, $J_{1b,2}$ 7.1, H-1b), 3.56 (1H, dd, $J_{5a,5b}$ 11.3, $J_{5a,4}$ 6.7, H-5a), 3.67 (1H, dd, $J_{5b,5a}$ 11.3, $J_{5b,4}$ 4.7, H-5b), 3.95-3.99 (1H, m, H-2), 4.05 (1H, dd, J 4.6, J 2.6, H-3). δ_C (**100.6MHz, D₂O**): 22.2 ($NHCOCH_3$), 48.8 (C-1), 58.1 (C-2), 60.3 (C-5), 61.6 (C-4), 76.3 (C-3), 174.3 ($NHCOCH_3$). **m/z (ES-):** 149.0 (81%), 173.1 ($[M-H]^-$, 100%), 205.1 ($[M+MeO]^-$, 71%).

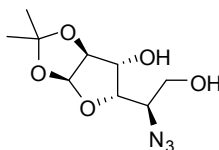
L-XylNAc**5-Azido-5-deoxy-1,2-O-isopropylidene- β -D-idurono-3,6-lactone 84**

Alcohol **83** (4.76g, 22.0mmol) was suspended in DCM (50ml) and cooled to -30°C. Pyridine (5.35ml, 66.1mmol) and trific anhydride (4.44ml, 26.5mmol) were added and the reaction stirred at -30°C. After 1h, t.l.c. analysis (cyclohexane:ethyl acetate 2:1) indicated complete conversion of starting material (R_f 0.11) into product (R_f 0.45). The mixture was washed with HCl (2M, 25ml) and the aqueous layer extracted using DCM (2x30ml). The combined organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*.

The crude triflate (assumed quant) was dissolved in DMF (20ml), cooled to -20°C and sodium azide (1.43g, 22.0mmol) was added. After 2.5h, t.l.c. analysis (cyclohexane:ethyl acetate 2:1) indicated formation of product (R_f 0.58) from starting material. 10% aqueous brine (10ml) was used to wash the mixture and the product was extracted with DCM (3x20ml). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography (cyclohexane:ethyl acetate 4:1) to afford **84** (5.31g, quant) as a pale yellow solid.

Data: m.p.: 101-104°C. $[\alpha]_D^{25}$: -203.1 (c , 1.03 in $CHCl_3$).

5-Azido-5-deoxy-1,2-*O*-isopropylidene- β -D-idofuranose **86**



Azide **84** (5.31g, 22.0mmol) was dissolved in DCM (30ml). The mixture was cooled to -78°C and DIBAL in toluene (1.5M, 18ml, 26.4mmol) was added. After 2.5h, t.l.c. analysis (cyclohexane:ethyl acetate 2:1) indicated complete formation of product (R_f 0.39) from starting material (R_f 0.54). Aqueous sodium potassium tartrate solution

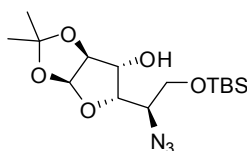
(200ml) was added along with DCM (150ml) and the mixture was stirred vigorously at room temperature for 18h. The organic layer was then separated and the aqueous layer extracted with DCM (6x150ml). The combined organic layers were dried (MgSO_4), filtered and concentrated to afford crude lactol **85** (3.65g, 68%) as a yellow oil.

Lactol **85** (3.65g, 15.0mmol) was dissolved in methanol (37ml) and cooled to -10°C . Sodium borohydride (170m, 4.50mmol) was added portionwise. After 1.5h, t.l.c. (ethyl acetate:cyclohexane 2:1) indicated no residual starting material (R_f 0.75) and a product (R_f 0.48). Acetic acid was used to quench the reaction and the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography (cyclohexane:ethyl acetate 3:1) to afford **86** (3.08g, 84%) as a yellow solid.

Data: m.p.: $102\text{--}108^\circ\text{C}$. $[\alpha]_D^{25}$: $+68.2$ (c , 0.86 in MeOH).

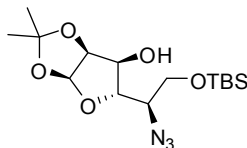
5-Azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene- β -D-idofuranose

87



Diol **86** (3.05g, 12.5mmol) was stirred with TBSCl (3.75g, 24.9mmol) in anhydrous pyridine (30ml) at 40°C for 2h under an argon atmosphere. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) revealed consumption of starting material (R_f 0.18) and product formation (R_f 0.65). The reaction was quenched with methanol (5ml) and the mixture concentrated. The product was purified by flash column chromatography (cyclohexane:ethyl acetate 4:1) to yield **87** (4.28g, 95%) as a pale yellow oil.

Data: $[\alpha]_D^{25}$: $+12.7$ (c , 0.80 in CHCl_3).

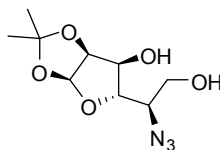
5-Azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene- β -D-talofuranose**88**

Protected ido alcohol **87** (4.28g, 11.9mmol) was dissolved in DCM (40ml) and added to a flame dried flask containing 3Å powdered molecular sieves. PCC (7.73g, 35.8mmol) was added and the mixture stirred under argon for 17h. The mixture was then filtered through a Celite® topped column of silica, eluting with cyclohexane:ethyl acetate 3:1 to give the crude ketone (3.96g, 93%) as a colourless oil.

The ketone was then dissolved in ethanol (30ml) and cooled to 0°C. Sodium borohydride (419mg, 11.1mmol) was added as a solution in ethanol:water 1:1 (10ml). After 2h, t.l.c. analysis (cyclohexane:ethyl acetate 2:1) revealed conversion of starting material (R_f 0.53) to product (R_f 0.62) and the reaction was quenched by acetic acid. After concentrating the mixture, sodium bicarbonate (10ml) and DCM (10ml) were added and the product separated. The aqueous layer was re-extracted with DCM (2x10ml). The product was concentrated after drying ($MgSO_4$) and purified by flash column chromatography (cyclohexane:ethyl acetate 4:1) to afford **88** (3.51g, 82%) as a colourless oil.

Data: $[\alpha]_D^{25}$: -41.6 (c , 1.00 in $CHCl_3$).

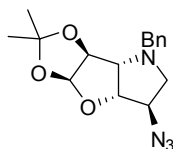
5-Azido-5-deoxy-1,2-*O*-isopropylidene- β -D-talofuranose **89**



Mono-protected diol **88** (2.61g, 7.27mmol) was dissolved in THF (20ml) and TBAF as a 1M solution in THF (8ml, 8.00mmol) was added. After 2h at room temperature, t.l.c. analysis (cyclohexane:ethyl acetate 1:1) showed complete formation of product (R_f 0.35) from starting material (R_f 0.11). Flash column chromatography (cyclohexane:ethyl acetate 1:1) afforded **89** (1.78g, quant) as a white solid.

Data: m.p.: 82-88°C. $[\alpha]_D^{25}$: -24.4 (c , 1.06 in CHCl_3).

5-Azido-*N*-benzyl-3,5,6-trideoxy-1,2-*O*-isopropylidene-3,6-imino- β -D-idofuranose **90**



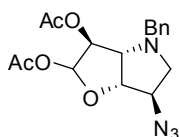
At -40°C, anhydrous pyridine (3.53ml, 43.6mmol) and trifluoromethanesulfonyl anhydride (3.66ml, 21.8mmol) were mixed in DCM (15ml). Diol **89** (1.78g, 7.27mmol) was added dropwise as a solution in DCM (50ml). The mixture was allowed to warm to -25°C and was stirred for 2h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated conversion of starting material (R_f 0.35) to product (R_f 0.75). HCl (2M, 10ml) was added and the organic layer was separated. The aqueous layer was re-extracted with DCM (2x10ml). The combined organic layer was dried (MgSO_4), filtered and concentrated.

The crude di-triflate (assumed quant) was suspended in THF (35ml) and benzylamine (2.38ml, 21.8mmol) was added. After 2h, LRMS indicated formation of

product. The reaction was concentrated and purified by flash column chromatography (cyclohexane:acetone 70:1). This gave pyrrolidine **90** (1.77g, 77%) as a colourless oil.

Data: $[\alpha]_D^{25}$: +5.3 (*c*, 1.26 in CHCl₃).

1,2-Di-*O*-acetyl-5-azido-*N*-benzyl-3,5,6-trideoxy-3,6-imino-D-idofuranose **92**

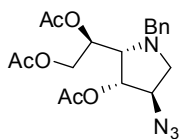


Pyrrolidine **90** (1.75g, 5.54mmol) was stirred in TFA:water 4:1 for 4.5h. Analysis by t.l.c. (cyclohexane:ethyl acetate 1:1) then revealed formation of product (R_f 0.02) and no remaining starting material (R_f 0.75). The mixture was concentrated *in vacuo* and partitioned between DCM (15ml) and sodium bicarbonate (10ml). The aqueous layer was re-extracted with more DCM (2x15ml). The organic layer was dried (MgSO₄), filtered and concentrated to afford crude diol **91** (1.50g, 98%) as an oil (R_f 0.36).

Diol **91** (1.50g, 5.43mmol) was dissolved in DCM (15ml) and DIPEA (3.86ml, 22.2mmol) was added followed by DMAP (70mg, 0.57mmol) then acetic anhydride (3.14ml, 33.2mmol). After stirring under argon for 18h, t.l.c. analysis (cyclohexane:ethyl acetate 2:1) indicated product formation (R_f 0.52) along with some baseline material. The mixture was concentrated and purified by flash column chromatography (cyclohexane:ethyl acetate 4:1) to give **92** (1.61g, 82%) as a yellow oil.

Data: $[\alpha]_D^{25}$: -91.1 (*c*, 1.21 in CHCl₃).

3,5,6-Tri-*O*-acetyl-2-azido-*N*-benzyl-1,2,3-trideoxy-1,4-imino-D-idofuranose **93**

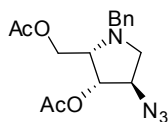


Diacetate **92** (1.56g, 4.33mmol) was dissolved in ethanol (31ml) and cooled to 0°C. Sodium borohydride (491mg, 13.0mmol) was added portionwise. The reaction was allowed to warm to room temperature and after 20h t.l.c. (cyclohexane:ethyl acetate 1:1) indicated complete conversion of starting material (R_f 0.80) to baseline product. The reaction was quenched with acetic acid and concentrated, co-evaporating with MeOH five times.

The crude triol (assumed quant) was suspended in DCM (15ml) and DIPEA (3.02ml, 17.3mmol) was added, followed by DMAP (106mg, 0.87mmol) and acetic anhydride (2.46ml, 26.0mmol). After 17h, t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated conversion of baseline starting material to a product (R_f 0.62). The mixture was concentrated and purified by flash column chromatography (cyclohexane:ethyl acetate 4:1) to afford **93** (1.48g, 85%) as a pale yellow oil.

Data: $[\alpha]_D^{25}$: +12.4 (c , 0.94 in CHCl_3).

3,5-Di-*O*-acetyl-2-azido-*N*-benzyl-1,2,4-trideoxy-1,4-imino-L-xylitol **94**



Method 1: Diol **91** (154mg, 0.56mmol), as obtained for **92**, was suspended in methanol:water 3:1 (1.4ml) at 0°C and sodium periodate (144mg, 0.67mmol) was added. After 3h, LRMS analysis indicated presence of product material and no remaining

starting material with masses 246 and 276, respectively. Still at 0°C sodium borohydride (42mg, 1.12mmol) was added to the mixture. After a further 3h LRMS indicated conversion to product with a mass of 248. The reaction was quenched by acetic acid and concentrated, co-evaporating with methanol.

The residue was dissolved in DCM (1.5ml) and DIPEA (0.39ml, 2.24mmol), DMAP (14mg, 0.11mmol) and acetic anhydride (0.32ml, 3.30mmol) were added. LRMS analysis after 17h indicated formation of a product with mass 332 so the mixture was concentrated *in vacuo*. Purification by flash column chromatography (cyclohexane:ethyl acetate 5:1) afforded **94** (82mg, 44%) as an oil.

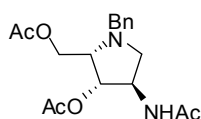
Method 2: Triacetate **93** (191mg, 0.47mmol) was dissolved in methanol (2ml). Sodium methoxide (5mg, 0.09mmol) was added and the mixture was stirred under argon for 2h. Analysis by t.l.c. (cyclohexane:ethyl acetate 1:1) indicated complete conversion of starting material (R_f 0.62) to a baseline product. Water (0.7ml) was added to the mixture followed by sodium periodate (121mg, 0.56mmol). After a further 2h of vigorous stirring, t.l.c. analysis (ethyl acetate) indicated conversion of the triol (R_f 0.24) into product (R_f 0.76). The mixture was cooled to 0°C and sodium borohydride (36mg, 0.94mmol) was added portionwise. After 3h, t.l.c (ethyl acetate) showed formation of product (R_f 0.57) from aldehyde starting material (R_f 0.79). Acetic acid was used to quench the reaction and the mixture was concentrated. The residue was partitioned between ethyl acetate (10ml) and water (10ml) and the aqueous layer extracted with ethyl acetate (2x10ml). The organic layer was dried ($MgSO_4$), filtered and concentrated.

The crude diol was dissolved in DCM (1ml) and DIPEA (0.33ml, 1.88mmol) was added followed by DMAP (11mg, 0.09mmol) and acetic anhydride (0.27ml, 2.82mmol).

After 18h LRMS indicated the presence of a compound with mass 248 which corresponded to the diol so further additions of DIPEA (0.33ml, 1.88mmol), DMAP (11mg, 0.09mmol) and acetic anhydride (0.27ml, 2.82mmol) were made. After a further 22h, t.l.c. (cyclohexane:ethyl acetate 1:1) indicated conversion to product (R_f 0.66) from baseline starting material. The mixture was purified by flash column chromatography (cyclohexane:ethyl acetate 5:1) to afford **94** (118mg, 76% over 4 steps) as a pale yellow oil.

Data: $[\alpha]_D^{25}$: +30.5 (c , 1.06 in CHCl_3).

2-Acetamido-3,5-di-*O*-acetyl-*N*-benzyl-1,2,4-trideoxy-1,4-imino-L-xylitol **95**

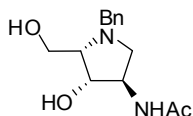


Azide **94** (118mg, 0.36mmol) was dissolved in 1,4-dioxane (1ml) and palladised carbon (10%, 38mg, 0.04mmol) was added. The flask was degassed and flushed with hydrogen. After 2h, t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated a baseline product and no remaining starting material (R_f 0.63). The mixture was filtered through Celite®, eluting with 1,4-dioxane, and concentrated to afford crude amine (quant).

The amine (110mg, 0.36mmol) was dissolved in DCM (1ml) and stirred with DIPEA (0.25ml, 1.44mmol), DMAP (9mg, 0.07mmol) and acetic anhydride (0.20ml, 2.16mmol) for 16h. T.l.c. analysis (ethyl acetate) indicated product formation (R_f 0.25). The mixture was purified by flash column chromatography (ethyl acetate:cyclohexane 7:1→1:0) to afford amide **95** (82mg, 66% over 2 steps) as a white solid.

Data: m.p: 66°C. $[\alpha]_D^{25}$: +35.2 (c , 1.03 in MeOH).

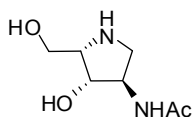
2-Acetamido-1,2,4-trideoxy-N-benzyl-1,4-imino-L-xylitol **96**



Amide **95** (71mg, 0.20mmol) was suspended in methanol (1ml) and stirred with sodium methoxide (2mg, 0.04mmol). After 2h, t.l.c. analysis (ethyl acetate:methanol 4:1) indicated complete conversion of starting material (R_f 0.54) to product (R_f 0.15). The mixture was concentrated and purified by flash column chromatography (acetone) to afford **96** (41mg, 77%) as a white solid.

Data: m.p: 123-126°C. $[\alpha]_D^{25}$: +39.9 (c , 0.99 in MeOH).

2-Acetamido-1,2,4-trideoxy-1,4-imino-L-xylitol (L-XylINAc) **82**



Pyrrolidine **96** (19mg, 0.08mmol) was suspended in 1,4-dioxane:water 1:1 (0.4ml) and palladium black (4mg) was added. The flask was degassed and flushed with argon twice, then degassed and flushed with hydrogen twice. After 1.5h stirring under a hydrogen atmosphere, t.l.c. analysis (ethyl acetate:methanol 4:1) showed baseline product and no remaining starting material (R_f 0.15). The mixture was filtered through Celite®, eluting with 1,4-dioxane and water. Concentration *in vacuo* afforded **82** (13mg, quant) as a pale yellow solid.

Data: m.p: 160°C, decomposed. $[\alpha]_D^{25}$: +5.3 (c , 0.61 in H₂O).

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Chapter 3: 2-Acetamido Piperidine Iminosugars

As outlined in Chapter 1, inhibitors are often designed to resemble the natural substrate of the enzyme as this increases the likelihood that the synthetic substrate will interact in a similar way to the natural substrate and therefore behave as an inhibitor. The natural substrates of *N*-acetylhexosaminidase are glycosides terminating with *N*-acetylgalactosamine **7** or *N*-acetylglucosamine **97** sugars (figure 47). Iminosugars containing an anomeric hydroxyl such as **98** are unstable compounds owing to their hemi-aminal functionality. Therefore the obvious alternative is to make the 1-deoxy iminosugar derivatives which are much more stable. These are known as 2-acetamido-1,5-imino-1,2,5-trideoxy-D-galactitol (DGJNAc) **99** and 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol (DNJNAc) **8** because of their similarities to the per-hydroxylated versions. The first half of this chapter concerns the synthesis of the enantiomer of **8**. In the second half, synthetic attempts to obtain the 6-deoxy analogue of **99** are described.

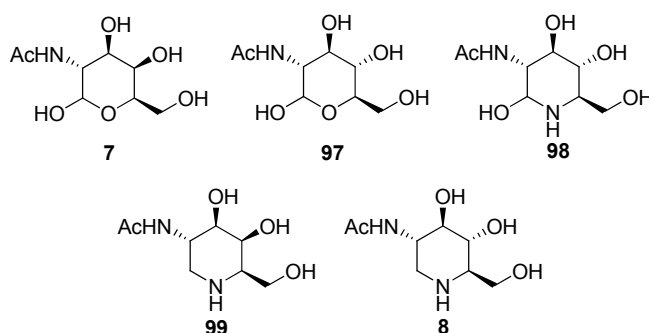


Figure 47

Iminosugar **8** has been studied both biologically and synthetically.¹⁻⁶ It displays potent and specific inhibition against β -*N*-acetylglucosaminidases. Interestingly, Kappes

et al synthesised **98** and it displayed 100-fold better inhibition than **8** against a range of β -*N*-acetylglucosaminidases (table 7).

Enzyme	98	DNJNAc 8
	$K_i/\mu\text{M}$	
Jack bean	0.0012	0.14
Bovine kidney	0.003	0.6
Human placenta	ND	0.9

Table 7: ND not determined

L-DNJNAc

This half of the chapter concerns the enantiomeric synthesis of DNJNAc. DNJNAc **8** itself has been synthesised using a number of routes but the most recent and most efficient synthesis was completed by the Fleet group.⁷ This route was based on the recent synthesis of DGJNAc **99** reported by the Fleet group.⁸ As explained in the introduction, the synthesis of unnatural sugars is interesting as some L-iminosugars display surprising biological activity. Therefore, to complete the picture it was considered necessary to synthesise L-DNJNAc. The synthesis of L-DNJNAc described here followed that of the Fleet enantiomeric route and used L-glucuronolactone as starting material.

Starting from mono-silylated diol **87**, which can be synthesised as shown in Chapter 2, the free hydroxyl group was protected using 1.5 eq each of benzyl bromide and sodium hydride in DMF (figure 48). 88% of product **100** was isolated following purification. The use of a benzyl protecting group was to allow orthogonality to the *tert*-

butyldimethylsilyl (TBS) group. A one pot deprotection of the TBS and acetonide groups and methyl glycoside formation was achieved using acidic conditions. This was effected by mixing acetyl chloride with methanol to produce anhydrous HCl prior to the addition of the starting material. Solid sodium bicarbonate was used to quench the reaction which was complete after 1h. Two products were formed which were identified as the α - and β -anomers. It was necessary to separate the anomers; the reason being because of their different reactivities exhibited in the following step. Careful purification by flash column chromatography, eluting with a varying ratio of toluene:acetone, allowed the anomers to be separated. The β -anomer had a slightly lower polarity in this solvent system so it eluted first. The anomers were present in a roughly 1:1 ratio. The α -product was an oil, however the β -product crystallised on standing.

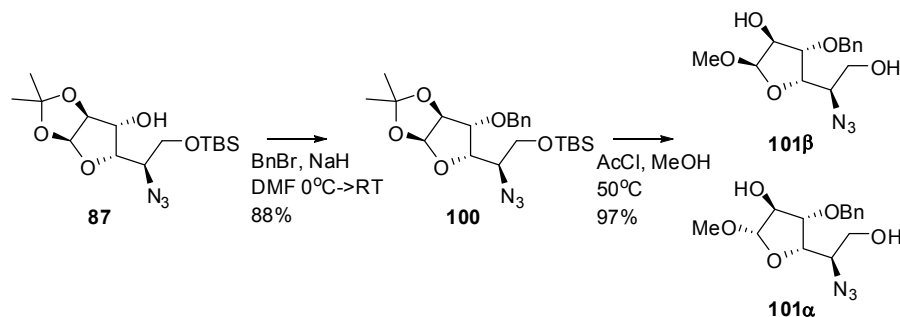


Figure 48

The next stage required activation of the two free hydroxyl groups to allow formation of the 6-membered ring by the double displacement method utilised for the LyxNAc and XylNAc syntheses. In this system di-triflation had previously been found to lead to tetrahydrofuran formation by intramolecular attack of the OBn of C-3 on the primary triflate followed by debenzoylation therefore mono-mesylation was performed instead (figure 49). This proceeded smoothly for the β -diol using 1 eq of mesyl chloride

with 2,4,6-collidine as the base in DCM. Di-mesylation was observed if an excess of reagent was used. For the α -anomer, however, mesylation was not selective for the primary group. The lack of a bulky group adjacent to OH-2 may be a possible explanation for this. To remedy this, mono-tosylation using 2 eq tosyl chloride with 2,4,6-collidine was necessary, even though the reaction required 26h. The mesylate and tosylate products were both washed with 2M HCl and purified by column chromatography. The different behaviour of the anomers under mesylation conditions merited their separation at the end of the previous step.

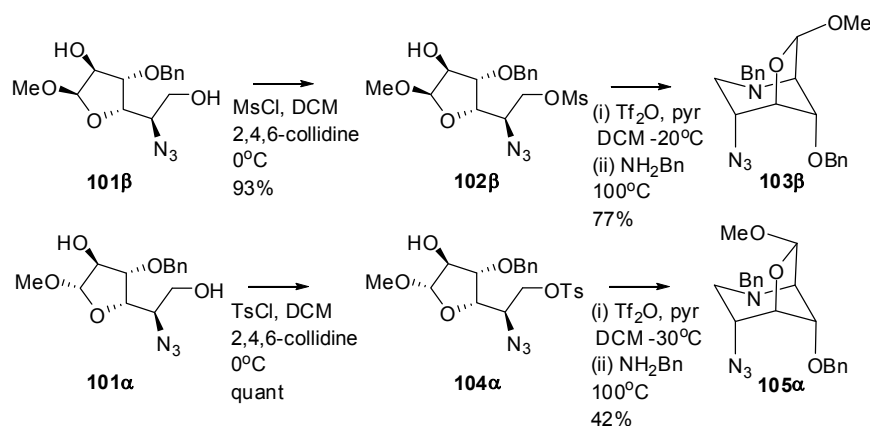


Figure 49

The mono-activated methyl glycosides were now separately treated with 1.3 eq triflic anhydride and 3 eq pyridine in DCM at -20°C to activate OH-2. To enable formation of the ring, the di-activated species were separately heated to 100°C in benzylamine overnight. Following column chromatography 77% of bicycle **103β** and 42% of bicycle **105α** were isolated. A test scale ring closure of the di-mesylate side product from the β -anomer was attempted however only attack on the primary centre had proceeded after two days at 150°C which justified the extra step required to form the more activated triflate.

Still keeping the epimers separate, the bicycles were opened by acetolysis using acetic anhydride, with boron trifluoride as the Lewis acid. It is interesting to note at this point how differently the two bicycles behaved under these conditions. Bicycle **105 α** required 3 eq of boron trifluoride to form **106** in 3h, however for **103 β** it was necessary to use 5 eq of boron trifluoride and a reaction time of 36h to form **106**. Both reactions proceeded in a good yield, 79% and 61% respectively, and resulted in a roughly 3:1 ratio of epimers of the acetyl methyl acetal **106** (figure 50).

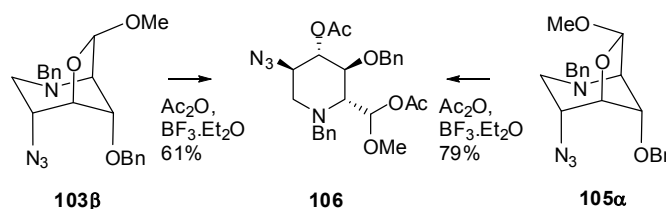


Figure 50

From this point on there were no parallel reactions of epimers as in the previous step the same products were formed from both bicycles. It was necessary to remove the methoxide group which was performed by a stepwise reduction (figure 51). First, DIBAL at -78°C in DCM was used to reduce the acetyl esters. After 1h, t.l.c. analysis indicated the formation of four apparent products. Following work up by saturated potassium sodium tartrate solution the intermediates collapsed into one product. A second reduction using 0.7 eq sodium borohydride in methanol at -10°C resulted in the formation of the 6-hydroxymethyl group. Per-acetylation was performed to facilitate purification by column chromatography, which yielded fully protected pyrrolidine **107** as a white solid in a yield of 54% over 3 steps.

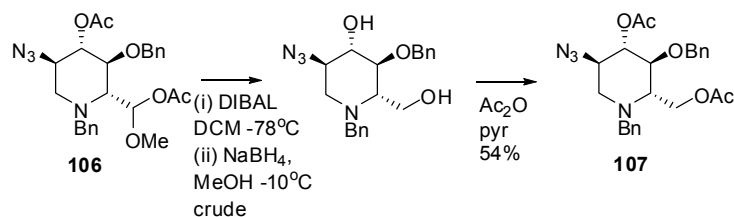


Figure 51

The remaining step in the synthesis, aside from global deprotection, was introduction of the *N*-acetyl group. This was achieved by using zinc powder activated by copper sulfate solution in a solvent mixture consisting of THF, acetic acid and acetic anhydride. Amide **108** was isolated in a yield of 65% as a white solid (figure 52). It was important to obtain clean material at this stage as purification of **109** by conventional column chromatography was not possible. First, Zemplén conditions were employed to remove the esters. The reaction mixture was concentrated *in vacuo* and used crude for the hydrogenation step. Water:2M HCl:1,4-dioxane 13:3:4 was used as the solvent system and 10% palladised carbon was sufficient to remove the two benzyl groups in the presence of hydrogen in 23h. The palladium was removed by filtration through Celite®, eluting with water, to yield the HCl salt of L-DNJNAc. A proton NMR of this compound correlated to that of the enantiomer.⁷ To liberate the free amine the salt was loaded onto a dowex (50W-X8, H⁺) column. Impurities were washed out using water and the product was eluted with 2M ammonia. L-DNJNAc **109** was isolated as a white solid in quantitative yield over the two deprotection steps.

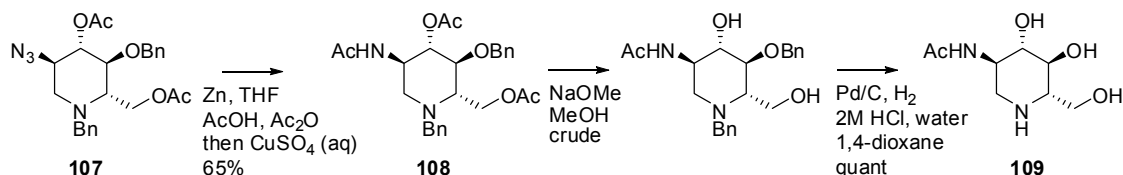


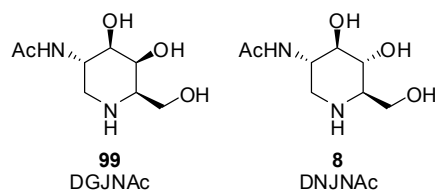
Figure 52

Biological evaluation

L-DNJNac **109**, and its enantiomer **8** synthesised by a co-worker, were assayed against a variety of enzymes by Professor Atsushi Kato. A summary of the inhibition observed is given in Table 8. Unlike the pyrrolidines, and despite the *gluco*-configuration, inhibition of α -N-acetylgalactosaminidase by **8** was observed, albeit very weak. Enantiomer **109** displayed no significant inhibition.

Enzyme		DNJNac 8	L-DNJNac 109
		IC ₅₀ /μM	
α -N-acetyl galactos aminidase	<i>Aspergillus oryzae</i>	998	-(0%)
	HL60	ND	-(14.8%)
β -N-acetylglucosaminidase	Jack bean	2.9	-(28.8%)
	Bovine kidney	7.4	-(24.4%)
	Human placenta	7.0	-(31.2%)
	HL60	ND	-(25.9%)
	<i>Aspergillus oryzae</i>	891	-(0.7%)

Table 8: - no significant inhibition () inhibition at 1000μM; ND not determined

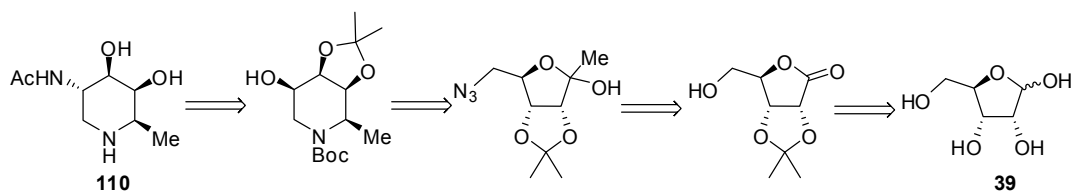
6-Deoxy DGJNAc (2-Acetamido-1,2,5,6-tetra-deoxy-1,5-imino-D-galactitol)**Figure 53**

This half of the chapter concerns the synthesis towards 6-deoxy DGJNAc. As mentioned earlier in the chapter, the synthesis of DNJNAc **8** was based upon that of DGJNAc **99** by a member of the Fleet group (figure 53). Biological results of this compound synthesised by the Fleet group previously had already given promising results and the latest, most efficient synthesis confirmed that,⁸ the results from the assays, performed by Atsushi Kato, are summarised in Table 9 below.⁷ In contrast to the other iminosugars mentioned in this thesis, DGJNAc **99** is the only potent α -N-acetylgalactosaminidase inhibitor. It is competitive against the chicken liver derived enzyme with a K_i of $0.08\mu\text{M}$ and against *Charonia lampas* α -N-acetylgalactosaminidase with a K_i of $0.14\mu\text{M}$. Unfortunately it is not selective as it is also a very good inhibitor of β -N-acetylglucosaminidases, inhibiting human placenta competitively with a K_i of $2.2\mu\text{M}$. Modest inhibition against α -galactosidase was also observed with an IC_{50} value against Coffee bean of $64\mu\text{M}$. The enantiomer displayed no significant inhibitory properties except for weak non-competitive inhibition of human placenta derived β -N-acetylhexosaminidase, with a K_i of 1.1mM .⁹

Enzyme		DGJNAc 99	L-DGJNAc
		IC ₅₀ /μM	
α-N-acetyl galactosa minidase	Chicken liver	0.32	-(22.6%)
	Jack bean	1.8	-(46.3%)
β-N- acetylglucosaminidase	Bovine kidney	4.2	-(46.8%)
	Human placenta	8.3	830

Table 9: - no significant inhibition () inhibition at 1000μM

Considering the potential biological significance of DGJNAc **99**, it was considered that the 6-deoxy version of this compound **110** would make an interesting target to gauge the importance of the hydroxymethyl group on the ability of the molecule to act as an inhibitor. The retrosynthetic analysis in figure 54 identified D-ribose **39** as a suitable starting material.

**Figure 54**

Following the work of previous Fleet group members,¹⁰ ribose **39**, which is a cheap and readily available starting material, was protected with an isopropylidene group to give **111** as the major product, which was isolated by column chromatography (figure 55).¹¹ An oxidation using bromine with barium carbonate as base afforded lactone **112**. Introduction of a nitrogen atom to allow formation of the piperidine ring was performed

by use of a mesylate/azide displacement because of the instability of the corresponding triflate.¹⁰ 1.1 Eq mesyl chloride was used, with triethylamine (TEA) as the accompanying base. The subsequent displacement with sodium azide proceeded in excellent yield, affording **113** in 96% over two steps.¹²

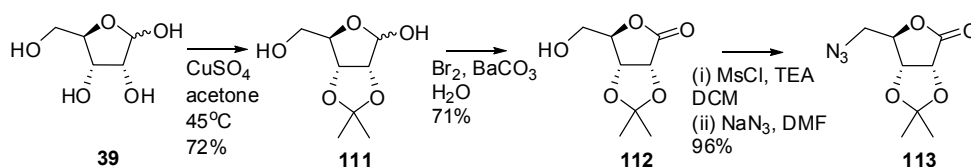


Figure 55

The next stage was nucleophilic addition of a methyl group to the carbonyl group (figure 56). This was initially attempted with the Grignard reagent methyl magnesium bromide. Incomplete reactions, however, lead to the use of the more reactive reagent methyl lithium. Lactol **114** was formed in 87% and was used without further purification. In order to obtain the intermediate iminosugar **115** reductive amination using palladium black under a hydrogen atmosphere was employed. T.l.c. analysis clearly revealed a major product and difficulties purifying **115** by conventional methods meant that it was decided to take the reaction mixture onto the next stage crude.

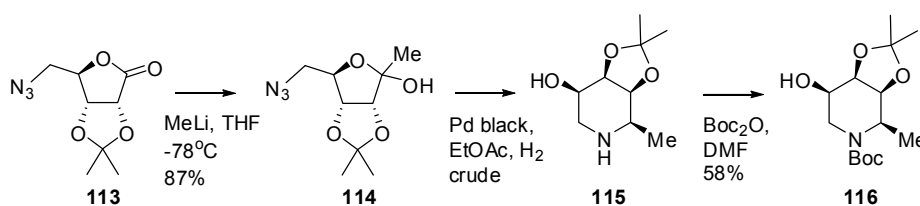


Figure 56

To enable the NAc group to be formed later an azide group needed to be introduced at C-2 as a precursor, facilitated by formation of a triflate or other sulfonate leaving group followed by displacement by sodium azide. However it was thought that

the ring nitrogen would cause difficulties by neighbouring group participation to form an aziridine with loss of triflate if it was not first protected. Boc protection was chosen as, being acid labile, it could be removed in the last step of the sequence together with the isopropylidene group. Purification of **116** via column chromatography was complicated as the desired product did not stain well in a selection of common t.l.c. visualisation aids. A moderate yield of 58% was obtained.

Unfortunately, attempts at subsequent azide introduction proved difficult. Neither mesylation nor triflation of the free hydroxyl group went to completion. No azide peak was observed by IR after stirring overnight with 1.5 eq sodium azide. The tosylate derivative was easier to isolate, in up to 78% yield, as it was more stable at room temperature. However, it did not readily displace with sodium azide overnight at room temperature. Heating with 10 eq sodium azide in DMF to 100°C yielded a product which would correspond to a 10% yield if it was the desired azide, along with 28% of unreacted tosylate starting material, however the mass (10mg) was insufficient to proceed with the synthesis so the identity of the product was not probed further. A variation on the Mitsunobu reaction, using DPPA and DIAD, was trialled on alcohol **116** but was unsuccessful.¹³

Second route

In light of these difficulties it was envisaged that the order of introduction of the azide group and ring formation could be reversed. Intermediate **113**, synthesised in the first route, was subjected directly to reducing conditions, palladium black with hydrogen, forming lactam **117** (figure 57).¹⁴ This was then used crude to afford corresponding azide

118. A large excess of sodium azide, 10 eq, was required for the reaction to proceed at a reasonable rate. The yield of **118** over two steps, though poor at 34%, was comparable to the literature value of 47%.¹⁵

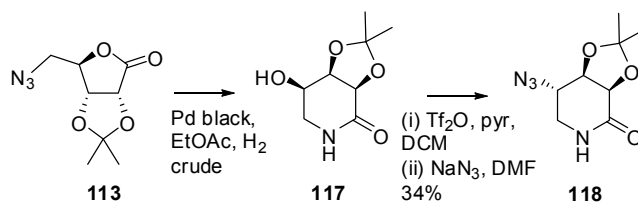


Figure 57

The methyl group could now be introduced as the ring had been formed. The reaction of methyl lithium with lactam **118** was, perhaps unsurprisingly, unsuccessful so the ring-nitrogen was benzylated as compound **119**, in a moderate yield of 49%, to prevent deprotonation. Reacting methyl lithium with this molecule yielded a number of products, only one of which, alkene **120** arising from addition followed by dehydration, was isolated (figure 58). Various temperatures and reaction times were tried in an effort to minimise side product formation. Initial attempts to reduce **120** using palladium black and hydrogen gave a complex mixture of products. Lack of time precluded further investigation.

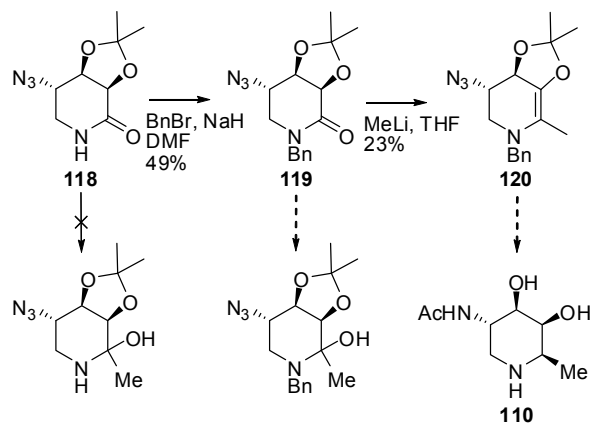
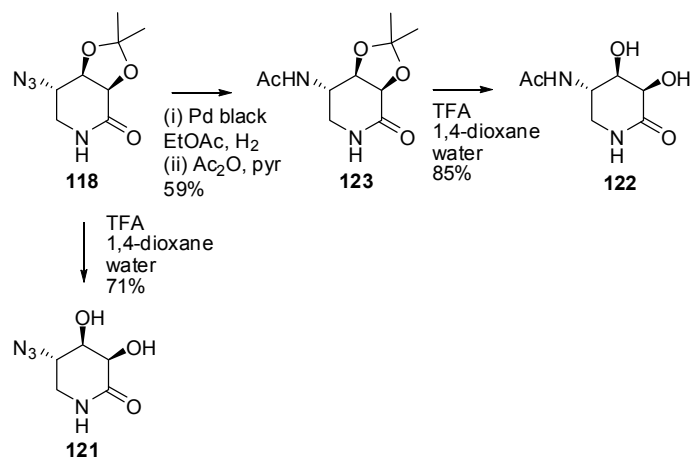


Figure 58

Other targets

Although the desired product 6-deoxy DGJNAc **110** was not accessible by this route it was possible to synthesise some related molecules from intermediates in the scheme which were thought might be biologically active in their own right. The first, **121**, was derived from azido-lactam **118** by direct deprotection of the acetonide group using TFA in water and 1,4-dioxane (figure 59). The second, **122**, differs from **121** by having an *N*-acetyl group in place of the azide. Reduction of the azide moiety to the amine via hydrogenation was facile as was the acetylation step using acetic anhydride in pyridine as the solvent. Amide **123** was purified by flash column chromatography using a polar solvent system comprising ethyl acetate, IPA and water, giving a yield of 59% over 2 steps.

**Figure 59**

For the subsequent deprotection of **123**, first 80% acetic acid at room temperature was tried, however heating at reflux was required before the starting material was consumed. Unfortunately this led to partial decomposition and the product could not be purified. Hence the reaction was repeated using 33% TFA in water:1,4-dioxane 1:1 at room temperature. After 23h, this afforded the desired deprotected product **122** in a good

yield of 85%. Lactams **121** and **122** were both biologically evaluated in the Oxford Glycobiology Institute against α -N-acetylgalactosaminidase (*Charonia Lampas*) and β -N-acetylhexosaminidase (HL60). Unfortunately, no significant inhibition was observed.

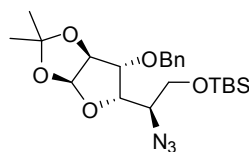
Summary

The synthesis of target compound L-DNJNAc **109** was successfully achieved from L-glucuronolactone in good yield overall. The synthetic procedure partly involved a parallel strategy because of the differing reactivity of the methyl glycoside anomers, via a bicyclic intermediate. Although no significant inhibition against a number of α -N-acetylgalactosaminidase and β -N-acetylglucosaminidase enzymes was observed this fact in itself provides important information in relation to structure activity relationships.

In addition, work towards the synthesis of 6-deoxy DGJNAc (2-Acetamido-1,2,5,6-tetra-deoxy-1,5-imino-D-galactitol) **110** was carried out. This compound was considered to have potential as an α -N-acetylgalactosaminidase inhibitor. The introduction of a NAc precursor at C-2 was found to be non-facile. Attempts at azide introduction via a range of sulfonates and a variant of the Mitsunobu reaction were found to be unsuccessful. An alternative strategy incorporating azide at an earlier point in the synthesis also proved unfruitful because of the difficulties introducing the methyl group at C-6. Time prevented the successful completion of this target, however a couple of related targets were accessed, albeit displaying no biological activity.

Chapter 3: Experimental

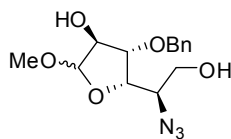
L-DNJNAc

5-Azido-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene- β -D-idofuranose **100**

Alcohol **87** (4.27g, 11.9mmol) was dissolved in anhydrous DMF (25ml) and benzyl bromide (2.13ml, 17.9mmol) was added. The mixture was cooled to 0°C under argon and sodium hydride (714mg, 17.9mmol) was added. The reaction was allowed to warm to room temperature. After 1h t.l.c. analysis (cyclohexane:ethyl acetate 3:1) indicated product formation (R_f 0.55) and no remaining starting material (R_f 0.30). Ethyl acetate (100ml) and water:brine 1:1 (100ml) were added and the organic layer removed and re-extracted with water:brine 1:1 (2x50ml). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography (cyclohexane:ethyl acetate 50:1 $\rightarrow$ 1:1) to afford **100** (4.71g, 88%) as a colourless oil.

Data: $[\alpha]_D^{25}$: +30.1 (c , 1.21 in $CHCl_3$) [Lit.⁷ enantiomer $[\alpha]_D^{25}$: -39.4 (c , 1.78 in $CHCl_3$)].

δ_H (400MHz, $CDCl_3$): 0.01, 0.04 (2x3H, 2xs, $SiCH_3$), 0.88 (3x3H, s, $C(CH_3)_3$), 1.34, 1.50 (2x3H, 2xs, $C(CH_3)_2$), 3.54 (1H, dd, $J_{6a,6b}$ 11.3, $J_{6a,5}$ 6.1, H-6a), 3.69-3.73 (2H, m, H-5, H-6b), 3.90 (1H, a-d, $J_{3,4}$ 3.2, H-3), 4.29 (1H, dd, $J_{4,5}$ 8.4, $J_{4,3}$ 3.2, H-4), 4.45 (1H, d, J_{gem} 11.8, CH_2Ph), 4.68 (1H, d, $J_{2,1}$ 3.9, H-2), 4.72 (1H, d, J_{gem} 11.8, CH_2Ph), 5.98 (1H, d, $J_{1,2}$ 3.8, H-1), 7.31-7.39 (5H, m, 5xArCH).

Methyl 5-azido-3-O-benzyl-5-deoxy-D-idofuranoside 101

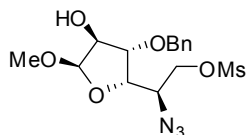
Acetyl chloride (1.6ml) and methanol (35ml) were premixed and then added to protected lactol **100** (4.71g, 10.5mmol). The temperature was increased to 50°C. After 1h t.l.c. analysis (cyclohexane:ethyl acetate 3:1) indicated complete consumption of starting material (R_f 0.60) and product formation (R_f 0.03). Sodium bicarbonate was added to quench the reaction and the mixture was then filtered, and concentrated *in vacuo*. Following column chromatography (toluene:acetone 98:2→92:8) the α -methyl glycoside (1.32g, 41%) was isolated as a yellow oil and the β -anomer (1.55g, 48%) was isolated as a yellow oil which crystallised on standing, plus some mixed fractions (254mg, 8%).

Data: β anomer m.p.: 43-44°C [Lit.⁷ enantiomer m.p.: 46-50°C]. $[\alpha]_D^{25}$: -73.2 (*c*, 1.32 in CHCl_3) [Lit.⁷ enantiomer $[\alpha]_D^{25}$: +79.1 (*c*, 1.39 in CHCl_3)]. δ_H (200MHz, CDCl_3): 2.00 (1H, br-s, *OH*-6), 2.84 (1H, br-s, *OH*-2), 3.51 (3H, s, OCH_3), 3.57 (1H, dd, $J_{6a,6b}$ 11.4, $J_{6a,5}$ 6.4, H-6a), 3.67 (1H, dd, $J_{6b,6a}$ 11.4, $J_{6b,5}$ 4.0, H-6b), 3.83 (1H, dt, $J_{5,4+6a}$ 6.4, $J_{5,6b}$ 4.0, H-5), 3.99 (1H, dd, $J_{3,4}$ 5.6, $J_{3,2}$ 3.6, H-3), 4.28 (1H, t, $J_{4,3+5}$ 6.0, H-4), 4.37 (1H, dd $J_{2,1}$ 4.7, $J_{2,3}$ 3.7, H-2), 4.55 (1H, d, J_{gem} 11.8, CH_2Ph), 4.82 (1H, d, J_{gem} 11.8, CH_2Ph), 5.06 (1H, d, $J_{1,2}$ 4.7, H-1), 7.31-7.39 (5H, m, 5xArCH).

α anomer $[\alpha]_D^{25}$: +33.6 (*c*, 1.03 in CHCl_3) [Lit. $[\alpha]_D^{25}$: -41.4 (*c*, 0.92 in CHCl_3)]. δ_H (400MHz, CDCl_3): 2.11 (1H, a-t, $J_{\text{OH},6}$ 6.3, *OH*-6), 2.35 (1H, d, $J_{\text{OH},2}$ 4.4, *OH*-2), 3.47 (3H, s, OCH_3), 3.52 (1H, dd, $J_{6a,6b}$ 11.7, $J_{6a,5}$ 5.9, H-6a), 3.66 (1H, ddd, $J_{6b,6a}$ 11.6, $J_{6b,\text{OH}}$ 5.7, $J_{6b,5}$ 4.0, H-6b), 3.89-3.93 (1H, m, H-5), 3.94 (1H, dd, $J_{3,4}$ 5.6, $J_{3,2}$ 2.4, H-3), 4.34

(1H, br-s, H-2), 4.36 (1H, dd $J_{4,5}$ 8.3, $J_{4,3}$ 5.7, H-4), 4.50 (1H, d, J_{gem} 11.8, CH_2Ph), 4.73 (1H, d, J_{gem} 11.8, CH_2Ph), 4.86 (1H, d, $J_{1,2}$ 1.3, H-1), 7.18-7.40 (5H, m, 5xArCH).

Methyl 5-azido-3-O-benzyl-5-deoxy-6-O-methanesulfonyl- β -D-idofuranoside **102 β**

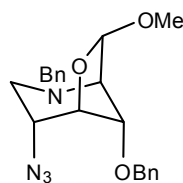


β -Methyl glycoside **101 β** (520mg, 1.68mmol) was dissolved in DCM (5ml) and cooled to 0°C. 2,4,6-Collidine (0.44ml, 3.36mmol) and methanesulfonyl chloride (0.13ml, 1.68mmol) were added. T.l.c. analysis (toluene:acetone 4:1) after 2h indicated formation of product (R_f 0.61) from starting material (R_f 0.49). HCl (2M, 20ml) was added and the organic layer was removed. After washing with HCl for a second time (2M, 20ml) the organic layer was dried (MgSO_4), filtered and concentrated. The residue was purified by flash column chromatography (toluene:acetone 96:4 $\rightarrow$ 88:12) to afford mesylate **102 β** (605mg, 93%) as a colourless oil.

Data: $[\alpha]_{\text{D}}^{25}$: -65.5 (c , 0.87 in CHCl_3) [Lit.⁷ enantiomer $[\alpha]_{\text{D}}^{25}$: +72.6 (c , 0.97 in CHCl_3)].

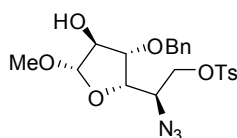
δ_{H} (400MHz, CDCl_3): 2.73 (1H, d, $J_{\text{OH},2}$ 7.9, OH-2), 3.02 (3H, a-d, J 0.7, SCH_3), 3.50 (3H, a-d, J 0.7, OCH_3), 3.96 (1H, a-dt, J 6.0, J 4.2, H-5), 4.05 (1H, dd, $J_{3,4}$ 6.1, $J_{3,2}$ 4.2, H-3), 4.19-4.28 (3H, m, H-4, H-6a, H-6b), 4.38 (1H, a-dt, $J_{2,\text{OH}}$ 7.7, $J_{2,1+3}$ 4.5, H-2), 4.58 (1H, d, J_{gem} 11.6, CH_2Ph), 4.84 (1H, d, J_{gem} 11.5, CH_2Ph), 5.02 (1H, d, $J_{1,2}$ 4.7, H-1), 7.30-7.40 (5H, m, 5xArCH).

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



Mesylate **102 β** (605mg, 1.56mmol) was suspended in DCM (6ml) and pyridine (0.38ml, 4.68mmol). The mixture was cooled to -20°C and trifluoromethanesulfonyl anhydride (0.34ml, 2.03mmol) was added. After 2h the starting material (R_f 0.51) had been consumed and product had formed (R_f 0.74) as indicated by t.l.c. analysis (toluene:acetone 3:1). HCl (2M, 15ml) was added and the organic layer was removed, re-washed with more HCl (2M, 10ml) and then dried (MgSO_4), filtered and concentrated. Benzylamine (3ml) was added and the mixture heated to 100°C . After 17h LRMS revealed formation of product and the mixture was concentrated *in vacuo*. Bicycle **103 β** (455mg, 77%) was isolated following flash column chromatography (cyclohexane:ethyl acetate:TEA 93:6:1 $\rightarrow$ 87:12:1) as a yellow oil which crystallised on standing.

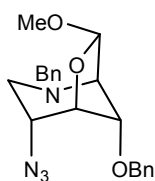
Data: m.p.: $48\text{--}50^{\circ}\text{C}$ [Lit.⁷ enantiomer m.p.: $50\text{--}54^{\circ}\text{C}$]. $[\alpha]_D^{25}$: -109.2 (c , 0.67 in CHCl_3) [Lit.⁷ enantiomer $[\alpha]_D^{25}$: $+120.3$ (c , 0.46 in CHCl_3)]. δ_{H} (400MHz, CDCl_3): 3.03-3.04 (2H, m, H-6a, H-6b), 3.18 (1H, d, $J_{2,3}$ 4.3, H-2), 3.34 (3H, s, OCH_3), 3.49-3.52 (1H, m, H-5), 3.73 (2H, a-s, CH_2Ph), 4.16 (1H, a-t, $J_{3,2+4}$ 4.5, H-3), 4.26 (1H, a-t, $J_{4,3+5}$ 4.7, H-4), 4.51 (1H, d, J_{gem} 12.0, $\text{CH}_2\text{Ph}'$), 4.63 (1H, d, J_{gem} 11.9, $\text{CH}_2\text{Ph}'$), 4.94 (1H, a-s, H-1), 7.27-7.42 (10H, m, 10xArCH).

Methyl 5-azido-3-O-benzyl-5-deoxy-6-O-*p*-toluenesulfonyl- α -D-idofuranoside 104a

α -Methyl glycoside **101a** (421mg, 1.36mmol) was dissolved in DCM (4ml) and after cooling to 0°C 2,4,6-collidine (1.07ml, 8.16mmol) and *p*-toluenesulfonyl chloride (519mg, 2.72mmol) were added. The reaction was allowed to warm to room temperature. T.l.c. analysis (toluene:acetone 3:1) after 26h indicated formation of a single product (R_f 0.42) and no remaining starting material (R_f 0.22). DCM (15ml) was added and the mixture was washed with HCl (2M, 2x10ml). The organic layer was dried ($MgSO_4$), filtered, concentrated and purified by flash column chromatography (toluene:acetone 96:4→9:1). Tosylate **104a** (526mg, quant) was isolated as a colourless oil.

Data: $[\alpha]_D^{25}$: +11.0 (*c*, 1.15 in $CHCl_3$) [Lit.⁷ enantiomer $[\alpha]_D^{25}$: -7.0 (*c*, 1.32 in $CHCl_3$)].

δ_H (400MHz, $CDCl_3$): 1.97 (1H, br-s, OH-2), 2.45 (3H, s, $PhCH_3$), 3.41 (3H, s, OCH_3), 3.91-3.96 (2H, m, H-3, H-5), 4.05 (1H, dd, $J_{6a,6b}$ 10.4, $J_{6a,5}$ 5.8, H-6a), 4.09 (1H, dd, $J_{6b,6a}$ 10.3, $J_{6b,5}$ 3.7, H-6b), 4.29-4.30 (1H, m, H-2), 4.31 (1H, dd, J 7.6, J 5.8, H-4), 4.46 (1H, d, J_{gem} 11.7, CH_2Ph), 4.67 (1H, d, J_{gem} 11.7, CH_2Ph), 4.82 (1H, d, $J_{1,2}$ 1.6, H-1), 7.27-7.39 (7H, m, 7xArCH), 7.75-7.77 (2H, m, 2xArCH).

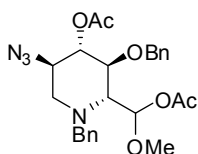
Methyl 5-azido-2-*N*-benzyl-3-*O*-benzyl-2,5,6-trideoxy-2,6-imino- α -D-gulofuranoside 105a

Tosylate **104a** (526mg, 1.36mmol) was stirred in DCM (5ml) with anhydrous pyridine (0.33ml, 4.08mmol) and trifluoromethanesulfonyl anhydride (0.30ml, 1.77mmol) at -30°C for 1h. T.l.c. analysis (toluene:acetone 3:1) indicated starting material disappearance (R_f 0.42) and product formation (R_f 0.69). The mixture was washed with HCl (2M, 2x10ml) and the organic layer was dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The crude was suspended in benzylamine (2ml) and heated at 100°C for 18h. LRMS confirmed conversion to product by the appearance of a peak at 381 and disappearance of the starting material peak at 464. The mixture was concentrated and purified by flash column chromatography (cyclohexane:ethyl acetate:TEA 97:2:1→93:7:1). Bicycle **105a** (215mg, 42% over 2 steps) was isolated as a yellow oil.

Data: $[\alpha]_D^{24}$: -40.0 (*c*, 1.58 in $CHCl_3$) [Lit.⁷ enantiomer $[\alpha]_D^{25}$: +39.2 (*c*, 1.05 in $CHCl_3$)].

δ_H (400MHz, $CDCl_3$): 3.01 (1H, a-d, $J_{6a,6b}$ 13.2, H-6a), 3.28 (1H, a-t, $J_{2,1+3}$ 3.4, H-2), 3.50 (3H, s, OCH_3), 3.57 (1H, a-t, $J_{5,4+6b}$ 4.8, H-5), 3.77 (1H, dd, $J_{6b,6a}$ 13.2, $J_{6b,5}$ 5.6, H-6b), 3.84 (1H, a-t, $J_{3,2+4}$ 4.4, H-3), 3.92 (1H, d, J_{gem} 13.4, CH_2Ph), 4.06 (1H, d, J_{gem} 13.4, CH_2Ph), 4.25 (1H, a-t, $J_{4,3+5}$ 4.5, H-4), 4.47 (1H, d, J_{gem} 12.1, CH_2Ph'), 4.65 (1H, d, J_{gem} 11.9, CH_2Ph'), 5.03 (1H, d, $J_{1,2}$ 3.0, H-1), 7.26-7.49 (10H, m, 10xArCH).

4-O-Acetyl-5-azido-2-N,3-O-dibenzyl-2,5,6-trideoxy-2,6-imino-D-gulose acetyl methyl acetal 106



Method 1: Bicycle **103b** (455mg, 1.20mmol) was stirred in acetic anhydride (5ml) with boron trifluoride diethyl etherate (0.74ml, 6.00mmol) for 36h. After this time t.l.c.

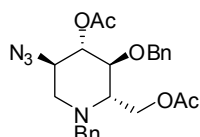
analysis (cyclohexane:ethyl acetate 4:1) indicated complete conversion of starting material (R_f 0.51) to two products (R_f 0.42, 0.47). The mixture was concentrated and the residue partitioned between ethyl acetate (15ml) and saturated sodium bicarbonate solution (15ml). The organic layer was re-washed with bicarbonate solution (10ml) and the product extracted from the combined aqueous layer with ethyl acetate (5ml). The organic layer was dried ($MgSO_4$), filtered and concentrated. The resulting crude was purified by flash column chromatography (cyclohexane:ethyl acetate:TEA 84:14:2) to afford piperidine **106** (354mg, 61%) as a solid in an anomeric ratio of A:B 3:1.

Method 2: Bicycle **105a** (88mg, 0.23mmol) was dissolved in acetic anhydride (1ml) and stirred with boron trifluoride diethyl etherate (86 μ l, 0.69mmol). After 3h analysis by t.l.c. (cyclohexane:ethyl acetate 4:1) indicated formation of product (R_f 0.45) and no remaining starting material (R_f 0.50). The mixture was concentrated and partitioned between ethyl acetate (10ml) and saturated aqueous sodium bicarbonate solution (5ml). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Following purification by flash column chromatography (cyclohexane:ethyl acetate:TEA 84:14:2) pure **106** (88mg, 79%) was yielded as a colourless oil in an anomeric ratio of A:B ~3:1.

Data: δ_H (400MHz, $CDCl_3$): 2.07, 2.15 (2x3H, 2xs, $COCH_3^B$), 2.08, 2.10 (2x3H, 2xs, $COCH_3^A$), 2.18-2.27 (2H, m, H-6a^{A+B}), 2.83-2.86 (2H, m, H-2^{A+B}), 2.97 (1H, dd, $J_{6b,6a}$ 12.3, $J_{6b,5}$ 4.8, H-6b^A), 2.97 (1H, dd, $J_{6b,6a}$ 11.7, $J_{6b,5}$ 4.4, H-6b^B), 3.36 (3H, m, OCH_3^B), 3.40 (3H, m, OCH_3^A), 3.43 (1H, d, J_{gem} 14.1, NCH_2Ph^B), 3.47-3.53 (2H, m, H-5^{A+B}), 3.58 (1H, d, J_{gem} 13.4, NCH_2Ph^A), 3.65 (1H, t, J 8.2, H-3^B), 3.71 (1H, t, J 8.7, H-3^A), 4.30 (1H, d, J_{gem} 13.5, NCH_2Ph^A), 4.39 (1H, d, J_{gem} 14.1, NCH_2Ph^B), 4.63-4.77 (4H, m,

$\text{OCH}_2\text{Ph}^{\text{A+B}}$), 5.09-5.14 (2H, m, $\text{H-4}^{\text{A+B}}$), 6.16 (1H, d, $J_{1,2}$ 1.8, H-1^{B}), 6.24 (1H, d, $J_{1,2}$ 2.2, H-1^{A}), 7.23-7.40 (20H, m, 20xArCH).

3,6-Di-O-acetyl-2-azido-1-N,4-O-dibenzyl-1,2,5-trideoxy-1,5-imino-L-glucitol **107**

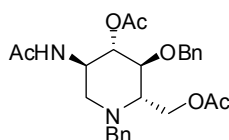


Acetal **106** (159mg, 0.33mmol) was suspended in DCM (2ml) and cooled to -78°C . DIBAL in toluene (1.5M, 1.1ml, 1.65mmol) was added and the reaction was stirred for 1h. T.l.c. analysis (cyclohexane:ethyl acetate 3:2) indicated formation of four product (R_f 0.40, 0.47, 0.56, 0.70) from starting material (R_f 0.60). The reaction was quenched with ethyl acetate (10ml) and stirred vigorously with saturated aqueous sodium potassium tartrate (10ml) for 2h. The organic layer was then separated and the aqueous layer washed with ethyl acetate (2x10ml). The organic layer was dried (MgSO_4), filtered and concentrated.

The residue was suspended in methanol (1ml) at 0°C and sodium borohydride (9mg, 0.23mmol) was added. After 2h, t.l.c. analysis (cyclohexane:ethyl acetate 3:2) indicated no remaining starting material (R_f 0.59) and one product (R_f 0.43). The mixture was concentrated *in vacuo* and co-evaporated with methanol three times. The residue was then suspended in acetic anhydride (1.5ml) and anhydrous pyridine (1.5ml). After 17h, t.l.c. analysis (cyclohexane:ethyl acetate 3:2) indicated product formation (R_f 0.67). The mixture was concentrated and purified by flash column chromatography (cyclohexane:ethyl acetate 9:1) to afford pyrrolidine **107** (81mg, 54%) as a white solid.

Data: m.p.: 90-92°C [Lit.⁷ enantiomer m.p.: 89-91°C]. $[\alpha]_D^{25}$: -4.3 (*c*, 2.2 in CHCl₃) [Lit.⁷ enantiomer $[\alpha]_D^{25}$: +3.3 (*c*, 1.20 in CHCl₃)]. δ_H (400MHz, CDCl₃): 2.07 (1H, m, H-1a), 2.08, 2.09 (2x3H, s, CO₂CH₃), 2.55 (1H, dd, $J_{5,4}$ 9.4, $J_{5,6}$ 2.0, H-5), 2.98 (1H, dd, $J_{1b,1a}$ 11.7, $J_{1b,2}$ 4.7, H-1b), 3.27 (1H, d, J_{gem} 13.5, NCH₂Ph), 3.48 (1H, dt, $J_{2,1a+3}$ 10.0, $J_{2,1b}$ 4.8, H-2), 3.66 (1H, a-t, $J_{4,3+2}$ 9.3, H-4), 4.10 (1H, d, J_{gem} 13.4, NCH₂Ph), 4.31 (1H, br-d, $J_{6a,6b}$ 12.7, H-6a), 4.59 (1H, d, J_{gem} 10.4, OCH₂Ph), 4.62-4.66 (1H, m, H-6b), 4.67 (1H, d, J_{gem} 10.7, OCH₂Ph), 5.09 (1H, a-t, $J_{3,2+4}$ 9.6, H-3), 7.26-7.38 (10H, m, 10xArCH).

2-Acetamido-3,6-di-*O*-acetyl-1-*N*-benzyl-4-*O*-benzyl-1,5-imino-1,2,5-trideoxy-L-glucitol **108**

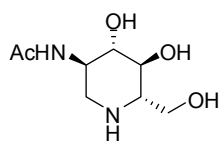


Azide **107** (137mg, 0.30mmol) was suspended in THF:AcOH:Ac₂O 3:2:1 (4.5ml) and zinc powder (396mg, 6.06mmol) was added. Finally, saturated aqueous copper sulfate (1ml) was added and the reaction was stirred for 1h. T.l.c. analysis (cyclohexane:ethyl acetate 4:1) indicated conversion of starting material (*R_f* 0.28) to product (*R_f* 0.02). The mixture was filtered and concentrated. Flash column chromatography (ethyl acetate:cyclohexane 2:1) afforded amide **108** (91mg, 65%) as a white solid.

Data: m.p.: 146-148°C [Lit.⁷ enantiomer m.p.: 149-151°C]. $[\alpha]_D^{25}$: -2.6 (*c*, 1.18 in CO(CH₃)₂) [Lit.⁷ enantiomer $[\alpha]_D^{25}$: +0.9 (*c*, 0.92 in CO(CH₃)₂)]. δ_H (400MHz, (CD₃)₂CO): 1.75 (3H, s, NHCOCH₃), 1.99, 2.07 (2x3H, 2xs, CO₂CH₃), 2.14 (1H, dd, $J_{1a,1b}$ 11.7, $J_{1a,2}$ 10.8, H-1a), 2.64 (1H, dt, $J_{5,4}$ 8.8, $J_{5,6a+6b}$ 3.0, H-5), 2.86 (1H, dd, $J_{1b,1a}$ 11.7, $J_{1b,2}$ 4.5, H-1b), 3.35 (1H, d, J_{gem} 13.6, NCH₂Ph), 3.74 (1H, a-t, $J_{4,3+5}$ 8.7, H-4), 4.07

(1H, dq, $J_{2,1a+3+NH}$ 9.8, $J_{2,1b}$ 4.5, H-2), 4.14 (1H, d, J_{gem} 13.6, NCH_2Ph), 4.36 (1H, dd, $J_{6a,6b}$ 12.5, $J_{6a,5}$ 3.4, H-6a), 4.65 (1H, dd, $J_{6b,6a}$ 12.5, $J_{6b,5}$ 2.7, H-6b), 4.66 (1H, d, J_{gem} 11.0, OCH_2Ph), 4.73 (1H, d, J_{gem} 11.1, OCH_2Ph), 4.91 (1H, a-t, $J_{3,2+4}$ 9.2, H-3), 6.80 (1H, d, $J_{NH,2}$ 9.1, NH), 7.22-7.37 (10H, m, 10xArCH).

2-Acetamido-1,5-imino-1,2,5-trideoxy-L-glucitol (L-DNJNAc) **109**



Fully protected piperidine **108** (145mg, 0.31mmol) was dissolved in methanol (5ml) and stirred with sodium methoxide (5mg, 0.09mmol) for 16h. T.l.c. analysis (ethyl acetate) indicated complete conversion of starting material (R_f 0.36) to product (R_f 0.11). The mixture was concentrated *in vacuo*. The resulting residue was suspended in water:2M HCl:1,4-dioxane 13:3:4 (5ml) with palladised carbon (10% 5mg, 0.3mmol). The flask was degassed and flushed with hydrogen. The flask was left under a hydrogen atmosphere for 23h. LRMS then indicated no remaining starting material, with mass 384, and product formation, corresponding to a mass of 204. The mixture was filtered on Celite®, eluting with water, and concentrated to afford the HCl salt. The salt was loaded onto a dowex (50W-X8, H^+) column using 1M HCl. Water was run through and the free amine was eluted using 2M aqueous ammonia to give **109** (63mg, quant) as a white solid.

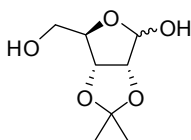
Data: Free base m.p: 220-222°C [Lit.⁷ enantiomer m.p.: 224-226°C]. $[\alpha]_D^{25}$: -18.8 (*c*, 0.57 in H_2O) [Lit.⁷ enantiomer $[\alpha]_D^{25}$: +14.6 (*c*, 0.86 in H_2O)]. δ_H (400MHz, D_2O): 1.88 (3H, s, $NHCOCH_3$), 2.34 (1H, a-t, $J_{1a,1b+2}$ 12.0, H-1a), 2.47 (1H, ddd, $J_{5,4}$ 9.5, $J_{5,6a}$ 5.9, $J_{5,6b}$ 2.8, H-5), 2.97 (1H, dd, $J_{1b,1a}$ 12.7, $J_{1b,2}$ 4.8, H-1b), 3.21 (1H, a-t, J 9.4, H-4), 3.29

(1H, a-t, J 9.5, H-3), 3.55 (1H, dd, $J_{6a,6b}$ 11.8, $J_{6a,5}$ 6.0, H-6a), 3.63 (1H, a-dt, $J_{2,1a+3}$ 10.7, $J_{2,1b}$ 4.8, H-2), 3.71 (1H, dd, $J_{6b,6a}$ 11.7, $J_{6b,5}$ 2.8, H-6b).

HCl salt δ_H (400MHz, D₂O): 1.93 (3H, s, NHCOCH_3), 2.89 (1H, a-t, $J_{1a,1b+2}$ 12.5, H-1a), 3.11-3.16 (1H, m, H-5), 3.39 (1H, dd, $J_{1b,1a}$ 12.8, $J_{1b,2}$ 4.9, H-1b), 3.53 (1H, a-t, $J_{3,2+4}$ 9.1, H-3), 3.57 (1H, a-t, $J_{4,3+5}$ 9.2, H-4), 3.79 (1H, dd, $J_{6a,6b}$ 12.8, $J_{6a,5}$ 5.1, H-6a), 3.86 (1H, dd, $J_{6b,6a}$ 12.8, $J_{6b,5}$ 3.1, H-6b), 3.97 (1H, ddd, $J_{2,1a}$ 12.4, $J_{2,3}$ 9.4, $J_{2,1b}$ 4.8, H-2).

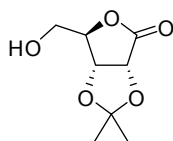
2-Acetamido-1,2,5,6-tetra-deoxy-1,5-imino-D-galactitol (6-deoxy DGJNAc)

2,3-*O*-Isopropylidene-D-ribose **111**



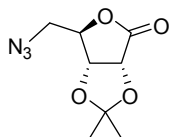
D-Ribose **39** (10.0g, 66.6mmol) was stirred in acetone (400ml) under nitrogen. Anhydrous copper(II) sulfate (17.0g, 106mmol) was added and the temperature was raised to 45°C. After 16h, t.l.c. analysis (ethyl acetate) indicated conversion of starting material (R_f 0.05) to a major product (R_f 0.67) and two minor products (R_f 0.86, 0.33). After cooling, the mixture was filtered through Celite®, eluting with acetone, and concentrated *in vacuo*. Flash column chromatography (ethyl acetate:cyclohexane 1:1) yielded **111** (9.15g, 72%) as a pale yellow oil.

Data: $[\alpha]_D^{21}$: -20.2 (c , 0.96 in CHCl_3) [Lit.¹⁰ $[\alpha]_D^{24}$: -23.4 (c , 0.71 in CHCl_3)]. **δ_H (400MHz, CDCl₃):** 1.33, 1.50 (2x3H, 2xs, $\text{C}(\text{CH}_3)_2$), 1.80 (1H, br-s, OH), 3.48 (1H, br-s, OH), 3.69-3.78 (2H, m, 2xH-5), 4.43 (1H, br-s, H-4), 4.59 (1H, a-d, $J_{2,3}$ 5.9, H-2), 4.85 (1H, a-d, $J_{3,2}$ 5.9, H-3), 5.43 (1H, d, $J_{1,2}$ 5.4, H-1).

2,3-O-Isopropylidene-D-ribo-1,4-lactone 112

Lactol **111** (4.00g, 21.0mmol) was dissolved in water (80ml) and cooled to 0°C. Barium carbonate (6.23g, 31.6mmol) was added portionwise and then bromine (1.62ml, 31.6mmol) was added dropwise. The temperature was allowed to increase to room temperature over the course of the reaction. T.l.c. (ethyl acetate:cyclohexane 1:1), after 18h, indicated complete conversion of starting material (R_f 0.18) to product (R_f 0.27). After quenching with saturated sodium thiosulfate solution (5ml) the product was extracted using ethyl acetate (3x80ml). The mixture was concentrated *in vacuo* and purified by flash column chromatography to afford **112** (2.80g, 71%) as white crystals.

Data: m.p.: 132-135°C [Lit.¹⁶ m.p.: 138-139°C]. $[\alpha]_D^{25}$: -54.5 (*c*, 0.97 in MeCN) [Lit.¹⁰ $[\alpha]_D^{25}$: -54.1 (*c*, 0.69 in MeCN)]. δ_H (400MHz, CDCl₃): 1.40, 1.50 (2x3H, 2xs, C(CH₃)₂), 1.98 (1H, br-s, OH), 3.83 (1H, ddd, $J_{5a,5b}$ 12.1, $J_{5a,OH}$ 5.4, $J_{5a,4}$ 1.8, H-5a), 4.00 (1H, ddd, $J_{5b,5a}$ 12.1, $J_{5b,OH}$ 5.1, $J_{5b,4}$ 2.4, H-5b), 4.64 (1H, a-t, $J_{4,5}$ 1.8, H-4), 4.79 (1H, d, $J_{2,3}$ 5.6, H-2), 4.84 (1H, a-d, $J_{3,2}$ 5.6, H-3).

5-Azido-5-deoxy-2,3-O-isopropylidene-D-ribo-1,4-lactone 113

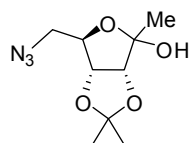
Lactone **112** (2.68g, 14.2mmol) was suspended in DCM (15ml) and TEA (2.18ml, 15.7mmol) was added. The temperature was reduced to -15°C and methanesulfonyl chloride (1.21ml, 15.7mmol) was added dropwise. After stirring for 16h at room

temperature under nitrogen, t.l.c. analysis (ethyl acetate) indicated no starting material (R_f 0.57), only product (R_f 0.60). Water was added (15ml) and the product was separated. The aqueous layer was re-extracted with DCM (2x10ml) and the combined organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford crude mesylate (3.04g, quantitative), which was used without further purification.

The mesylate (3.04g, 14.2mmol) was stirred in DMF (15ml) and sodium azide (1.20mg, 18.5mmol) was added. After stirring for 19h under a nitrogen atmosphere t.l.c. (cyclohexane:ethyl acetate 2:1) indicated complete conversion of starting material (R_f 0.14) to product (R_f 0.41). Solvent was removed *in vacuo*, co-evaporating with toluene, and the residue was partitioned between DCM (15ml) and water (15ml). The aqueous layer was re-extracted using DCM (2x15ml) and after drying ($MgSO_4$) the combined organic layers were filtered and concentrated. Flash column chromatography (cyclohexane:ethyl acetate 2:1) afforded azide **113** (2.92g, 96%) as a yellow oil which crystallised on standing.

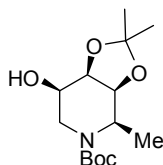
Data: m.p.: 32-33°C: [Lit.¹⁷ m.p.: 39°C]. $[\alpha]_D^{21}$: +19.2 (*c*, 1.19 in $CHCl_3$) [Lit.¹⁰ $[\alpha]_D^{23}$: +18.9 (*c*, 1.66 in $CHCl_3$)]. δ_H (400MHz, $CDCl_3$): 1.39, 1.48 (2x3H, 2xs, $(C(CH_3)_2)$), 3.67 (1H, dd, $J_{5a,5b}$ 13.2, $J_{5a,4}$ 2.3, H-5a), 3.79 (1H, dd, $J_{5b,5a}$ 13.2, $J_{5b,4}$ 3.1, H-5b), 4.64 (1H, a-d, $J_{3,2}$ 5.7, H-3), 4.67 (1H, m, H-4), 4.86 (1H, d, $J_{2,3}$ 5.7, H-2).

6-Azido-1,6-dideoxy-3,4-*O*-isopropylidene-D-psicofuranose **114**



Lactone **113** (1.41g, 5.35mmol) was dissolved in anhydrous THF (6ml) and cooled to -78°C. 1.6M MeLi solution in diethyl ether (4.34ml, 6.95mmol) was added dropwise. After 1h t.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated the reaction had proceeded to completion with product evident (R_f 0.65) and no remaining starting material (R_f 0.63). IR analysis confirmed this by the absence of a peak at around 1700cm^{-1} . Water was added (15ml) and the product was extracted using DCM (2x25ml). The combined organic layers were dried (MgSO_4), filtered and concentrated to give **114** (1.07g, 87%) as a yellow oil, which was used without further purification. The ratio of anomers A:B was 2:1.

Data: HRMS: $\text{C}_9\text{H}_{15}\text{O}_4\text{N}_3\text{Na}$ ($\text{M}+\text{Na}^+$) calculated 252.0955, found 252.0952. ν_{max} (NaCl)/ cm^{-1} : 2108 (m, N_3), 3417 (m, br, OH). δ_{H} (400MHz, CDCl_3): 1.35, 1.51 (2x3H, 2xs, $\text{C}(\text{CH}_3)_2^{\text{A}}$), 1.40, 1.53 (2x3H, 2xs, 2xCCH₃^B), 1.56 (3H, s, 3xH-1^A), 1.61 (3H, s, 3xH-1^B), 2.87 (1H, br-s, OH^A), 3.35 (1H, dd, $J_{6a,6b}$ 13.2, $J_{6a,5}$ 4.1, H-6a^B), 3.42 (1H, dd, $J_{6a,6b}$ 12.5, $J_{6a,5}$ 5.8, H-6a^A), 3.58 (1H, dd, $J_{6b,6a}$ 13.1, $J_{6b,5}$ 4.0, H-6b^B), 3.61 (1H, dd, $J_{6b,6a}$ 12.5, $J_{6b,5}$ 7.3, H-6b^A), 4.02 (1H, s, OH^B), 4.20 (1H, a-q, $J_{5,4+6a+6b}$ 4.2, H-5^B), 4.25 (1H, ddd, $J_{5,6b}$ 7.3, $J_{5,6a}$ 5.9, $J_{5,4}$ 1.4, H-5^A), 4.49 (1H, d, $J_{3,4}$ 7.7, H-3^B), 4.51 (1H, d, $J_{3,4}$ 6.0, H-3^A), 4.69 (1H, dd, $J_{4,3}$ 7.4, $J_{4,5}$ 4.8, H-4^B), 4.70 (1H, dd, $J_{4,3}$ 5.8, $J_{4,5}$ 1.4, H-4^A). δ_{C} (100.6MHz, CDCl_3): 22.9 (C-1^A), 25.0, 26.0 (2xCCH₃^B), 25.2, 26.6 (2xCCH₃^A), 26.5 (C-1^B), 51.8 (C-6^B), 52.0 (C-6^A), 80.3, 81.6, 84.1 (C-3^B, C-4^B, C-5^B), 83.0 (C-4^A), 84.5 (C-5^A), 86.0 (C-3^A), 107.6 ($\text{C}(\text{CH}_3)_2^{\text{A}}$), 113.0 ($\text{C}(\text{CH}_3)_2^{\text{B}}$). m/z (ES-): 228.1 ($[\text{M}-\text{H}]^-$, 36%), 264.1 ($[\text{M}+\text{Cl}]^-$, 40%), 288.1 ($[\text{M}+\text{AcO}]^-$, 61%), 457.2 ($[\text{2M}-\text{H}]^-$, 100%).

N-Boc-1,5-imino-1,5,6-trideoxy-3,4-O-isopropylidene-D-talitol 116

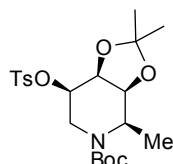
Azide **114** (500mg, 2.18mmol) was suspended in ethanol (13ml), palladium black (100mg) was added and the flask was degassed and flushed with hydrogen. The mixture was left to stir under a hydrogen atmosphere for 24h. T.l.c. (cyclohexane:ethyl acetate 1:1) indicated conversion of starting material (R_f 0.60) to product (R_f 0.40). After filtering through Celite®, eluting with ethanol, the mixture was concentrated *in vacuo* to afford **115** (408mg, assumed quant) which was used without further purification.

Crude **115** (327mg, 1.75mmol) was dissolved in DMF (2ml), di-*tert*-butyl dicarbonate (441μl, 1.92mmol) was added dropwise and the mixture was stirred under nitrogen at room temperature for 8h. T.l.c. analysis (cyclohexane:ethyl acetate 1:1) indicated product formation (R_f 0.68) and consumption of starting material (R_f 0.50). The product was concentrated *in vacuo*, co-evaporating with toluene, and purified by flash column chromatography (cyclohexane:ethyl acetate 5:1→4:1→3:1). This afforded **116** (292mg, 58%) as a colourless oil which crystallised on standing.

Data: HRMS: $C_{14}H_{25}NO_5Na$ ($M+Na^+$) calculated 310.1625; found 310.1619. **m.p.:** 53-55°C. $[\alpha]_D^{23}$: +2.6 (*c*, 0.69 in $CHCl_3$). ν_{max} (NaCl)/ cm^{-1} : 3455 (m, br, OH), 1692 (s, C=O). δ_H (400MHz, $CDCl_3$): 1.34 (3H, d, $J_{6,5}$ 6.7, 3xH-6), 1.39 (3H, s, $C(CH_3)$), 1.46 (9H, s, $C(CH_3)_3$), 1.53 (3H, s, $C(CH_3)$), 2.68 (1H, d, $J_{OH,2}$ 9.0, OH), 2.99 (1H, a-t, J 12.0, H-1a), 3.57-3.64 (1H, m, H-2), 3.88 (1H, dd, $J_{1b,1a}$ 12.8, $J_{1b,2}$ 4.1, H-1b), 4.00 (1H, a-quin, $J_{5,4+6a+6b+6c}$ 6.2, H-5), 4.35-4.41 (2H, m, H-3, H-4). δ_C (100.6MHz, $CDCl_3$): 16.7 (C-6), 24.7, 26.3 ($C(CH_3)_2$), 28.4 ($C(CH_3)_3$, C-1), 47.3 (C-5), 66.3 (C-2), 72.7, 76.0 (C-3, C-4),

80.0 ($C(CH_3)_3$), 108.7 ($C(CH_3)_2$), 155.0 (CO_2Me_3). **m/z (ES⁺):** 288.4 ($M+H^+$, 62%); 310.3 ($M+Na^+$, 23%); 351.3 ($M+MeCN+Na^+$, 100%), 597.3 ($2M+Na^+$, 57%).

***N*-Boc-1,5-imino-1,5,6-trideoxy-2-*O*-*p*-toluenesulfonyl-3,4-*O*-isopropylidene-D-talitol**

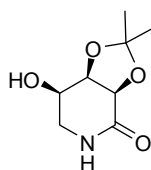


A solution of **116** (36mg, 0.13mmol) in pyridine (anhydrous 0.4ml) was cooled to 0°C and *p*-toluenesulfonyl chloride (60mg, 0.31mmol) was added. After 19h t.l.c. analysis (cyclohexane: ethyl acetate 3:1) appeared to indicate remaining starting material (R_f 0.25), hence more *p*-toluenesulfonyl chloride (48mg, 0.26mmol) was added. After a further 2h, t.l.c. (cyclohexane:ethyl acetate 3:1) indicated a major product (R_f 0.29). The product was concentrated, co-evaporating with toluene, and the residue suspended in DCM (10ml), washed with water (10ml) and the product was extracted using DCM (2x10ml). The organic layer was dried, filtered, concentrated and the residue was purified by flash column chromatography (cyclohexane:ethyl acetate 4:1→3:1→2:1) to afford tosylate (36mg, 63%) as a colourless oil.

Data: HRMS: $C_{21}H_{31}NO_7SNa$ ($M+Na^+$) calculated 464.1713, found 464.1709. **$[\alpha]_D^{23}$:** +15.9 (*c*, 0.74 in $CHCl_3$). **ν_{max} (NaCl)/ cm^{-1} :** 1695 (m, C=O). **δ_H (400MHz, $CDCl_3$):** 1.24-1.27 (2x3H, m, $C(CH_3)_3$, 3xH-6), 1.41 (9H, s, $C(CH_3)_3$), 1.50 (3H, s, $C(CH_3)_3$), 2.44 (3H, s, $PhCH_3$), 3.20 (1H, a-t, $J_{1a,1b+2}$ 11.5, H-1a), 3.84 (1H, a-d, $J_{1b,1a}$ 12.8, H-1b), 4.10 (1H, br-s, H-5), 4.27-4.35 (2H, m, H-3, H-4), 4.41 (1H, br-s, H-2), 7.34 (2H, d, J 6.7, 2xArCH), 7.83 (2H, d, J 5.9, 2xArCH). **δ_C (100.6MHz, $CDCl_3$):** 16.1 (CH_3), 21.7

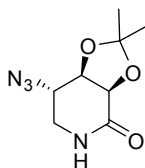
(PhCH₃), 24.9, 25.9 (C(CH₃)₂), 28.2 (C(CH₃)₃), 38.3 (C-1), 47.5 (C-5), 72.2, 74.0, 74.9 (C-2, C-3, C-4), 80.5 (C(CH₃)₃), 109.7 (C(CH₃)₂), 127.9, 129.9, 133.7 (ArCH), 145.0 (Ar_{quat}), 154.5 (CO₂Me₃). **m/z (ES⁺):** 442.2 (M+H⁺, 35%), 459.2 (M+NH₄⁺, 100%), 464.2 (M+Na⁺, 36%), 505.2 (M+MeCN+Na⁺, 27%), 900.3 (2M+NH₄⁺, 80%).

5-Amino-5-deoxy-2,3-*O*-isopropylidene-D-ribo-1,5-lactam **117**



Palladium black (194mg) was added to azide **113** (1.94g, 9.10mmol) suspended in ethyl acetate (50ml). The flask was degassed and flushed with hydrogen. The mixture was left to stir under a hydrogen atmosphere. After 18h, t.l.c. (ethyl acetate:cyclohexane 1:1) indicated conversion of starting material (R_f 0.56) to product (R_f 0.12). Filtration through Celite®, eluting with ethyl acetate, removed the palladium and concentration *in vacuo* afforded lactam **117** (1.70g, assumed quant) as a colourless oil. A small portion (100mg) was purified by flash column chromatography (ethyl acetate:IPA:water 11:3:1) for characterisation purposes to afford pure amide (32mg, 32%) as white crystals.

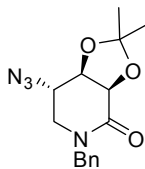
Data: m.p.: 136-138°C [Lit.¹⁴ m.p.: 139-140°C]. [**α**]_D²⁵: +18.1 (*c*, 1.47 in CHCl₃) [Lit.¹⁴ [**α**]_D²⁰: +26.8 (*c*, 1.02 in CHCl₃)]. **δ_H (400MHz, D₂O):** 1.43, 1.46 (2x3H, 2xs, C(CH₃)₂), 3.32 (1H, dd, *J*_{5a,5b} 12.7, *J*_{5a,4} 4.0, H-5a), 3.44 (1H, dd, *J*_{5b,5a} 12.7, *J*_{5b,4} 8.0, H-5b), 4.24 (1H, ddd, *J*_{4,5b} 7.9, *J*_{4,5a} 4.0, *J*_{4,3} 2.9, H-4), 4.50-4.55 (2H, m, H-2, H-3).

5-Amino-4-azido-4,5-dideoxy-2,3-O-isopropylidene-L-lyxono-1,5-lactam 118

Crude alcohol **117** (744mg, 3.97mmol) was suspended in DCM (6ml) with anhydrous pyridine (0.96ml) and cooled to 0°C. Trifluoromethanesulfonyl anhydride (0.87ml, 5.17mmol) was added dropwise under a nitrogen atmosphere. After stirring for 1h t.l.c. analysis (ethyl acetate:IPA:water 16:3:1) indicated complete conversion of starting material (R_f 0.24) to product (R_f 0.55). Water (15ml) was added and the product was extracted using DCM (3x20ml). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford the triflate as a yellow oil.

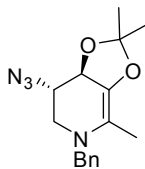
Crude triflate (assumed quant) was suspended in DMF (2ml) and sodium azide (722mg, 11.1mmol) was added. T.l.c. analysis (ethyl acetate:IPA:water 16:3:1) indicated conversion to product (R_f 0.70) from starting material (R_f 0.65) after 16h. The mixture was concentrated *in vacuo* and washed with water (15ml) and ethyl acetate (20ml). The aqueous layer was re-extracted with ethyl acetate (2x20ml). After drying ($MgSO_4$), the combined organic layers were filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate:cyclohexane 4:1→3:1) to afford **118** (283mg, 34%) as a yellow solid.

Data:¹⁵ **m.p.:** 69-71°C. $[\alpha]_D^{23}$: +48.1 (*c*, 0.70 in $CHCl_3$). δ_H (400MHz, $CDCl_3$): 1.41, 1.52 (2x3H, 2xs, $C(CH_3)_2$), 3.24 (1H, ddd, $J_{5a,5b}$ 13.2, $J_{5a,NH}$ 6.4, $J_{5a,4}$ 3.6, H-5a), 3.60 (1H, a-dt, $J_{5b,5a}$ 13.2, $J_{5b,4}$ 3.2, H-5b), 3.87 (1H, a-dt, $J_{4,3}$ 5.9, $J_{4,5}$ 3.2, H-4), 4.40 (1H, a-t, $J_{3,2+4}$ 6.1, H-3), 4.55 (1H, d, $J_{2,3}$ 6.9, H-2), 6.40 (1H, br-s, NH).

N-Benzyl-5-amino-4-azido-4,5-dideoxy-2,3-O-isopropylidene-L-lyxono-1,5-lactam**119**

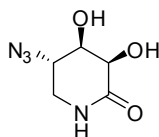
Lactam **118** (491mg, 2.31mmol) was suspended in DMF (4ml) in a flame dried flask and stirred with 4Å molecular sieves under a nitrogen atmosphere having been degassed. Sodium hydride (111mg, 2.78mmol) was added followed by benzyl bromide (0.33ml, 2.78mmol). After 1h, t.l.c. analysis (ethyl acetate:IPA:water 16:3:1) indicated complete consumption of starting material (R_f 0.71) to form a major product (R_f 0.82). The solvent was removed, co-evaporating with toluene, and the residue washed with water (20ml) and DCM (20ml). The aqueous layer was re-extracted with more DCM (2x20ml). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash column chromatography (cyclohexane:ethyl acetate 3:1) afforded pure **119** (341mg, 49%) as a yellow oil.

Data: HRMS: $C_{15}H_{18}N_4O_3Na$ ($M+Na^+$) calculated 325.1271; found 325.1270. $[\alpha]_D^{25}$: +72.6 (c , 1.28 in $CHCl_3$). ν_{max} (NaCl)/ cm^{-1} : 2112 (s, N_3), 1663 (s, C=O amide I), 1553 (m, C=O amide II). δ_H (400MHz, $CDCl_3$): 1.41, 1.48 (2x3H, 2xs, $C(CH_3)_2$), 3.13 (1H, dd, $J_{5a,5b}$ 13.3, $J_{5a,4}$ 5.9, H-5a), 3.50 (1H, dd, $J_{5b,5a}$ 13.3, $J_{5b,4}$ 3.0, H-5b), 3.79 (1H, dt, $J_{4,5a+3}$ 5.6, $J_{4,5b}$ 3.1, H-4), 4.37 (1H, a-t, $J_{3,2+4}$ 5.9, H-3), 4.55 (1H, d, J_{gem} 14.6, CH_2Ph), 4.65 (1H, d, $J_{2,3}$ 6.8, H-2), 4.71 (1H, d, J_{gem} 14.6, CH_2Ph), 7.27-7.35 (5H, m, 5xArCH). δ_C (100.6MHz, $CDCl_3$): 24.7, 26.8 ($C(CH_3)_2$), 45.1 (C-5), 50.4 (CH_2Ph), 58.2 (C-4), 73.9 (C-2), 75.6 (C-3), 110.8 ($C(CH_3)_2$), 127.9, 128.3, 128.8 (ArCH), 135.7 (Ar_{quat}), 166.1 (C-1). m/z (ES⁺): 198.5 (100%), 303.3 ($M+H^+$, 61%).

N-Benzyl-1,5-imino-1,2,5,6-tetra-deoxy-2-azido-3,4-O-isopropylidene-4,5-ene-D-galactitol 120

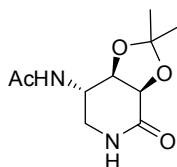
Benzyl amide **119** (72mg, 0.24mmol) was suspended in anhydrous THF (0.3ml) and cooled to -50°C . 1.6M MeLi in diethyl ether (0.18ml, 0.29mmol) was added and after 10 minutes the temperature was allowed to rise to -30°C . T.l.c. (cyclohexane:ethyl acetate 1:1) after 30 minutes indicated consumption of starting material (R_f 0.54) and formation of a major product (R_f 0.64). Water (5ml) was added to the reaction and the product extracted using ethyl acetate (3x5ml). The organic fractions were combined, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was partially purified by flash column chromatography (cyclohexane:ethyl acetate 8:1), from which only **120** (17mg, 23%) was isolated, as an orange oil.

Data: HRMS: $\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_2\text{H}$ ($\text{M}+\text{H}^+$) calculated 301.1659; found 301.1650. $[\alpha]_{\text{D}}^{25}$: +64.3 (*c*, 1.70 in CHCl_3). ν_{max} (NaCl)/ cm^{-1} : 2105 (s, N_3), 1642 (w, $\text{C}=\text{C}$). δ_{H} (400MHz, CDCl_3): 1.43, 1.50 (2x3H, 2xs, $\text{C}(\text{CH}_3)_2$), 1.93 (3H, d, $J_{6,3}$ 1.8, 3xH-6), 2.45 (1H, a-t, $J_{1a,1b+2}$ 12.0, H-1a), 2.96 (1H, dd, $J_{1b,1a}$ 11.9, $J_{1b,2}$ 4.3, H-1b), 3.60 (1H, ddd, $J_{2,1a}$ 12.2, $J_{2,3}$ 8.0, $J_{2,1b}$ 4.2, H-2), 3.79 (1H, d, J_{gem} 14.7, CH_2Ph), 4.19 (1H, d, J_{gem} 14.7, CH_2Ph), 4.42 (1H, dq, $J_{3,2}$ 8.0, $J_{3,6}$ 1.7, H-3), 7.26-7.37 (5H, m, 5xArCH). δ_{C} (100.6MHz, CDCl_3): 12.7 (C-6), 25.8, 27.0 ($\text{C}(\text{CH}_3)_2$), 49.2 (C-1), 55.4 (CH_2Ph), 60.4 (C-2), 74.9 (C-3), 110.9 ($\text{C}(\text{CH}_3)_2$), 119.1 (C-5), 127.4, 128.2, 128.6, 128.6 (C-4, ArCH), 137.8 (Arquat). m/z (**ES**⁺): 301.3 ($\text{M}+\text{H}^+$, 100%), 333.3 ($\text{M}+\text{MeOH}+\text{H}^+$, 62%), 601.3 ($2\text{M}+\text{H}^+$, 48%), 633.3 ($2\text{M}+\text{MeOH}+\text{H}^+$, 41%).

Other targets**5-Amino-4-azido-4,5-dideoxy-L-lyxono-1,5-lactam 121**

Lactam **118** (104mg, 0.49mmol) was suspended in 1,4-dioxane:water 1:1 (2ml) and TFA (1ml) was added. After 8 days analysis by t.l.c. (ethyl acetate:IPA:water 10:3:1) indicated conversion of starting material (R_f 0.75) to product (R_f 0.10) which was concentrated *in vacuo*. The residue was purified by ion-exchange chromatography (Dowex 50WX8 H^+) to afford **121** (60mg, 71%) as a yellow amorphous solid.

Data: HRMS (FI+): $C_5H_9N_4O_3$ ($M+H^+$) calculated 173.0669, found 173.0674. **m.p.:** 102-105°C. $[\alpha]_D^{23}$: -21.5 (*c*, 0.70 in H_2O). ν_{max} (**Ge**)/ cm^{-1} : 3174 (m, br, OH), 2122 (m, N_3), 1673 (m, C=O amide I), 1592 (m, C=O amide II). δ_H (**400MHz, D₂O**): 2.94 (1H, dd, $J_{5a,5b}$ 13.3, $J_{5a,4}$ 9.4, H-5a), 3.12 (1H, dd, $J_{5a,5b}$ 13.3, $J_{5b,4}$ 3.6, H-5b), 3.81 (1H, a-t, $J_{3,2+4}$ 5.2, H-3), 3.88 (1H, a-dt, $J_{4,5a}$ 8.7, $J_{4,3+5b}$ 4.4, H-4), 3.96 (1H, d, $J_{2,3}$ 5.6, H-2). δ_C (**100.6MHz, D₂O**): 40.9 (C-5), 60.9 (C-4), 73.5, 74.0 (C-2, C-3), 178.7 (C-1).

5-Amino-4-acetamido-4,5-dideoxy-2,3-O-isopropylidene-L-lyxono-1,5-lactam 123

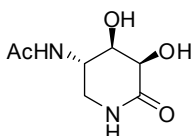
Azide **118** (234mg, 1.10mmol) was dissolved in ethyl acetate (6ml) and palladium black (23mg) was added. The flask was degassed, flushed with hydrogen and the reaction stirred under a hydrogen atmosphere for 5h. T.l.c. analysis (ethyl acetate:IPA:water

10:3:1) indicated conversion of starting material (R_f 0.64) to product (R_f 0.04). The mixture was filtered through Celite®, eluting with ethyl acetate, and concentrated to afford amine (196mg, 96%) as a pale yellow foam.

Crude amine (100mg, 0.54mmol), was suspended in anhydrous pyridine (1ml). Acetic anhydride (56μl, 0.59mmol) was added dropwise under nitrogen. After 3h t.l.c. analysis (ethyl acetate:IPA:water 16:3:1) indicated conversion of starting material (R_f 0.03) to product (R_f 0.17). The product was concentrated *in vacuo*, co-evaporating with toluene, and purified using flash column chromatography (ethyl acetate:IPA:water 10:3:1). This afforded **123** (75mg, 61%) as a yellow glassy solid.

Data: HRMS: $C_{10}H_{16}N_2O_4Na$ ($M+Na^+$) calculated 251.1002; found 251.1007. **m.p.:** 89-91°C. $[\alpha]_D^{24}$: +24.0 (*c*, 1.15 in MeOH). ν_{max} (NaCl)/ cm^{-1} : 3264 (w, br, NH), 1659 (m, C=O amide I), 1555 (m, C=O amide II). δ_H (400MHz, $CDCl_3$): 1.37, 1.47 (2x3H, 2xs, $C(CH_3)_2$), 2.02 (3H, s, $NHCOCH_3$), 3.20 (1H, a-br-d, $J_{5a,5b}$ 12.8, H-5a), 3.70 (1H, a-br-d, $J_{5b,5a}$ 12.2, H-5b), 4.31-4.33 (1H, m, H-4), 4.44-4.50 (2H, m, H-2, H-3), 6.89 (1H, br-s, NH), 7.32 (1H, d, $J_{NH,4}$ 7.6, $NHAc$). δ_C (100.6MHz, $CDCl_3$): 23.1 ($NHCOCH_3$), 24.5, 26.6 ($C(CH_3)_2$), 41.2 (C-5), 46.9 (C-4), 73.1, 75.1 (C-2, C-3), 110.5 ($C(CH_3)_2$), 169.8, 170.9 (C-1, $NHCOCH_3$). **m/z (ES+):** 229.4 ($M+H^+$, 37%), 251.3 ($M+Na^+$, 24%), 292.3 ($M+MeCN+Na^+$, 100%), 479.2 ($2M+Na^+$, 67%).

5-Amino-4-acetamido-4,5-dideoxy-L-lyxono-1,5-lactam **122**



Lactam **123** (72mg, 0.32mmol) was suspended in 1,4-dioxane:water (1:1, 1.2ml) and TFA (0.6ml) was added dropwise. After 23h t.l.c. analysis (ethyl acetate:IPA:water 10:3:1) indicated full consumption of starting material (R_f 0.45) to form a single product (R_f 0.04). The solvents were removed *in vacuo* and the residue was purified by ion-exchange chromatography (Dowex 50WX8 H^+) to afford **122** (51mg, 85%) as a pale yellow amorphous solid.

Data: HRMS: $C_7H_{11}N_2O_4$ ($[M-H]^+$) calculated 187.0724; found 187.0728. **m.p.:** 135-139°C. $[\alpha]_D^{25}$: +1.35 (*c*, 1.26 in H_2O). ν_{max} (**Ge**)/ cm^{-1} : 3164 (br, OH), 1677 (s, C=O), 1655 (m, C=O), 1560 (m, C=O amides II). δ_H (**400MHz, D₂O**): 1.92 (1H, s, $NHCOCH_3$), 3.00 (1H, dd, $J_{5a,5b}$ 13.2, $J_{5a,4}$ 9.2, H-5a), 3.12 (1H, dd, $J_{5b,5a}$ 13.2, $J_{5b,4}$ 3.9, H-5b), 3.67-3.72 (2H, m, H-2, H-3), 4.25-4.27 (1H, m, H-4). δ_C (**100.6MHz, D₂O**): 22.3 (CH_3), 42.0 (C-5), 48.7 (C-4), 72.0, 72.4 (C-2, C-3), 175.4 ($COCH_3$), 179.1 (C-1). **m/z (ES-):** 149.1 (M-39, 100%), 187.3 ($[M-H]^+$, 83%), 223.3 (M+ Cl^- , 56%).

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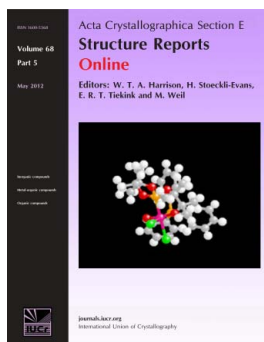
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2-Acetamido-*N*-benzyl-1,4-imino-1,2,4-trideoxy-*L*-xylitol (*N*-benzyl-*L*-XYLNAC)

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2-Acetamido-*N*-benzyl-1,4-imino-1,2,4-trideoxy-L-xylitol (*N*-benzyl-L-XYLNAC)

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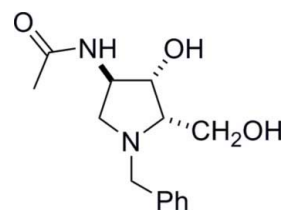
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Key indicators: single-crystal X-ray study; $T = 150$ K; mean $\sigma(\text{C}-\text{C}) = 0.004$ Å; R factor = 0.051; wR factor = 0.130; data-to-parameter ratio = 10.4.

X-ray crystallography defines the relative configuration at the three-stereogenic centres in the title compound *N*-benzyl-L-XYLNAC, $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_3$. The five-membered pyrrolidine ring adopts an envelope conformation with the N atom lying out of the plane of the other four atoms. In the crystal structure, intermolecular $\text{O}-\text{H}\cdots\text{O}$, $\text{N}-\text{H}\cdots\text{O}$ and $\text{O}-\text{H}\cdots\text{N}$ hydrogen bonds link the molecules into chains along [100]. The carbonyl group O atom acts as an acceptor for a bifurcated hydrogen bond. The absolute configuration is determined by the use of L-glucuronolactone as the starting material for the synthesis.

Related literature

For iminosugars see: Asano *et al.* (2000); Watson *et al.* (2001). For the inhibition of hexosaminidases, see: Liu, Numa *et al.* (2004); Reese *et al.* (2007); Liu, Iqbal *et al.* (2004); Woynarowska *et al.* (1992). For piperidine hexosaminidase inhibitors, see: Tatsuta *et al.* (1997); Fleet *et al.* (1986, 1987); Steiner *et al.* (2009); Ho *et al.* (2010); For furanose hexosaminidase inhibitors, see: Usuki *et al.* (2009); Rountree *et al.* (2007, 2009); Boomkamp *et al.* (2010). For strategies for cancer treatment, see: Kato *et al.* (2010); Greco *et al.* (2009). For the use of glucuronolactone as a starting material for the synthesis of iminosugars, see: Best, Wang *et al.* (2010); Best, Chairatana *et al.* (2010).



Experimental

Crystal data

$\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_3$
 $M_r = 264.32$
Orthorhombic, $P2_12_12_1$
 $a = 4.9731$ (1) Å
 $b = 10.0145$ (3) Å
 $c = 26.9297$ (7) Å
 $V = 1341.18$ (6) Å³
 $Z = 4$
Mo $K\alpha$ radiation
 $\mu = 0.09$ mm⁻¹
 $T = 150$ K
 $0.50 \times 0.15 \times 0.05$ mm

Data collection

Nonius KappaCCD area-detector diffractometer
Absorption correction: multi-scan (DENZO/SCALEPACK; Otwinowski & Minor, 1997)
 $T_{\min} = 0.77$, $T_{\max} = 1.00$
7494 measured reflections
1788 independent reflections
1471 reflections with $I > 2\sigma(I)$
 $R_{\text{int}} = 0.040$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.051$
 $wR(F^2) = 0.130$
 $S = 0.95$
1788 reflections
172 parameters
H-atom parameters constrained
 $\Delta\rho_{\max} = 0.33$ e Å⁻³
 $\Delta\rho_{\min} = -0.46$ e Å⁻³

Table 1

Hydrogen-bond geometry (Å, °).

$D-H\cdots A$	$D-H$	$H\cdots A$	$D\cdots A$	$D-H\cdots A$
$\text{O15}-\text{H151}\cdots\text{O19}^{\text{i}}$	0.85	1.94	2.790 (4)	173
$\text{N16}-\text{H161}\cdots\text{O19}^{\text{ii}}$	0.89	2.19	3.041 (4)	159
$\text{O1}-\text{H11}\cdots\text{N4}^{\text{ii}}$	0.85	2.29	3.121 (4)	167

Symmetry codes: (i) $x + \frac{1}{2}, -y + \frac{3}{2}, -z + 1$; (ii) $x + 1, y, z$.

Data collection: *COLLECT* (Nonius, 2001); cell refinement: *DENZO/SCALEPACK* (Otwinowski & Minor, 1997); data reduction: *DENZO/SCALEPACK* and Görbitz (1999); program(s) used to solve structure: *SIR92* (Altomare *et al.*, 1994); program(s) used to refine structure: *CRYSTALS* (Betteridge *et al.*, 2003); molecular graphics: *CAMERON* (Watkin *et al.*, 1996); software used to prepare material for publication: *CRYSTALS*.

Supplementary data and figures for this paper are available from the IUCr electronic archives (Reference: LH5029).

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supplementary materials

Acta Cryst. (2010). E66, o1147–o1148 [doi:10.1107/S1600536810014145]

2-Acetamido-*N*-benzyl-1,4-imino-1,2,4-trideoxy-L-xylitol (*N*-benzyl-L-XYLNac)

S. F. Jenkinson, E. V. Crabtree, A. F. G. Glawar, T. D. Butters, G. W. J. Fleet and D. J. Watkin

Comment

Iminosugars in which the oxygen of a sugar ring is replaced by nitrogen comprise a large family of inhibitors of carbohydrate processing enzymes (Asano *et al.*, 2000; Watson *et al.*, 2001). Specific inhibition of individual hexosaminidases may allow the investigation of a number of diseases including osteoarthritis (Liu, Numa *et al.*, 2004), allergy (Reese *et al.*, 2007), Alzheimer's disease (Liu, Iqbal *et al.*, 2004), and cancer (Woyrnarowska *et al.*, 1992). Inhibition of *N*-acetylglucosaminyltransferases (Kato *et al.*, 2010) and protection of macrophage activating factor (Greco *et al.*, 2009) may provide new strategies for the treatment of cancer. There are many piperidine hexosaminidase inhibitors, such as naturally occurring nagstatin (Tatsuta *et al.*, 1997) and DNJNac (Fleet *et al.*, 1986; Fleet *et al.*, 1987; Steiner *et al.*, 2009), some with picomolar inhibition (Ho *et al.*, 2010). Until very recently, potent furanose analogue inhibitors of hexosaminidases have been unknown. The first pyrrolizidine β -hexosaminidase inhibitor, pochonicine **1** (Fig. 1) [or its enantiomer], has been isolated from a fungal strain (Usuki *et al.*, 2009). A rare example of a pyrrolidine potent hexosaminidase inhibitor is the iminoarabinitol LABNac **2** (Rountree *et al.*, 2007; Rountree *et al.*, 2009) which has promise for the study of lysosomal storage of oligosaccharide and glycosphingolipid in iminosugar treated cells (Boomkamp *et al.*, 2010).

In a study of the hexosaminidase inhibition of diastereomers of LABNac **2** (Fig. 1), the L-xylo-epimer L-XYLNac **4** has been prepared from L-glucuronolactone **6**, a common constituent of the chiral pool for the preparation of imino sugars (Best, Wang *et al.*, 2010). The lactone **6** may be efficiently converted to the diol **5** (Best, Chairatana *et al.*, 2010) which has been further transformed to **4** via the *N*-benzyl L-XYLNac **3** of L-XYLNac. This paper reports the crystal structure of **3** which establishes the relative configuration and will allow modelling studies to rationalize enzyme inhibition by the diastereomeric 2-acetamido-pyrrolidine sugar mimics; the absolute configuration is determined by the use of L-glucuronolactone **6** as the starting material.

The pyrrolidine ring of the title compound adopts an envelope conformation with the nitrogen lying out of the plane (Fig. 2). The compound exists as chains of hydrogen-bonded molecules lying parallel to the *a*-axis (Fig. 3). Each molecule is a donor and acceptor for 3 hydrogen bonds and the hydrogen bond involving O19 is bifurcated. Only classical hydrogen bonding is considered.

Experimental

N-Benzyl-L-XYLNac **3** was crystallized from acetonitrile: m.p. 396–399 K; $[\alpha]_D^{25} +39.9$ (*c*, 0.99 in MeOH).

Refinement

In the absence of significant anomalous scattering, Friedel pairs were merged and the absolute configuration was assigned from the use of L-glucuronolactone as the starting material.

The relatively large ratio of minimum to maximum corrections applied in the multiscan process (1:1.29) reflect changes in the illuminated volume of the crystal. Changes in illuminated volume were kept to a minimum, and were taken into account (Göribitz, 1999) by the multi-scan inter-frame scaling (*DENZO/SCALEPACK*, Otwinowski & Minor, 1997).

The H atoms were all located in a difference map, but those attached to carbon atoms were repositioned geometrically. The H atoms were initially refined with soft restraints on the bond lengths and angles to regularize their geometry (C—H in the range 0.93–0.98, N—H in the range 0.86–0.89 N—H to 0.86 O—H = 0.82 Å) and $U_{\text{iso}}(\text{H})$ (in the range 1.2–1.5 times U_{eq} of the parent atom), after which the positions were refined with riding constraints.

Figures



Fig. 1. Synthetic Scheme

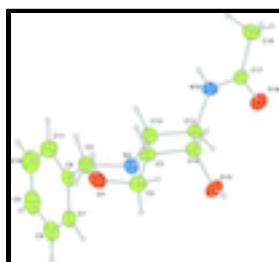


Fig. 2. The title compound with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

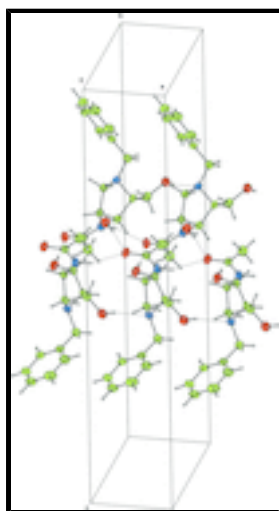


Fig. 3. Packing diagram of the title compound with hydrogen bonds shown by dotted lines.

2-Acetamido-*N*-benzyl-1,4-imino-1,2,4-trideoxy-L-xylitol

Crystal data

$\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_3$

$M_r = 264.32$

Orthorhombic, $P2_12_12_1$

Hall symbol: P 2ac 2ab

$a = 4.9731$ (1) Å

$b = 10.0145$ (3) Å

$F(000) = 568$

$D_x = 1.309$ Mg m $^{-3}$

Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å

Cell parameters from 1650 reflections

$\theta = 5\text{--}27^\circ$

$\mu = 0.09$ mm $^{-1}$

$c = 26.9297$ (7) Å
 $V = 1341.18$ (6) Å³
 $Z = 4$

$T = 150$ K
 Needle, colourless
 $0.50 \times 0.15 \times 0.05$ mm

Data collection

Nonius KappaCCD area-detector
 diffractometer
 graphite
 ω scans
 Absorption correction: multi-scan
 (DENZO/SCALEPACK; Otwinowski & Minor,
 1997)
 $T_{\min} = 0.77$, $T_{\max} = 1.00$
 7494 measured reflections
 1788 independent reflections

1471 reflections with $I > 2\sigma(I)$
 $R_{\text{int}} = 0.040$
 $\theta_{\max} = 27.5^\circ$, $\theta_{\min} = 5.1^\circ$
 $h = -6 \rightarrow 6$
 $k = -12 \rightarrow 12$
 $l = -34 \rightarrow 34$

Refinement

Refinement on F^2
 Least-squares matrix: full
 $R[F^2 > 2\sigma(F^2)] = 0.051$
 $wR(F^2) = 0.130$
 $S = 0.95$
 1788 reflections
 172 parameters
 0 restraints

Primary atom site location: structure-invariant direct
 methods
 Hydrogen site location: inferred from neighbouring
 sites
 H-atom parameters constrained
 Method = Modified Sheldrick $w = 1/[\sigma^2(F^2) +$
 $(0.07P)^2 + 0.9P]$,
 where $P = [\max(F_o^2, 0) + 2F_c^2]/3$
 $(\Delta/\sigma)_{\max} = 0.0003$
 $\Delta\rho_{\max} = 0.33 \text{ e \AA}^{-3}$
 $\Delta\rho_{\min} = -0.46 \text{ e \AA}^{-3}$

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å²)

	<i>x</i>	<i>y</i>	<i>z</i>	$U_{\text{iso}}^*/U_{\text{eq}}$
O1	0.9783 (4)	0.43009 (19)	0.61171 (7)	0.0319
C2	0.7716 (7)	0.4811 (3)	0.58063 (10)	0.0297
C3	0.6606 (6)	0.6164 (3)	0.59663 (10)	0.0231
N4	0.4652 (5)	0.6138 (2)	0.63782 (8)	0.0240
C5	0.5868 (6)	0.5705 (3)	0.68516 (10)	0.0294
C6	0.3890 (6)	0.5636 (3)	0.72762 (9)	0.0260
C7	0.1951 (6)	0.4640 (3)	0.72916 (10)	0.0290
C8	0.0221 (7)	0.4529 (3)	0.76921 (11)	0.0382
C9	0.0372 (7)	0.5432 (4)	0.80805 (11)	0.0431
C10	0.2268 (8)	0.6428 (4)	0.80709 (11)	0.0441
C11	0.4029 (7)	0.6540 (3)	0.76700 (11)	0.0359
C12	0.3731 (6)	0.7532 (3)	0.64027 (10)	0.0262
C13	0.3392 (6)	0.7942 (3)	0.58540 (9)	0.0240

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C14	0.5058 (6)	0.6899 (3)	0.55652 (9)	0.0259
O15	0.3165 (4)	0.6041 (2)	0.53220 (7)	0.0325
N16	0.4213 (5)	0.9324 (2)	0.57677 (8)	0.0258
C17	0.2483 (6)	1.0276 (3)	0.56297 (10)	0.0243
C18	0.3628 (7)	1.1653 (3)	0.55702 (12)	0.0344
O19	0.0046 (4)	1.0055 (2)	0.55648 (7)	0.0301
H22	0.8439	0.4905	0.5468	0.0376*
H21	0.6258	0.4171	0.5801	0.0378*
H31	0.8146	0.6719	0.6070	0.0290*
H51	0.6619	0.4808	0.6798	0.0390*
H52	0.7323	0.6330	0.6949	0.0386*
H71	0.1814	0.4027	0.7021	0.0362*
H81	−0.1097	0.3838	0.7705	0.0516*
H91	−0.0852	0.5371	0.8355	0.0554*
H101	0.2377	0.7039	0.8338	0.0523*
H111	0.5375	0.7242	0.7665	0.0449*
H122	0.2057	0.7596	0.6587	0.0343*
H121	0.5034	0.8116	0.6565	0.0343*
H131	0.1474	0.7860	0.5763	0.0293*
H141	0.6349	0.7323	0.5324	0.0339*
H181	0.2239	1.2282	0.5501	0.0525*
H183	0.4944	1.1658	0.5306	0.0528*
H182	0.4537	1.1924	0.5865	0.0527*
H151	0.3832	0.5671	0.5065	0.0524*
H161	0.5958	0.9521	0.5796	0.0324*
H11	1.0957	0.4903	0.6166	0.0526*

Atomic displacement parameters ($\text{\AA}^2$)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O1	0.0291 (11)	0.0288 (11)	0.0377 (10)	0.0048 (10)	0.0042 (10)	0.0039 (9)
C2	0.0294 (15)	0.0284 (15)	0.0313 (13)	0.0024 (14)	0.0030 (13)	−0.0026 (12)
C3	0.0188 (12)	0.0250 (13)	0.0255 (12)	−0.0023 (12)	0.0046 (11)	−0.0004 (11)
N4	0.0245 (12)	0.0263 (12)	0.0212 (10)	0.0009 (11)	0.0047 (10)	0.0021 (9)
C5	0.0259 (14)	0.0339 (16)	0.0285 (13)	0.0004 (14)	−0.0001 (13)	0.0041 (12)
C6	0.0275 (13)	0.0278 (15)	0.0228 (12)	0.0025 (13)	−0.0014 (12)	0.0050 (11)
C7	0.0293 (15)	0.0328 (16)	0.0250 (12)	0.0011 (13)	−0.0034 (12)	0.0055 (12)
C8	0.0334 (16)	0.050 (2)	0.0309 (14)	−0.0051 (16)	0.0010 (14)	0.0136 (14)
C9	0.0385 (17)	0.063 (2)	0.0279 (14)	0.0076 (19)	0.0069 (14)	0.0106 (15)
C10	0.058 (2)	0.047 (2)	0.0277 (14)	0.007 (2)	0.0017 (17)	−0.0042 (14)
C11	0.0422 (18)	0.0360 (17)	0.0295 (14)	−0.0026 (15)	−0.0002 (15)	−0.0017 (13)
C12	0.0263 (14)	0.0271 (15)	0.0251 (12)	0.0021 (13)	0.0047 (12)	0.0014 (11)
C13	0.0213 (14)	0.0231 (14)	0.0275 (13)	−0.0009 (12)	0.0012 (12)	0.0019 (11)
C14	0.0265 (14)	0.0282 (14)	0.0230 (12)	−0.0031 (13)	0.0035 (13)	−0.0009 (11)
O15	0.0286 (11)	0.0400 (12)	0.0288 (9)	−0.0021 (10)	−0.0029 (9)	−0.0099 (9)
N16	0.0213 (11)	0.0238 (12)	0.0322 (12)	−0.0029 (11)	−0.0003 (10)	0.0021 (10)
C17	0.0248 (13)	0.0254 (14)	0.0228 (12)	−0.0001 (12)	−0.0009 (12)	−0.0023 (11)
C18	0.0349 (17)	0.0257 (15)	0.0427 (16)	−0.0007 (14)	−0.0017 (16)	0.0013 (13)

O19 0.0213 (9) 0.0343 (11) 0.0345 (10) 0.0027 (10) −0.0024 (9) −0.0056 (9)

Geometric parameters (Å, °)

O1—C2	1.420 (4)	C9—H91	0.960
O1—H11	0.850	C10—C11	1.394 (5)
C2—C3	1.525 (4)	C10—H101	0.946
C2—H22	0.984	C11—H111	0.971
C2—H21	0.967	C12—C13	1.543 (4)
C3—N4	1.475 (3)	C12—H122	0.971
C3—C14	1.517 (4)	C12—H121	0.976
C3—H31	0.987	C13—C14	1.543 (4)
N4—C5	1.476 (3)	C13—N16	1.462 (3)
N4—C12	1.471 (4)	C13—H131	0.988
C5—C6	1.510 (4)	C14—O15	1.434 (3)
C5—H51	0.984	C14—H141	1.007
C5—H52	0.992	O15—H151	0.852
C6—C7	1.388 (4)	N16—C17	1.337 (4)
C6—C11	1.396 (4)	N16—H161	0.893
C7—C8	1.384 (4)	C17—C18	1.500 (4)
C7—H71	0.954	C17—O19	1.245 (4)
C8—C9	1.385 (5)	C18—H181	0.954
C8—H81	0.954	C18—H183	0.966
C9—C10	1.373 (5)	C18—H182	0.953
C2—O1—H11	109.5	C11—C10—H101	120.1
O1—C2—C3	114.5 (2)	C6—C11—C10	120.3 (3)
O1—C2—H22	108.4	C6—C11—H111	119.5
C3—C2—H22	108.0	C10—C11—H111	120.2
O1—C2—H21	108.3	N4—C12—C13	104.1 (2)
C3—C2—H21	108.7	N4—C12—H122	110.6
H22—C2—H21	108.9	C13—C12—H122	112.3
C2—C3—N4	115.8 (2)	N4—C12—H121	112.4
C2—C3—C14	114.5 (2)	C13—C12—H121	110.0
N4—C3—C14	102.1 (2)	H122—C12—H121	107.5
C2—C3—H31	107.5	C12—C13—C14	104.1 (2)
N4—C3—H31	107.9	C12—C13—N16	111.9 (2)
C14—C3—H31	108.8	C14—C13—N16	114.2 (2)
C3—N4—C5	112.6 (2)	C12—C13—H131	108.7
C3—N4—C12	102.8 (2)	C14—C13—H131	109.7
C5—N4—C12	111.6 (2)	N16—C13—H131	108.0
N4—C5—C6	113.6 (2)	C13—C14—C3	104.0 (2)
N4—C5—H51	107.2	C13—C14—O15	106.5 (2)
C6—C5—H51	108.5	C3—C14—O15	111.5 (2)
N4—C5—H52	110.0	C13—C14—H141	112.5
C6—C5—H52	107.7	C3—C14—H141	109.9
H51—C5—H52	109.8	O15—C14—H141	112.1
C5—C6—C7	120.5 (3)	C14—O15—H151	112.1
C5—C6—C11	120.9 (3)	C13—N16—C17	122.7 (2)
C7—C6—C11	118.5 (3)	C13—N16—H161	117.8

supplementary materials

C6—C7—C8	120.8 (3)	C17—N16—H161	119.4
C6—C7—H71	119.3	N16—C17—C18	116.2 (3)
C8—C7—H71	119.9	N16—C17—O19	122.6 (3)
C7—C8—C9	120.2 (3)	C18—C17—O19	121.2 (3)
C7—C8—H81	120.9	C17—C18—H181	110.7
C9—C8—H81	119.0	C17—C18—H183	109.9
C8—C9—C10	119.9 (3)	H181—C18—H183	110.0
C8—C9—H91	120.4	C17—C18—H182	110.7
C10—C9—H91	119.7	H181—C18—H182	108.6
C9—C10—C11	120.3 (3)	H183—C18—H182	106.8
C9—C10—H101	119.6		

Hydrogen-bond geometry ($\text{\AA}$, $^\circ$)

$D-H\cdots A$	$D-H$	$H\cdots A$	$D\cdots A$	$D-H\cdots A$
C5—H51 $\cdots$ O1	0.98	2.47	3.111 (4)	123
C14—H141 $\cdots$ O15 ⁱ	1.01	2.56	3.514 (4)	159
O15—H151 $\cdots$ O19 ⁱ	0.85	1.94	2.790 (4)	173
N16—H161 $\cdots$ O19 ⁱⁱ	0.89	2.19	3.041 (4)	159
O1—H11 $\cdots$ N4 ⁱⁱ	0.85	2.29	3.121 (4)	167

Symmetry codes: (i) $x+1/2, -y+3/2, -z+1$; (ii) $x+1, y, z$.

Fig. 1

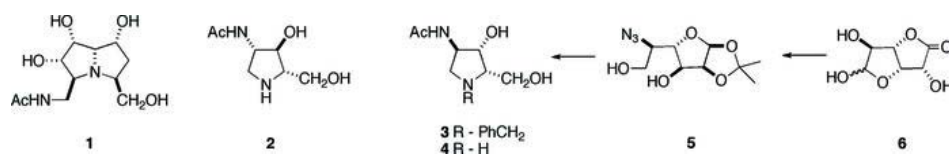


Fig. 2

