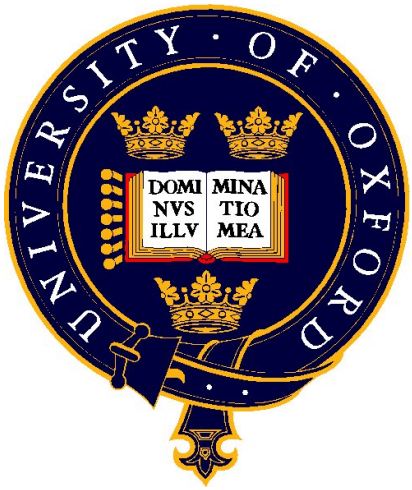
Synthesis and Biological Evaluation of Rare Monosaccharides and Their Mimics



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St John's College, University of Oxford

Hilary Term, 2016

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

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Zilei Liu, St John's College, University of Oxford
Doctor of Philosophy in Biochemistry, Hilary Term, 2016

This thesis describes work in the synthesis and biological evaluation of different carbohydrates and their derivatives.

Azetidine carboxylic acid (Aze), as a pharmacophore in medicinal chemistry, is attracting more and more researchers in recent years. As a four membered ring analogue of proline, this nature occurring compound can be mis-incorporated into proteins and can cause changes in protein conformation and therefore induce toxic effects. It is therefore useful to study how Aze and its derivatives affect protein confirmation. Previous research has identified some azetidine iminosugars to be promising inhibitors of certain glycosidases. Chapter 2 describes the synthesis, from D-glucose, of various novel 3-fluoro-Aze, 3-azido-Aze analogues and a *trans*,*trans*-3,4-difluoro-proline as novel peptidomimetics. Short oligomers of these amino acids were synthesized. A number of 3-fluoro-azetidine iminosugars were obtained to test their inhibition of glycosidases and cancer cell growth. A 3-fluoro-azetidine diol showed significant growth inhibition of several different human cancer cell lines compared with anti-cancer drugs 5-fluorouracil and gemcitabine.

Most rare sugars are still difficult to produce in quantity by either chemical or biotechnological methods. Some of them have interesting activities and potential applications; however, research on these rare sugars is constrained by their high price and inaccessibility. In Chapter 3 an approach to economically and easily produce rare sugars, in high purity, is developed. A number of rare sugars, including L-glucose, D-gulose, D-idose and 6-deoxy-hexoses, were synthesized from cheap D-glucose or its derivatives on large scales and in high yields. Complicated purification methods were avoided. The development of this methodology can contribute not only to the accessibility of these expensive sugars but also to increase their availability for biological evaluation.

The L-rhamnose inducible promoter system (L-rhamnose operon) in *E. coli*. is a commercially available gene expression system which is widely used for various purposes. L-Rhamnose regulates the gene expression of L-rhamnose operon in *E. coli.* Moreover, L-rhamnose processing enzymes play crucial roles in different pathogenic bacteria while they have no role in mammalian metabolism, suggesting L-rhamnose processing enzymes could be potential therapeutic targets. Searching for L-rhamnose analogues, which have induction activity for the L-rhamnose operon, or specific inhibitory activity towards L-rhamnose processing enzymes, is of both commercial and academic interest. In Chapter 4 the synthesis of a large number of L-rhamnose analogues, including 6-deoxy-hexoses and 6-deoxy-iminosugars, from L-rhamnose, is described. Biological assays identified five hexoses as non-metabolizable inducers for the L-rhamnose operon and several inhibitors of L-rhamnosidases and rhamnose isomerases.

Acknowledgement

First of all I would like to thank both Prof George W. J Fleet and Prof Mark Wormald for the opportunity to work with them and study in their labs. Professor Fleet's advice in organic chemistry is invaluable and Prof Wormald has always been kind to answer my questions in both biology and nuclear magnetic resonance technology. As an old Chinese saying goes, 'once a teacher, always a teacher': their valuable advises and spirit of scientific research will guide me to go further in my future career.

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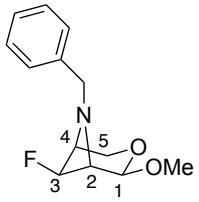
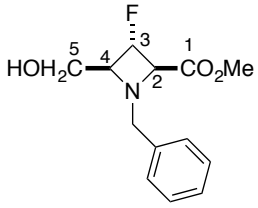
Concerning nomenclature and abbreviations

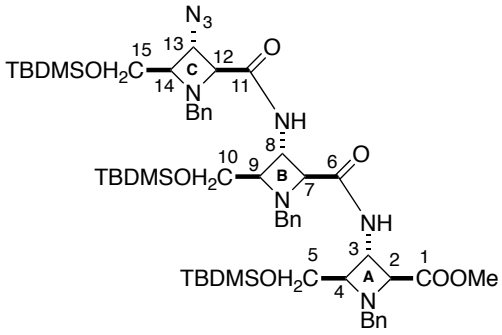
The spectroscopic data of compounds is assigned based upon a numbering scheme derived from the systematic naming of materials according to IUPAC recommendations on carbohydrate nomenclature.[1,2] The carbohydrate rings of oligomeric derivatives of carbohydrate amino acids are identified by labelling each residue alphabetically from 'A'. Examples are given below.



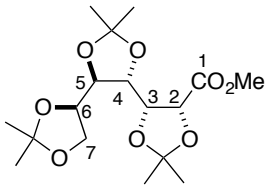
Methyl *N*-benzyl-3-fluoro-2,4-imino-2,3,4-trideoxy-β-L-ribo-pyranoside



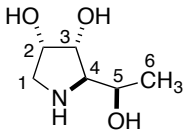
Methyl *N*-benzyl-3-fluoro-2,4-imino-2,3,4-trideoxy-L-ribonate



Methyl *N*-benzyl-3-(*N*-benzyl-8-(13-azido-*N*-benzyl-16-*O*-*(tert*-butyldimethylsilyl)-12,14-imino-12,13,14-trideoxy-L-ribonamido)-10-*O*-*(tert*-butyldimethylsilyl)-7,9-imino-7,8,9-trideoxy-L-ribonamido)-5-*O*-*(tert*-butyldimethylsilyl)-2,4-imino-2,3,4-trideoxy-L-ribonamide



Methyl 2,3:4,5:6,7-tri-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptonate



1,4-Imino-1,4,6-trideoxy-D-allitol

[1] McNaught, A. D.; Nomenclature of carbohydrates. Recommendations 1996. *Carbohydr. Res.* **1997**, 297,1-92

[2] Nomenclature and symbolism for amino acids and peptides. Recommendations 1983. *Eur. J. Biochem.* **1984**, 138, 9-37

Aze	Azetidine carboxylic acid
BBB	blood-brain barrier
CGT	ceramide glucosyltransferases
CMT	Chaperon-mediated therapy
DAB	1,4-Dideoxy-1,4-imino-D-arabinitol
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DFJ	Deoxyfuconojirimycin
DGDP	2,5-Dideoxy-2,5-imino-D-gulitol
DHAP	Dihydroxyacetone phosphate
DIBALH	Diisobutylaluminium hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DMDP	2,5-Dideoxy-2,5-imino-D-mannitol
DMJ	Deoxymannojirimycin
DMP	2,2-Dimethoxypropane
DNJ	Deoxynojirimycin
DTE	D-Tagatose-3-epimerase
ECM	Extracellular matrix
ERT	Enzyme replacement therapy
Gc protein	Serum vitamin D3-binding protein
GcMAF	Protein macrophage activating factor
HBTU	*N*,*N*,*N*',*N*'-Tetramethyl-*O*-(1H-benzotriazol-1-yl)uranium hexafluorophosphate
HMBC	Heteronuclear multiple-bond correlation relationship
HMOs	Human milk oligosaccharides
IC_{50}	Half maximal inhibitory concentration
KDRA	2-Keto-3-deoxy-L-rhamnonate aldolase
LAB	1,4-Dideoxy-1,4-imino-L-arabinitol
lit.	Literature
LPS	Lipopolysaccharides
LSDs	Lysosomal storage disorders
m.p.	Melting point
NMP-I	Non-phosphorylated metabolic pathway I
NMP-II	Non-phosphorylated metabolic pathway II
nOe	Nuclear overhauser effect spectroscopy
PMP	Phosphorylated metabolic pathway
R_f	Retention factor
RhaA	Rhamnose isomerase
RhaB	Rhamnulose kinase
RhaD	Rhamnulose-1-phosphate aldolase
RhaDH	L-Rhamnose-1-dehydrnase
RhaDT	L-Rhamnonate dehydratase
RHJ	Deoxyrhamnojirimycin
RmlA	Glucose-1-phophate thymidylyltransferase
RmlB	dTDP-Glucose-4,6-dehydratase
RmlC	dTDP-Glucose-D-xylo-hexulose 3,5-epimerase
RmlD	dTDP-L-lyxo-6-deoxy-hexulose reductase
RNAP	RNA polymerase
SAR	structure-activity-relationship
sGFP	Superfolder green fluorescent protein
S_N2	Bimolecular nucleophilic substitution
SRT	Substrate reduction therapy
TBAF	*tetra*-*N*-Butylammonium fluoride
TBSOTf	*tert*-Butyldimethysilyl triflate
TLC	Thin layer chromatography
TMSBr	Bromotrimethylsilane
TPPO	Triphenylphosphine oxide
TXNIP	Thioredoxin interacting protein
XI	D-Xylose isomerase
α-GalNAcase	α-*N*-Acetyl-galactosaminidase
β-GlcNAcase	β-*N*-Acetyl-glucosaminidase

Chapter 1 General introduction

This thesis describes the synthesis and biological evaluation of novel monosaccharides (iminosugars and rare sugars) and their derivatives. Three projects are included: i) synthesis of novel azetidine iminosugars, azetidine amino acids and difluoro proline derivatives (Chapter 2); ii) scalable syntheses of rare monosaccharides and derivatives from novel triacetonides of seven carbon sugars (Chapter 3); iii) synthesis of 6-deoxy hexoses, 6-deoxy iminosugars as potent inducers for L-rhamnose promoter system and potent inhibitors of L-rhamnosidase, rhamnose isomerase and other glycosidases (Chapter 4).

1.1 Iminosugars

1.1.1 Glycosidases and related diseases

Glycoside hydrolases, commonly named as glycosidases, are a major family of enzymes that catalyze the hydrolysis of the glycosidic bonds in complex carbohydrates. They are key enzymes in all living organisms relying on the processing of carbohydrates. In mammals, they are found to be vital to a wide range of biological activities including metabolism, immune response and post-transcriptional modifications. The malfunction of glycosidases leads to some serious diseases. Lysosomal storage disorders (LSDs), a group of inherited metabolic disorders, are caused by the deficiency of relevant glycosidases in the lysosome and lead to engorgement and vacuolization in cells and eventually cell death. Some LSDs, such as Sandhoff and Niemann–Pick disease, are fatal and treatments are very limited.[1] Moreover, abnormal glycosylation and secretion of glycosidases are observed in cancer cells and might facilitate cancer development.

Asbell *et al* reported that α-*N*-acetyl-galactosaminidase (α-GalNAcase), abnormally released into extracellular space by cancer cells, is responsible for the cleavage of the terminal *O*-linked *N*-acetyl-galactosamine sugar residue of Serum vitamin D3-binding protein (Gc protein) and further inhibition of the activation of Protein Macrophage Activating Factor (GcMAF) in cancer patients.[2] Some evidence also showed that β-*N*-acetyl-glucosaminidase (β-GlcNAcase) released in the extracellular medium by different cancer cells can digest the extracellular matrix (ECM) to facilitate cancer metastasis.[3] Currently, iminosugars have been the most common tools and candidates to study -and even treat- some diseases related with the malfunction of glycosidases.

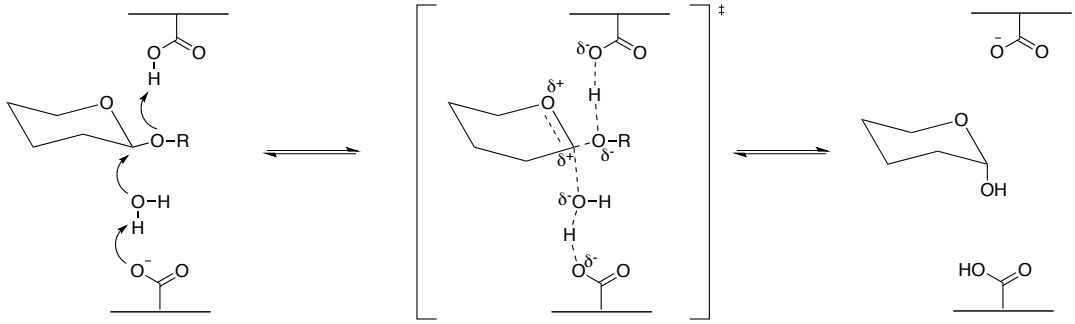
1.1.2 Iminosugars as glycosidase inhibitors

Iminosugars are a type of carbohydrate analogue with endocyclic nitrogen instead of oxygen in the ring (Figure 1.1A). Many protonated iminosugars are capable of inhibiting glycosidases as the transition state mimics (Figure 1.1B).[4] This type of carbohydrate analogue widely exists in various microorganisms, plants and animals and profoundly affects most living organisms. In the last decade, their importance has been recognized and their biological properties and synthesis have been intensively studied. According to their structures, they can be classified into five major types: pyrrolidine, piperidine, indolizidine, pyrrolizidine and *nor*-tropane.

A

Sugars **Iminosugars**

B **Mechanism of inverting glycosidases**



Mechanism of retaining glycosidases

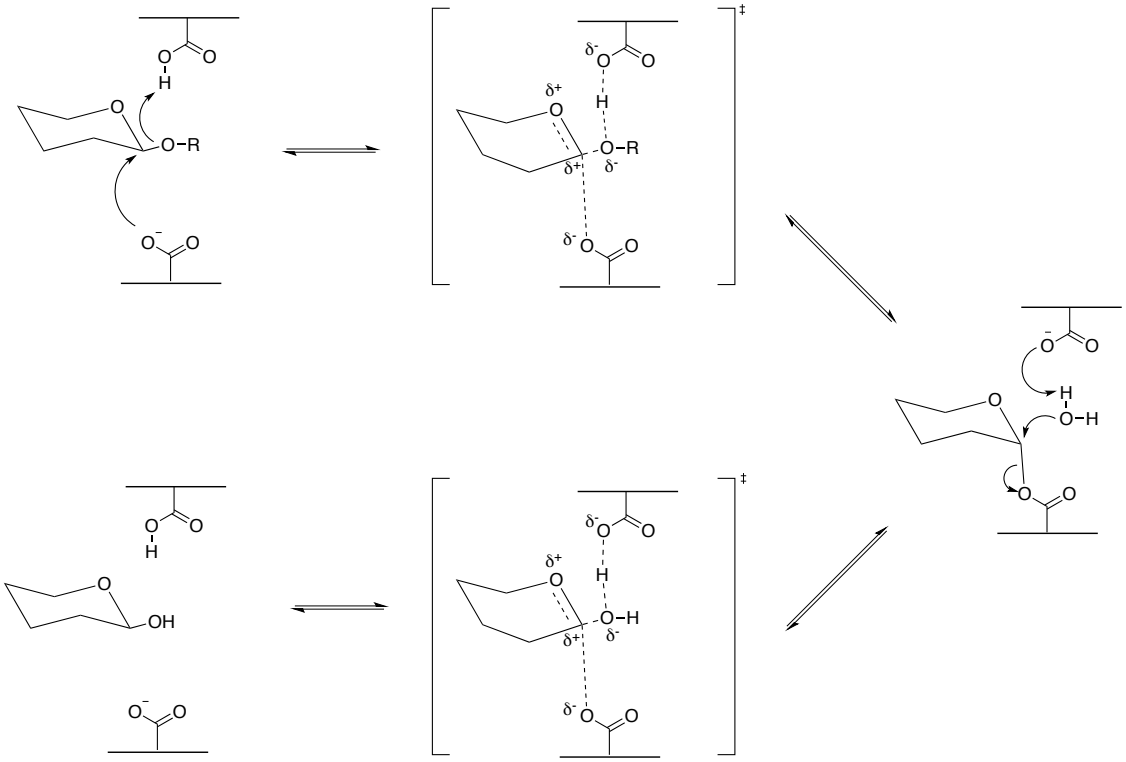


Figure 1.1 **A** Sugars and iminosugars; **B** Mechanism of inverting and retaining glycosidases

1.1.2.1 Piperidines

The first iminosugar, nojirimycin **1.2**, was isolated from *Streptomyces* as a novel antibiotic in the 1970s[5] (Figure 1.2). This polyhydroxylated piperidine equivalent of glucose is a competitive inhibitor of α- and β-glucosidases.[6] In the following years, more naturally occurring piperidine iminosugars were discovered including nojirimycin B **1.5** as an inhibitor of α-mannosidase[7] and β-galatcostatin **1.4** as an inhibitor of β-galactosidase.[8] The removal of the anomeric hydroxyl group does not destroy the bioactivity of Nojirimycin **1.2**. 1-Deoxynojirimycin (DNJ) **1.3**, as the 1-deoxy analogue of nojirimycin **1.2**, was fist synthesized by the reduction of nojirimycin **1.2** in 1968[9] and was also isolated from microorganisms and plants as a potent inhibitor of α-glucosidase.[6, 10] Other 1-deoxy analogues of iminosugars, including deoxymannojirimycin (DMJ) **1.6** and deoxyfuconojirimycin (DFJ) **1.7**, were made or discovered in the following years. Their inhibitory profiles were intensively explored.[6] DMJ **1.6** is a potent inhibitor of α-L-fucosidase but a weak inhibitor of α-D-mannosidase.[11] This initial research provides valuable information for understanding glycosidases and the design of novel specific glycosidase inhibitors.

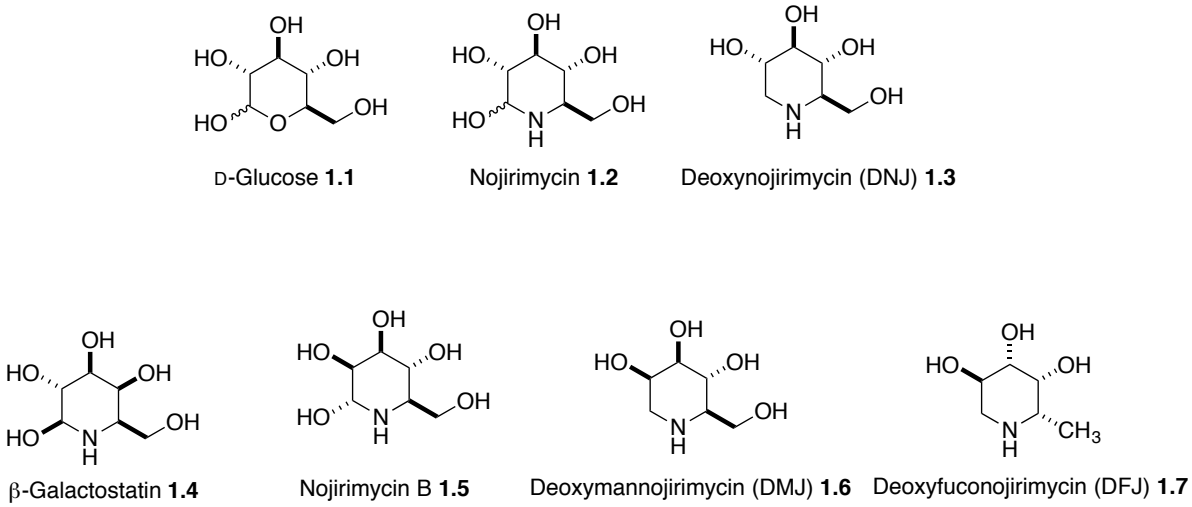


Figure 1.2 Examples of piperidine iminosugars

1.1.2.2 Pyrrolidines

Pyrrolidine iminosugars are also common in nature (Figure 1.3). Some of them are powerful glycosidase inhibitors. 2,5-Dideoxy-2,5-imino-D-mannitol (DMDP) **1.8**, a naturally occurring five membered ring iminosugar isolated from the leaves of *Derris elliptica*,[12] shows good inhibition of mammalian α-glucosidases[13] and human α-, β-glucosidases.[14] This is an interesting case of pyrrolidine iminosugars inhibiting glycosidases processing pyranose sugars. Since the first synthesis of DMDP **1.8** from L-sorbose was achieved in 1985,[15] a number of methods for its synthesis from carbohydrates and non-carbohydrates have been developed.[16] It's enantiomer L-DMDP **1.9**, which was first synthesized from D-tartrate[17], was found to be a more powerful inhibitor of α-D-glucosidases than DMDP **1.8**.[18] 1,4-Dideoxy-1,4-imino-D-arabinitol (DAB) **1.10** is a rare example of a naturally occurring iminosugar that inhibits yeast glycosidases.[19] Its enantiomer LAB **1.11** also showed potent competitive inhibition of yeast α-glucosidase.[20] The syntheses of DAB **1.10** and LAB **1.11** were first reported in 1986.[21]

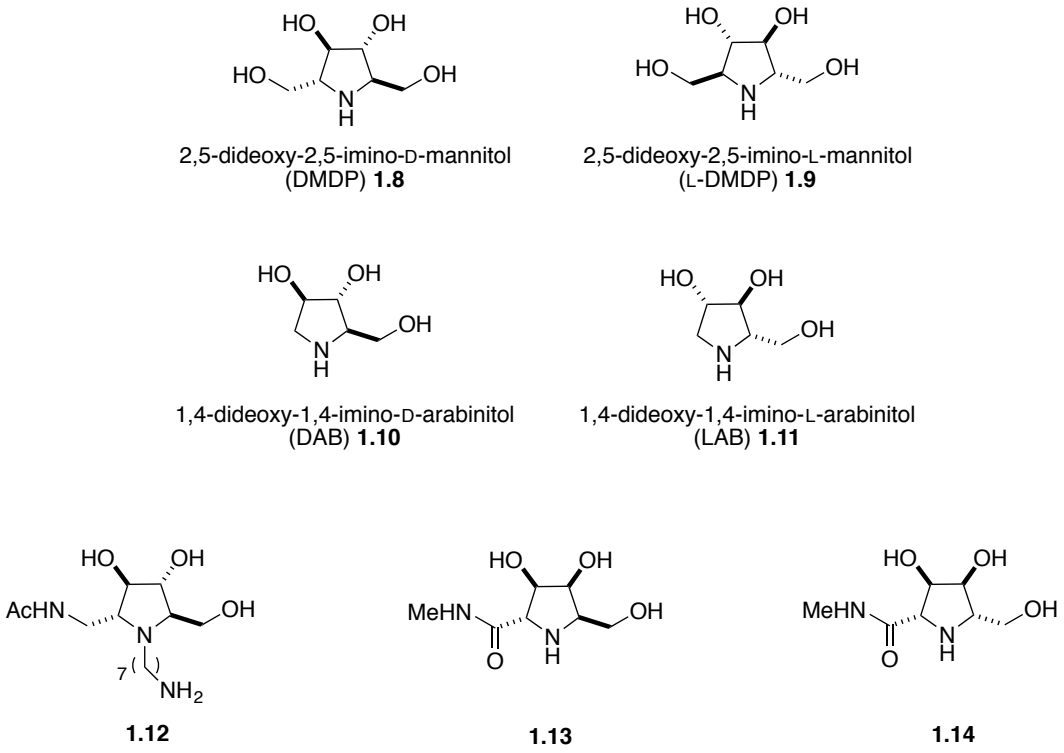


Figure 1.3 Examples of pyrrolidine iminosugars

More recently, the DMDP analogue **1.12** was synthesized by Wong *et al* and was found to be a very potent competitive inhibitor of β-*N*-acetyl-hexosaminidase (IC_{50} 2.6 nM).[22] Later a series of pyrrolidine iminosugars were synthesized, including **1.13** and **1.14** with acetamido side chains, **1.13** and **1.14** showed potent inhibition of β-*N*-acetyl-hexosaminidase (IC_{50} 0.3 – 10 μM).[23]

1.1.2.3 Indolizidines

Research on indolizidine iminosugars began with the discovery of swainsonine **1.15**, an indolizidine alkaloid isolated from *Swainsona canescens* in Australia.[24] (Figure 1.4) Swainsonine **1.15** was found to decrease lysosomal α-mannosidase function and affect the processing of glycoproteins causing toxic effects in livestock and humans.[24-25] Horisberger *et al.* showed that swainsonine **1.15** prevented the formation of complex glycoproteins in influenza virus.[26] As a potent lysosomal α-mannosidase inhibitor, swainsonine **1.15** has also been used as a tool for

understanding the mechanism of LSDs since the 1980s.[27] As shown in Figure 1.4, swainsonine **1.15** can be seen as a fusion of one piperidine ring and one pyrrolidine ring. Although the synthesis of swainsonine from D-mannose and D-glucose have been reported in 1984,[28] interest in the synthesis of swainsonine **1.15** and its analogues is still going.[29] Castanospermine **1.16** is another naturally occurring indolizidine iminosugar isolated from the seeds of *Castanospermum austral*.[30] It is a potent inhibitor of lysosomal α- and β-glucosidase and β-glucocerebrosidase.[31] This can be understood by the conformationally restricted polyhydroxylated piperidine moiety in castanospermine **1.16** mimicking glucose to interact with the corresponding glycosidases (Figure 1.4).

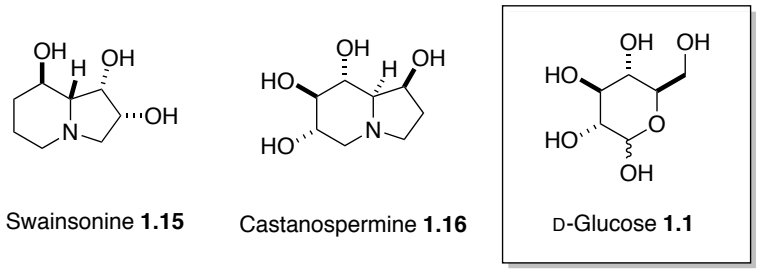


Figure 1.4 Examples of indolizidine iminosugars

1.1.2.4 Pyrrolizidines

One of the most well-known pyrrolizidine iminosugars is australine **1.17**, an amyloglucosidase inhibitor isolated from *Castanospermum austral*.[32] It can be considered as a ring-contracted version of castanospermine **1.16** containing a DMDP **1.8** moiety (Figure 1.5). The biological activity of australine **1.17** and its analogues were explored in 2003. **1.18** is a stronger and more specific inhibitor of α-glucosidases (IC_{50} 2 – 3 μM) than australine **1.17** (IC_{50} > 200 μM).[33] The synthesis of australine analogues has been intensively studied in the last two decades.[34] Notably, fluorinated and difluorinated of australine derivatives/analogues (**1.19** and **1.20**, Figure 1.5)

gave a significantly increase in inhibition of α-glucosidase.[35] This is a rare case of fluorination of iminosugars enhancing glycosidase inhibition; usually introduction of fluorine leads to loss of glycosidase inhibition.[4]

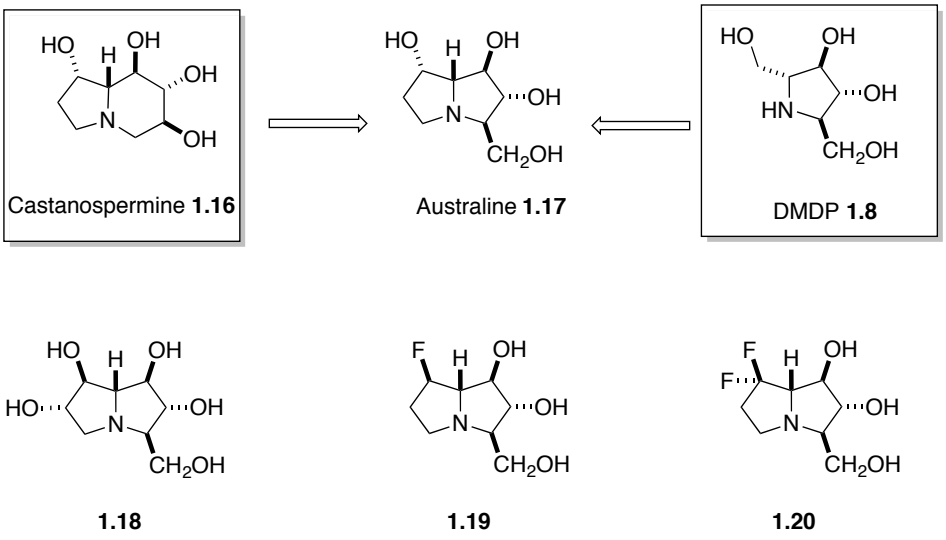
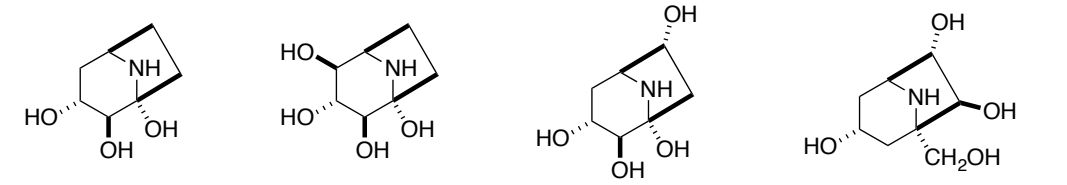


Figure 1.5 Australine **1.17** and its derivatives

1.1.2.5 *Nor*-tropanes

Calystegines are a group of polyhydroxylated *nor*-tropane alkaloids discovered as plant secondary metabolites.[36] (Figure 1.6) Many members of the calystegine family, e.g. calystegine A_3 **1.21**, calystegine B_2 **1.22** and calystegine B_1 **1.23**, can competitively inhibit various glycosidases including glucosidases and galatosidases.[37] Atsushi *et al.* recently examined the structure activity relationship of calystegines against β-glucocerebrosidase to show that the configuration of the hydroxyl groups on the *nor*-tropane rings was essential to the inhibition against β-glucocerebrosidase.[38] In the same report, 3(*S*)-hydroxy-labystegine **1.24**, which can be considered as a hybrid of LAB **1.11** and calystegine B_2 **1.22**, showed good inhibition of maltase (IC_{50} 0.15 μM), isomaltase (IC_{50} 2.8 μM) and sucrose (IC_{50} 0.18 μM).



Calystegine A_3 **1.21** Calystegine B_2 **1.22** Calystegine B_1 **1.23** 3(*S*)-Hydroxy-labystegine **1.24**

Figure 1.6 Example of *nor*-tropane iminosugars

1.1.3 Iminosugars as therapeutic agents

The potency of iminosugars in the area of medicine and drug-discovery has been recognized since the discovery of potential of DNJ in the treatment of diabetes.[39] Because of the diversity of the biological properties of iminosugars, they are studied and used in the treatment of a variety of ailments including LSDs, cancer, diabetes mellitus and virus infections (Figure 1.7).

As mentioned above, LSDs are caused by the malfunction of lysosomal glycosidases and result in metabolic disorders. Enzyme replacement therapy (ERT), involving the direct introduction of purified enzymes, bone marrow transplantation or gene delivery, is a well-established treatment for several LSDs including type I Gaucher disease and Fabry disease.[40] However, the inability of recombinant enzymes to cross the blood-brain barrier (BBB) and the high costs are the main drawbacks of ERT. More recently, two novel therapy approaches using iminosugars have been developed.[41] Substrate reduction therapy (SRT) is a strategy aimed to partially inhibit the biosynthesis of the substrates (usually glycolipids) of malfunctioning glycosidases and hence reduce the abnormal substrates accumulation in lysosome. The other therapy is Chaperon-mediated therapy (CMT) using iminosugars to work as pharmacological chaperones to induce the mutant glycosidases to fold and function correctly.[42] *N*-Butyl-DNJ (brand name

Zavesca) **1.26** inhibits ceramide glucosyltransferases (CGT) and has been used in SRT treatment of LSDs including type I Gaucher disease and Niemann-Pick type C disease.[43] *N*-nonyl-DNJ **1.27** was studied in CMT treatment of Gaucher disease as a β-glucosidase inhibitor. The therapeutic potential of *N*-butyl-DGJ **1.28** was also explored in both SRT (as a CGT inhibitor)[44] and CMT (as a α-galactosidase inhibitor)[45] strategies.

Iminosugars have also been studied for the treatment of other diseases. *N*-Hydroxyethyl-DNJ (Miglitol, brand name Glyset) **1.25** was the first marketed iminosugar drug for the treatment of diabetes.[46] Zavesca **1.26** was also found to inhibit the infection of HIV *in vitro* by affecting the formation of the viral envelope glycoprotein.[47] Unfortunately, treatment with Zavesca **1.26** is accompanied by serious side effects which limit its further application.[42a] Celgosivir **1.29**, a prodrug of Castanospermine **1.16** (Figure 1.4), was reported to reduce HIV activity[43a, 48] and is currently in clinical trials against dengue fever.[49] Swainsonine **1.15** (Figure 1.4) inhibits cancer cell metastasis but showed significant side-effects due to its cytotoxicity.[50] In summary, the family of iminosugars is a promising pool of drug candidates because of its simple structures and various biological activities. Nevertheless, the side effects in several cases caused problems and a new generation of iminosugars is required.

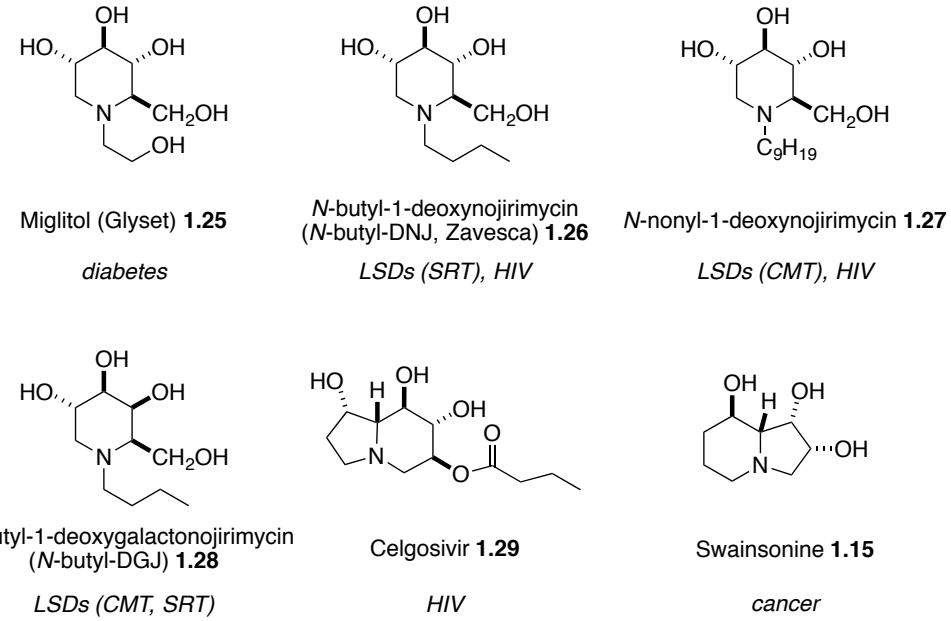


Figure 1.7 Examples of iminosugars as therapeutic agents

1.2 Rare sugars

A rare sugar is defined as a monosaccharide that exists in low abundance in nature. Normally rare sugars are difficult to access. Interest in the biological properties of rare sugars as low calorie sweeteners and the observation of unexpected biological activity in some rare sugars has lead to the need to access large amounts of rare sugars. The occurrence, application and synthesis of rare sugars is introduced in this section.

1.2.1 Occurrence of rare sugars in nature

Compared with D-sugars, most of L-sugars and their derivatives are not common in nature. The occurrence and importance of rare sugars in living organisms has long been recognized. The L-glucoside containing compound, Littoralisone **1.30**, was isolated from a traditional medicine, *Verbena Littoralis*.[51] L-Mannose **1.33** was found in the capsular polysaccharide S-88 **1.32** of bacteria *Sphingomonas*.[52] Kondakova *et al*. reported the lipopolysaccharide (LPS) of *Proteus*

vulgaris contained 2-acetamido-2,6-dideoxy-L-mannose (*N*-acetyl-L-rhamnosamine).[53] Alginates **1.34** (Figure 1.8), anionic polysaccharides containing L-guluronate moieties, are widely distributed in the cell walls of *brown algae* and other seaweeds.[54]

Besides L-sugars, many D-sugars and their derivatives are not common in nature either. For example, (−)-*epi*-α-bisabolol 6-deoxy-β-D-gulopyranoside **1.38** (Figure 1.8) was first isolated and identified from the glandular trichome exudate of *Brillantaisia owariensis* in 2012.[55] The 6-Deoxy-D-gulopyranose unite **1.39** was also found in Canescein **1.40** isolated from *Erysimum canescens* and *E. suffruticosum*.[56] D-Allose **1.37** was found in Rubropilosin **1.36** isolated from the leaves of *P.rubropilosa*.[57].

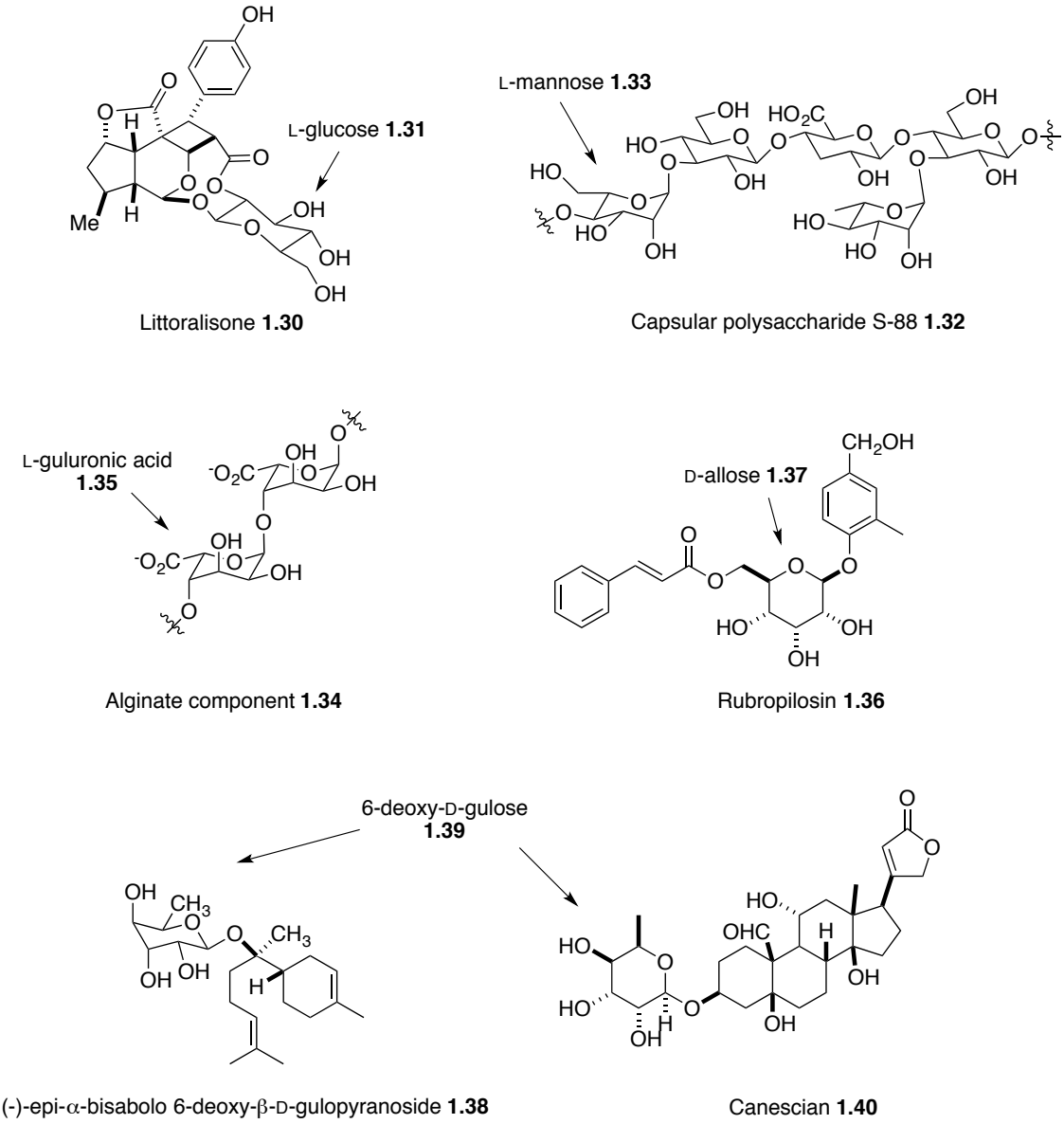


Figure 1.8 Examples of nature products containing rare sugars

1.2.2 Applications of rare sugars

Although occurring in low quantities, rare sugars have been used for the production of various useful chemicals. For example, the glycosylation of bentulinic acid by L-galactose **1.41** was reported to improve the divergence of its anti-tumor and anti-HIV properties.[58] L-Galactose **1.41** was also used for the synthesis of macrocyclic sialyl Lewis mimic 2.[59] O'Reilly *et al*. reported the total synthesis of a constrained polyfunctional bicyclic iminocyclitol scaffold from L-sorbose

1.42.[60] (Figure 1.9)

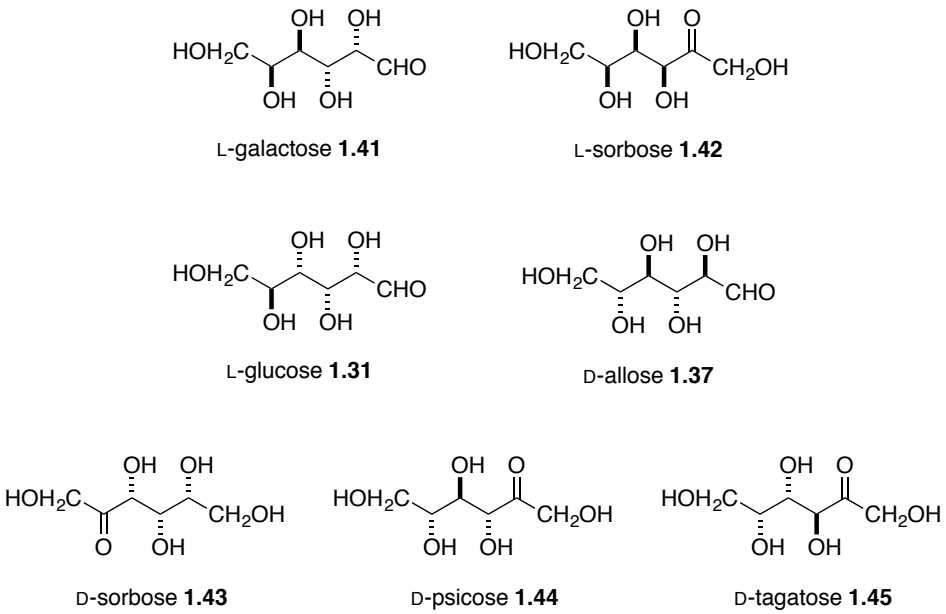


Figure 1.9 Examples of rare sugars used in chemical and biological studies

Rare sugars are not only useful in chemical synthesis but also have interesting biological properties. The inhibitory activity of D-allose **1.37** (Figure 1.9), the 3-epimer of D-glucose, towards cancer cell proliferation *in vitro* was reported in 2005.[61] This simple monosaccharide was also found to induced G1 cell cycle arrest by up-regulation of thioredoxin interacting protein (TXNIP) on gene expression.[62] Interestingly, its anti-oxidative effects, liver and renal protection effects in rats have also been discovered/discussed in the last decade.[63] In recent reports, dietary D-sorbose **1.43** was found to significantly lower serum insulin levels in rats[64], suggesting the possibility of D-sorbose **1.43** as a new dietary sweetener for preventing diabetes. D-Psicose **1.44**, which has 70% of the sweetness of sucrose, is a zero-calorie sweetener.[65] A series of its health benefits, including the prevention of diabetes and the control of fat accumulation, have been reported.[66] A successful market for dietary D-psicose **1.44** has been established in Japan. The U. S. Food and Drug Administration (FDA) approved D-psicose **1.44** as

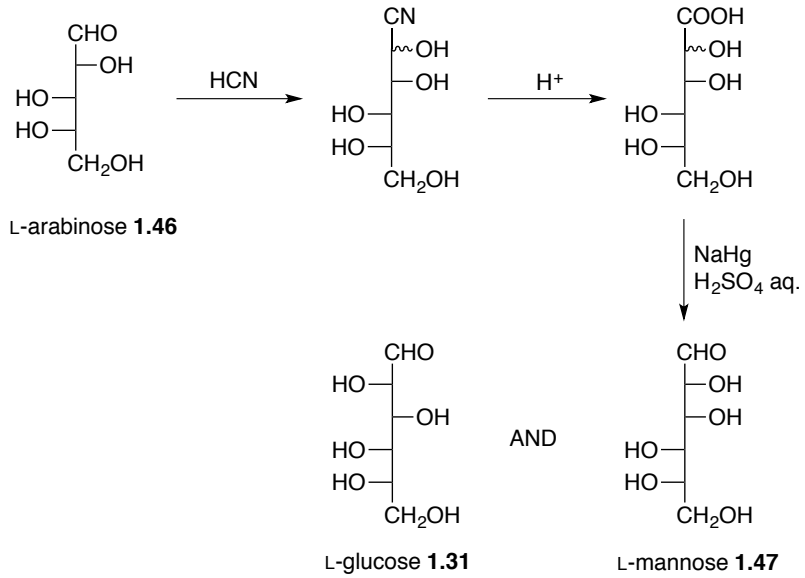
a generally recognized as safe (GRAS) product. Similarly, another sweetener, D-tagatose **1.45**, which only presents in small amounts in plants, was approved as a GRAS product in 2001.[67] Accordingly, the potential market of rare sugars has been recognized with public's growing concerns of dietary related diseases in recent years. Unfortunately, the efficient production of most rare sugars is limited and therefore many rare sugars remain expensive.[68]

1.2.3 Production of rare sugars

1.2.3.1 Chemical synthesis

Organic chemists have been pursuing simple and generalizable approaches for producing sugars and their derivatives since the dawn of carbohydrate chemistry. The Kiliani-Fischer synthesis is the classic approach for accessing various D- and L- sugars (Scheme 1.1). In the 1890s, Kiliani identified the formula of arabinose, a pentose isolated from plants, and found HCN could be added to arabinose to form a mixture of six carbon sugars containing 'unnatural' sugar components.[69] This Kiliani chain homologation method was further developed by Fischer: the addition of cyanide to arabinose, followed by the hydrolysis to the corresponding acids and reduction with sodium amalgam gave glucose and its 2-epimer mannose.[70] This approach was the main method of synthesizing unnatural L-glucose **1.31** and L-mannose **1.47** from L-arabinose **1.46** in the next decades (Scheme 1.1). In 1942, Sowden and Fischer modified this method by using nitromethane instead of cyanide.[71] Latterly, a modified Kiliani approach involving the reduction of cyanides by hydrogenation catalysed by palladium on barium carbonate was used to produce various rare sugars and their derivatives.[72] However, there remain problems: the chain extension gives a mixture of 2-epimers that are difficult to separate;

the yields of some rare sugars are low; reagents including cyanides are toxic and not environmental friendly.



Scheme 1.1 Synthesis of L-glucose and L-mannose by Kiliani-Fischer synthesis

In the following years, much effort was made to develop general procedures to synthesize rare sugars from carbohydrates or non-carbohydrates. In 1983, Sharpless reported the syntheses of a range of L-hexoses from 4-benzhydroxy-(*E*)-but-2-ene-1-ol **1.48** using asymmetric epoxidation as the key reaction (Figure 1.10A).[73] Enantioselective dihydroxylation has also been used to access L-hexoses by different groups.[74] Hasehira *et al*. used a fluorescence-labelling reagent, 2-aminopyridine **1.50**, as a Schiff-Base to promote the isomerization of ketohexoses to the corresponding aldohexoses however HPLC was required for the purification of the products (Figure 1.10B).[75] Recently, Frihed *et al*. reported an approach to produce eight L-glycopyranosyl donors *via* C-H activation of their corresponding 6-deoxy-L-hexoses on a gram scale, which was

particularly useful for the further functionalization and glycosylation of these L-sugars (Figure 1.10C).[76]

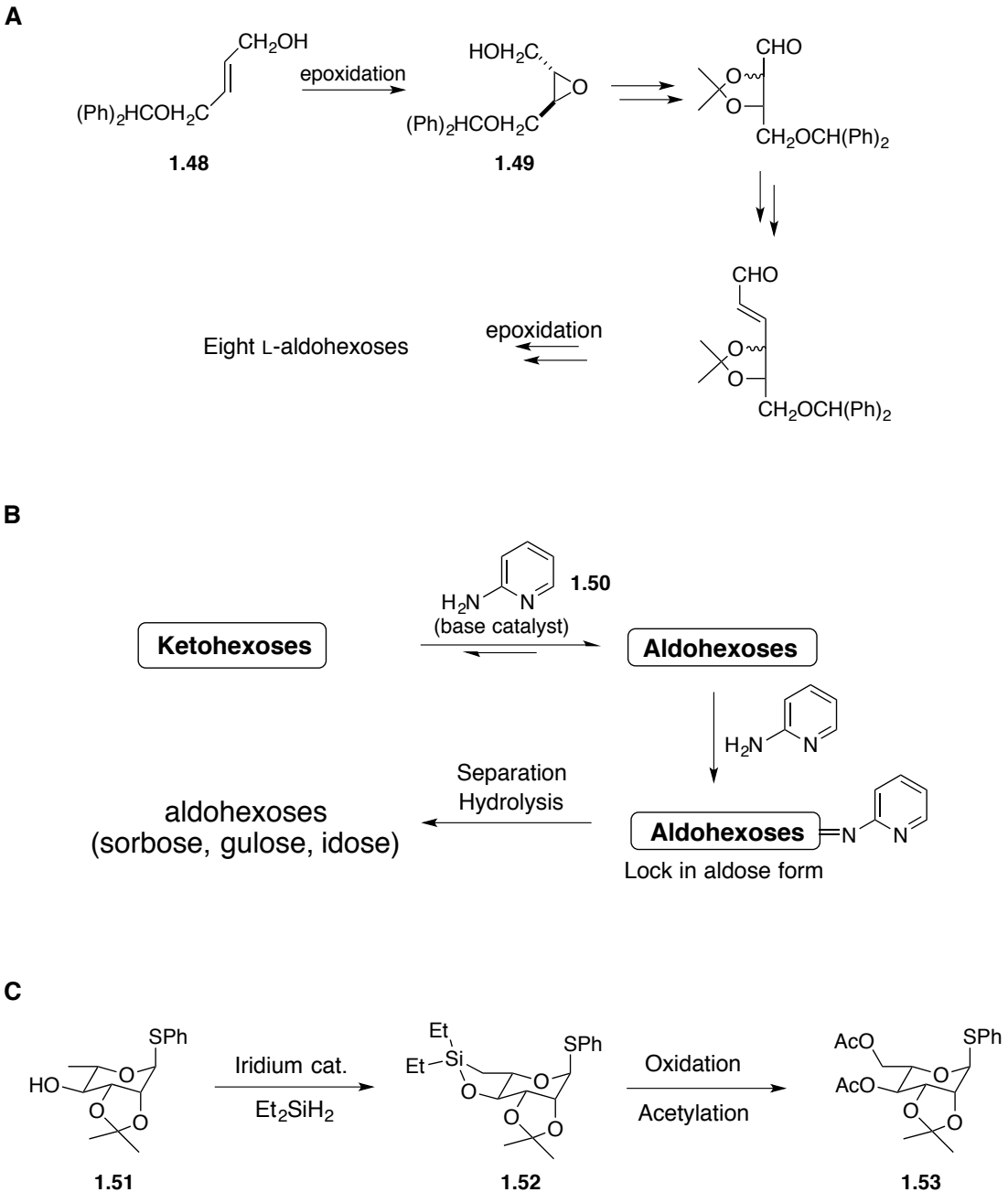


Figure 1.10 **A** Synthesis of L-hexoses *via* Sharpless epoxidation; **B** Production of aldohexoses using 2-aminopyridine; **C** Synthesis of L-glycopyranosyl donors *via* C-H activation

1.2.3.2 Biotechnological production of rare sugars

Enzymatic approaches are particularly powerful for the production of rare sugars and have been developing fast since the beginning of 21th century. Izumori's group has explored this area since the 1980s and several useful enzymes have been identified and developed as powerful tools. For example, D-tagatose **1.45** was made from D-galactitol **1.54** by microbial cells containing oxidoreductases which oxidize hexitols to ketohexoses (Figure 1.11).[77] Bioproduction of several rare hexitols including D-talitol **1.55** and D-iditol **1.56** by microbial cells were also explored.[78] On a large scale, ketoses are usually hydrogenated to give two epimeric hexitols. Therefore separation is required. In 1994, the same group purified and identified D-tagatose-3-epimerase (DTE) from *Pseudomonas sp*.[79] DTE is able to catalyze the epimerization of all ketohexoses at the C-3 position, accessing all of the ketohexoses. This enzyme presented a simple way to connect all ketohexoses. With those tools, D-sorbose **1.43**, D-psicose **1.44**, L-fructose **1.57** and L-tagatose **1.58** were produced in large quantities. Notably, this method significantly reduced the price of D-psicose and made D-psicose commercially available as a safe food in Japan.

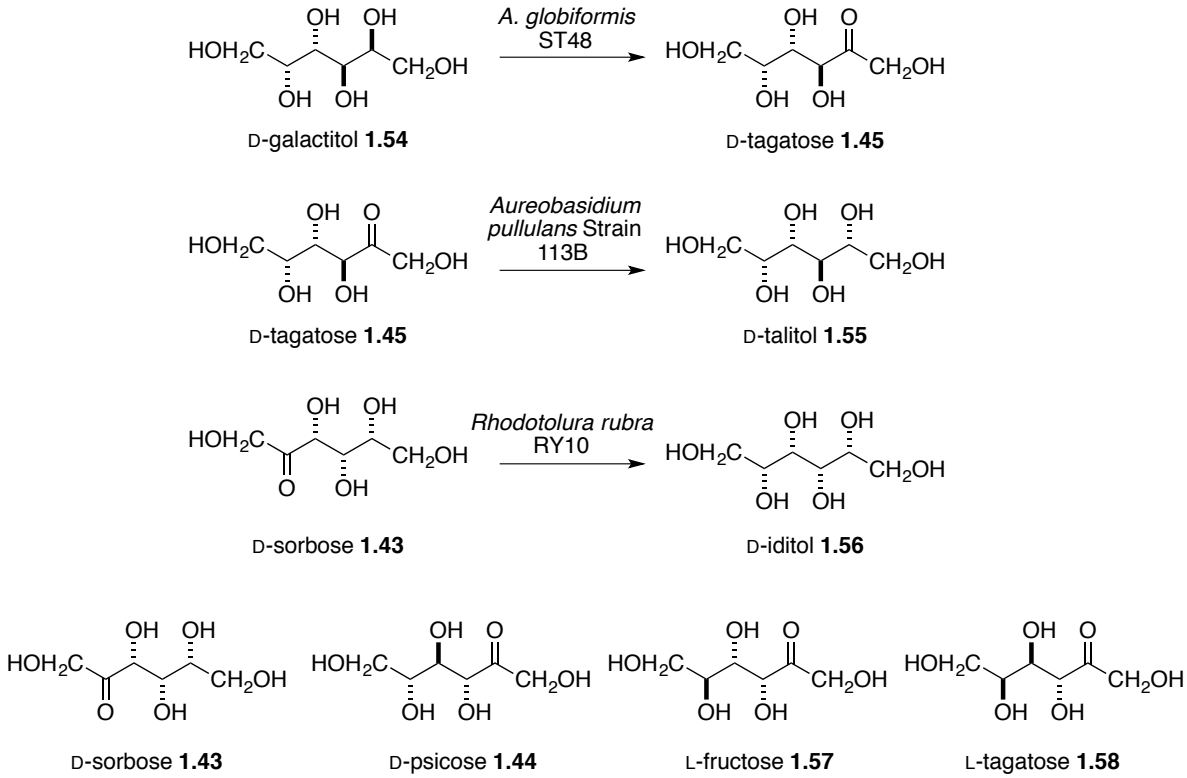


Figure 1.11 Examples of rare sugars produced by biotechnological approaches

A milestone was made in 2002 when Izumori proposed a biotechnological strategy for production of all hexoses.[80] Together with their following research, this strategy, named Izumoring, was successfully developed for the production of all hexose sugars and corresponding hexitols.[81] Three enzymatic tools are essential for the construction of Izumoring (Figure 1.12): i) DTE (converting ketohexose to 3-epimeric ketohexose); ii) oxidoreductases (converting ketohexose to hexitols); iii) aldose isomerases (converting aldohexoses to corresponding ketohexoses). There are three entrances to 'L-world' from 'D-world': D-gulitol/L-glucitol, D-glucitol/L-gulitol, galactitol and allitol. All ketohexoses, aldohexoses and hexitols are connected in an Izumoring map.[81a] Therefore expensive hexoses can be accessed from cheap sugars. The mass production of D-psicose **1.44** and D-tagatose **1.45** have been established using this methodology. Recently, the biotechnology of Izumoring was further developed: ten of the sixteen 1- or 6-deoxyketohexoses were produced from cheap L-rhamnose

(6-deoxy-L-mannose) by the treatment of DTE in water.[82]

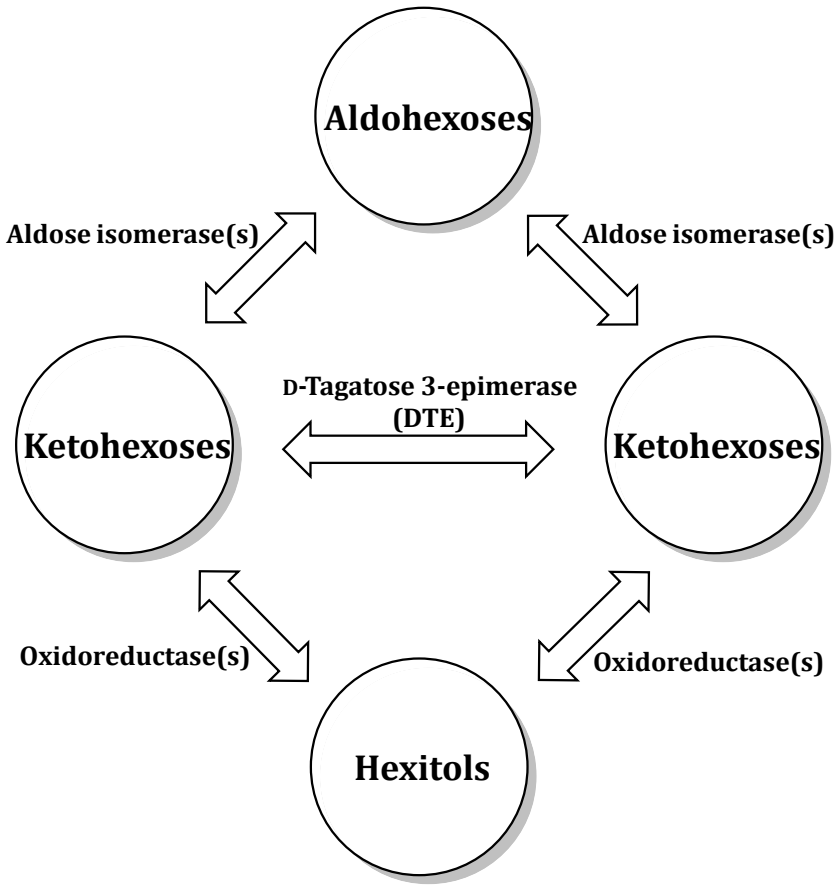


Figure 1.12 Izumoring strategy

Although the biotransformation of cheap sugars to expensive rare sugars shows exciting potential, there remain problems: some rare sugars such as L-glucose **1.31** and D-sorbose **1.43** are still expensive due to the complicated purification and production procedures; and the mass production of some rare sugars is still a challenge. Accordingly, chemical production of rare sugars with simple procedures and cheap materials is still required.

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Chapter 2 Synthesis of novel azetidine iminosugars, azetidine amino acids and difluoro proline derivatives

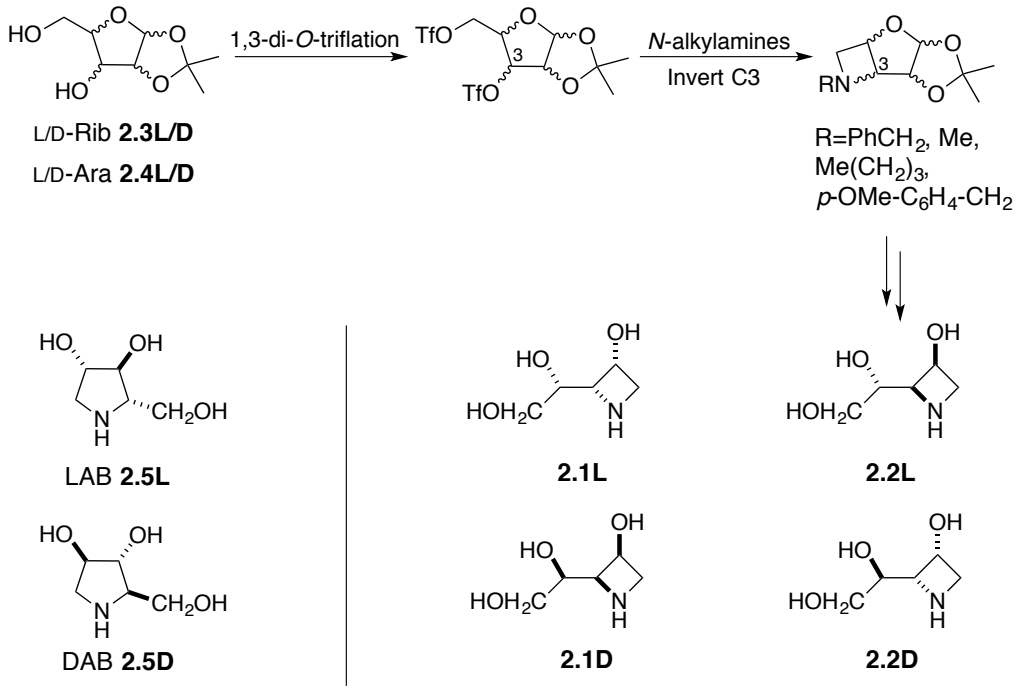
2.1 Introduction

2.1.1 Synthesis of novel azetidine iminosugars as potential glycosidase inhibitors

The development of novel iminosugars as specific glycosidase inhibitors has been actively pursued in carbohydrate chemistry for decades. Replacement of individual hydroxyl groups in iminosugars by alkyl/hydroxyalkyl/halogen/amide/acid with retention or inversion is a general strategy of the design of bioactive iminosugars.[1] Other strategies include *N*-alkylation, ring expansion and ring contractions.[2] Iminosugars with various ring sizes and substitutions have been synthesized and their structure-activity-relationship (SAR) was studied. However, studies on azetidine iminosugars are limited.

A convenient strategy for the synthesis of four 1,3-dideoxy-1,3-imino-pentitols **2.1L/D**, **2.2L/D** and their *N*-alkylated analogues was developed from the 3,5-di-*O*-triflation of corresponding protected pentoses **2.3L/D**, **2.4L/D** followed by the double S_N2 displacements by various primary alkylamines (Scheme 2.1).[3] Glycosidase inhibition studies confirmed 1,3-dideoxy-1,3-imino-L-xylitol **2.1L** was a specific inhibitor of *A. niger* amyloglucosidase (IC_{50} 25 μM) while its enantiomer **2.1D** showed no inhibition. Also, **2.2L** as an isomer of LAB **2.5L**, a specific glucosidase inhibitor, is a potent inhibitor of *A. niger* α-glucosidase (IC_{50} 39 μM), rat

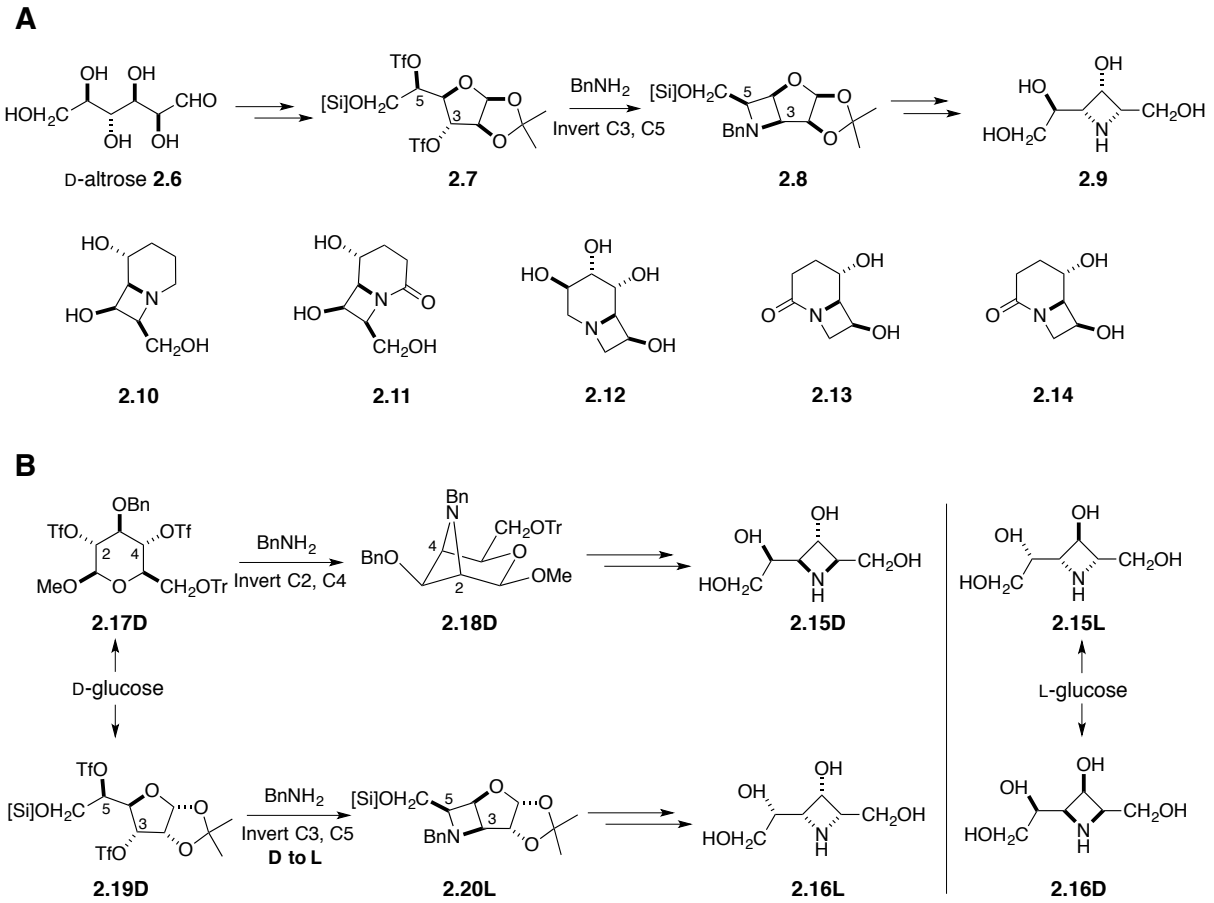
β-galactosidase (IC_{50} 70 μM) and *Rhizopus sp* amyloglucosidase (IC_{50} 19 μM). Its enantiomer **2.2D**, as an isomer of DAB **2.4D**, is a weak inhibitor of rat β-galactosidase (IC_{50} 241 μM).[3]



Scheme 2.1 Synthesis of azetidine **2.1L/D** and **2.2L/D** by a double S_N2 displacement of 3,5-di-*O*-triflates

The synthesis of novel azetidine iminosugars by a similar strategy emerged in the following years. Araujo *et al*. employed the 3,5-di-*O*-triflation of diacetonide D-altrose **2.6** (Scheme 2.2A), followed by displacement by benzylamine to synthesize 2,4-dideoxy-2,4-imino-D-glucitol **2.9**, two bicyclic swainsonine analogues **2.10** and lactam **2.11**. **2.9** does not inhibit any glycosidase while **2.10** and **2.11** are weak inhibitor of β-galactosidase (IC_{50} 492 μM and 341 μM respectively).[4] More bicyclic azetidines **2.12**, **2.13** and **2.14** were synthesized and their SAR was investigated (Scheme 2.2A).[5] This strategy involving the double S_N2 displacement of di-*O*-triflates was further developed to synthesize two pairs of azetidine enantiomers **2.15L/D** and **2.16L/D** from the 3,5-di-*O*-triflate of D/L-glucofuranosides and 2,4-di-*O*-triflate of D/L-glucopyranosides respectively (Scheme 2.2B).[6] The novel bicyclic azetidine **2.18D**, derived from ditriflate **2.17D**, could be efficiently hydrolyzed to corresponding lactol, the following

opening of pyranose ring allowed the formation of corresponding azetidine **2.15D**. Bioassays showed the *N*-benzyl derivative of **2.16D** was a potent inhibitor of rice and rat intestinal maltase with IC_{50} 27 μM and 71 μM respectively. **2.15L** is a moderate inhibitor of rice and rat intestinal maltase (IC_{50} 481 μM and 176 μM respectively) and **2.16L** weakly but selectively inhibits α-galactosidase (IC_{50} 416 μM).[6]



Scheme 2.2 **A** Synthesis of azetidine iminosugar **2.9** and bicyclic iminosugars **2.10** – **2.14** using a double S_N2 displacement strategy; **B** Synthesis of azetidine iminosugars **2.15L/D** and **2.16L/D** using a double S_N2 displacement strategy

In the following research, the hydrolysis of two bicyclic azetidines **2.18D** and **2.21** from protected D-glucose was followed by either reduction or oxidation leading to azetidine iminosugars **2.22**, **2.23L/D** and a pair of non-proteinogenic amino acids **2.24L/D** with *trans*,*trans*-configurations (Figure 2.13A).[7] *meso*-Azetidine triol **2.22** is one rare case of specific yeast α-glucosidase inhibitor (IC_{50} 9.5 μM). The *N*-nonyl analogue of **2.22**, a specific inhibitor of

ceramide glucosyltransferases (IC_{50} 44 μM), was previously synthesized by an approach involving Sharpless epoxidation, epoxide ring opening and recyclization.[2c] Azetidine amide **2.23L** is a good specific inhibitor of β-hexosaminidases (IC_{50} 1.4 – 48 μM). The amide **2.23L** can be considered as ring contraction analogue of pipecolic amide **2.25** (Figure 2.13B), a very potent competitive inhibitor of β-*N*-acetyl-glucosaminidase (IC_{50} 0.09 μM)[8], and proline amide **2.26**, a sub-micromolar inhibitor of *N*-acetyl-hexosaminidases (IC_{50} 0.033μM)[1b, 9]. Molecular modeling showed good overlap among **2.25**, **2.26** and **2.23L**.[10] These results showed azetidine iminosugars are a fertile field of the development of new generation of glycosidase inhibitors. However, there remain problems (Figure 2.13C): the β-hydroxy carbonyl moiety in **2.23L** makes the molecule vulnerable in basic conditions due to the retro-aldol fragmentation, which limits the further synthesis of its analogues as well as biological studies.[7]

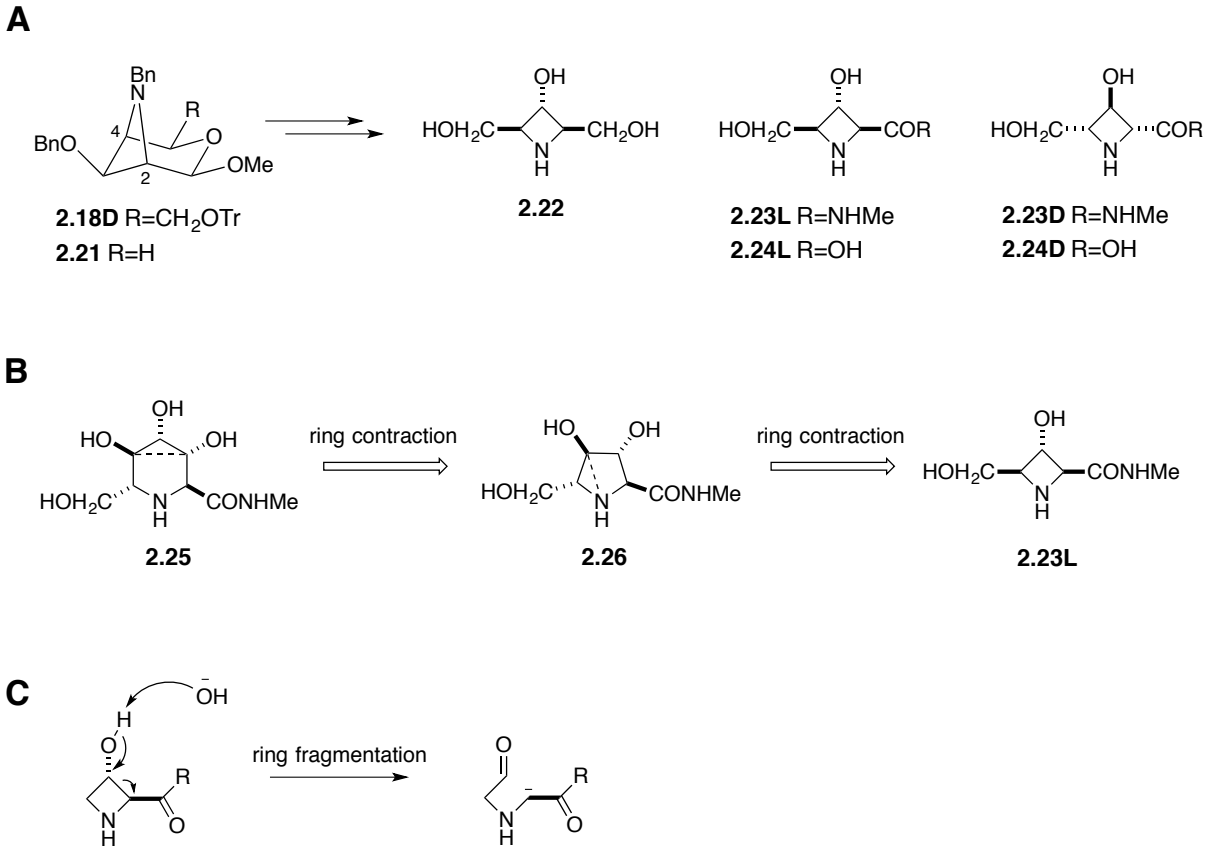
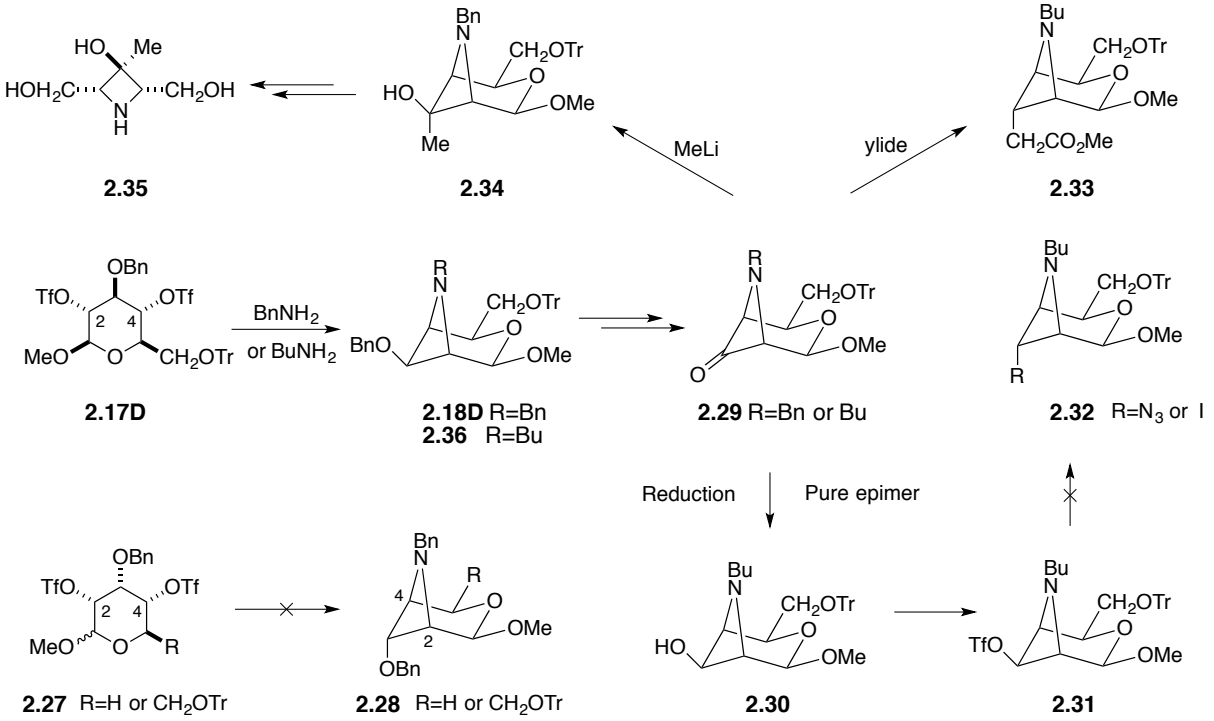


Figure 2.13 **A** Synthesis of azetidine iminosugars from bicyclic intermediates; **B** A ring

contraction strategy for rationalizing the activity of **2.23L**; **C** Retro-aldol ring fragmentation of β-hydroxy-carbonyl moiety

The formation of bicyclic azetidine scaffolds has been proved to be useful for accessing various azetidines with *trans,trans*-configurations (Figure 2.13A). Nevertheless, there are limitations (Scheme 2.3). Unlike **2.17D**, neither anomer of **2.27** is able to form corresponding bicyclic azetidines **2.28** under the same conditions, suggesting the bicyclic azetidines are only formed when all substitution groups are equatorial.[11] The reduction of *N*-butyl ketone **2.29** by various reagents (DIBALH, $NaBH_4$, $LiEt_3BH$) gave only one epimer **2.30** with 3-equatorial hydroxy group. The displacement of triflate **2.31** by azide or iodide was unable to give the corresponding 3-axial substituted bicyclic azetidines. However, the treatment of *N*-butyl ketone **2.29** with the stabilized ylide $Bu_3PCHCO2Me$ followed by hydrogenation formed **2.33** with 3-axial substitution. The treatment of *N*-benzyl protected ketone **2.29** with methyl lithium led to a 3-disubstituted azetidine **2.35**.[11]



Scheme 2.3 Exploration of the formation of various bicyclic azetidines.

There are other reported syntheses of polyhydroxylated azetidine rings. For instance, polyhydroxylated 1,2-oxazine **2.39** (Figure 2.14A), easily accessible from nitrones **2.37** and lithiated alkoxyallenes **2.38** by a [3 + 3] cyclization, was transformed to polyhydroxylated azetidines by a N-O cleavage – recyclization strategy.[12] More recently, four polyhydroxylated azetidines **2.41** - **2.44** and two bicyclic azetidines **2.45**, **2.46** were synthesized from 1,2:5,6-di-*O*-isopropylidene-3-oxo-α-D-glucofuranose **2.40** involving the Jocic-Reeve and Corey-Link approaches (Figure 2.14B).[1a] Biological assays of **2.41**, **2.42** and **2.43** showed weak inhibitions of *A. Niger* Amyloglucosidase (IC_{50} 415 μM, 522 μM, 201 μM, respectively) and **2.44**, **2.45** and **2.46** were good inhibitors of *A. Niger* Amyloglucosidase (IC_{50} 1.5 μM, 2.8 μM, 10.5 μM, respectively).

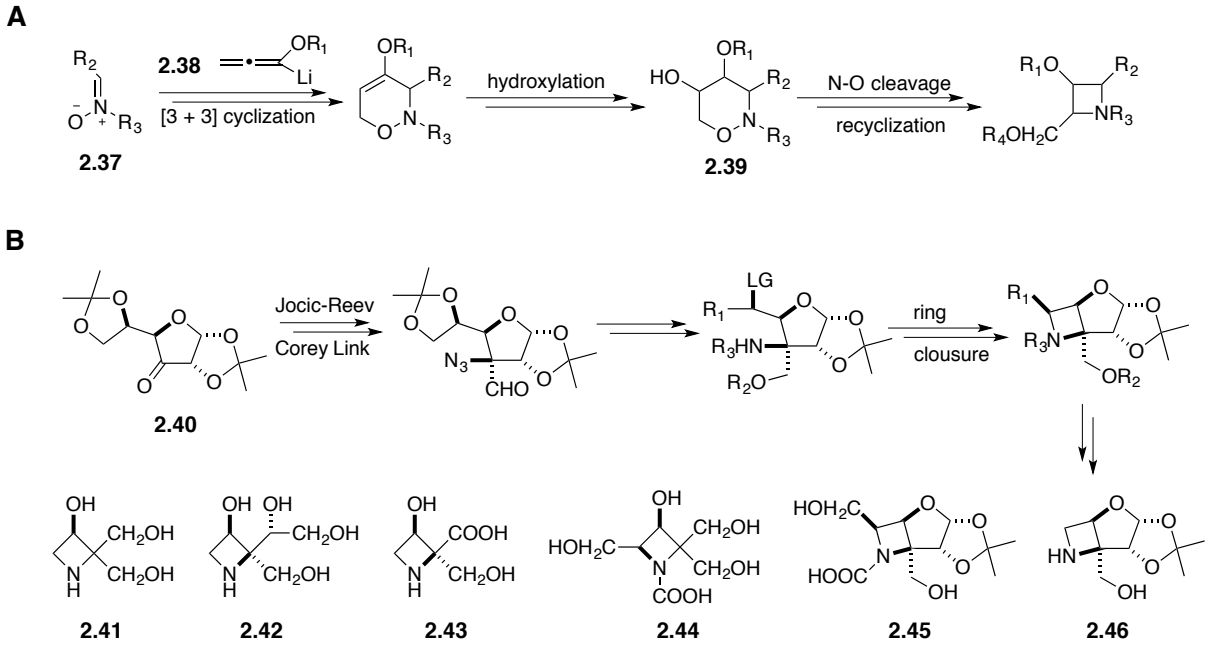


Figure 2.14 Strategies of accessing polyhydroxylated azetidines

2.1.2 Azetidine carboxylic acid

Azetidine carboxylic acid (Aze) **2.47** (Figure 2.15A), a four-membered ring amino acid analogue

of proline, was first isolated from *Convallaria majalis* in 1955.[13] This non-proteinogenic amino acid is widely distribute in many plants including sugar beet[14] and is toxic to livestock since it can affect the production of proteins as it can replace L-proline **2.48**.[15] Because of its rigid structure, L-proline plays an important role in the formation of secondary structures of proteins. During the biosynthesis of polypeptide chains, Aze forms Aze-tRNA complex as a substitute for L-proline. Evidence shows that Aze can alter the α-helix of the polypeptide chain to a smaller angle than that of L-proline and thus lead to changes of secondary and tertiary structures of proteins.[16] Additionally, the formation of OH-Aze-containing peptides from the oxidation by corresponding hydroxylases significant affects the stability of the peptide chain due to the retro-aldol decomposition (Figure 2.15B).[17] The conformational changes and degradation of proteins correlate with various toxic effects including teratogenesis and congenital malformation.[18] Reports also suggest there is a correlation of the occurrence of multiple sclerosis and the consumption of sugar beet. The problems of Aze **2.13** in the food chain are of increasing concern.[19]

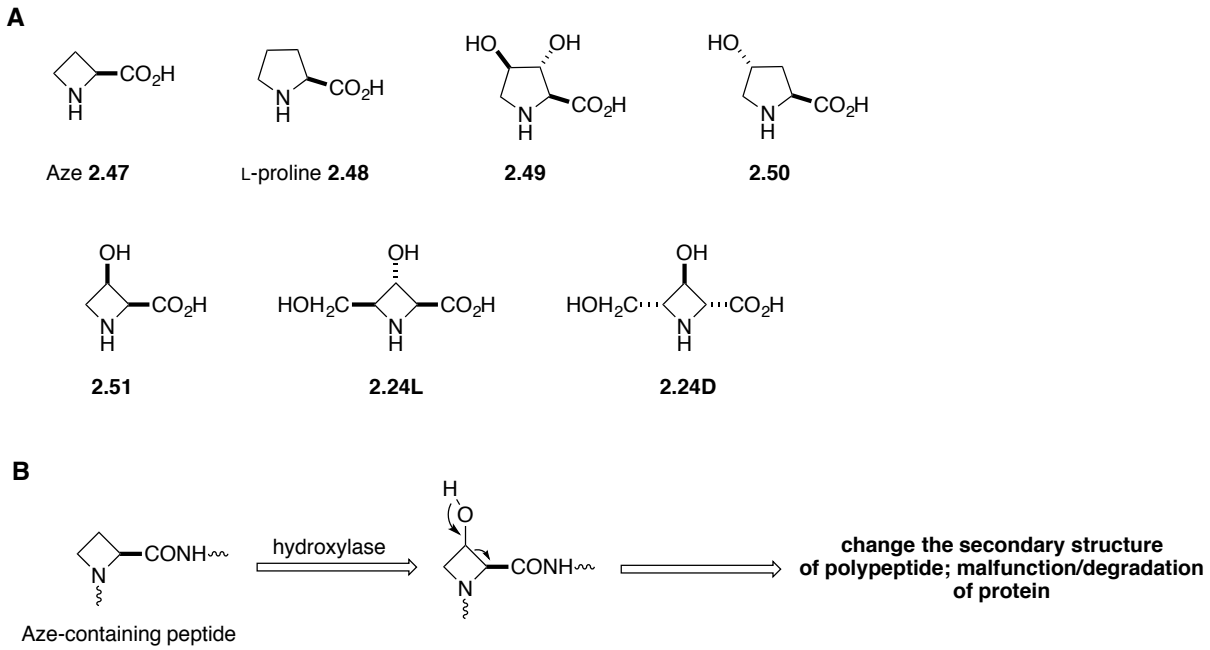


Figure 2.15 **A** Aze analogues and proline analogues; **B** The instability of Aze-containing peptide

Nevertheless, Aze **2.47** and its derivatives can be beneficial to certain organisms and also be useful for researchers. Yeung *et al*. reported *Enterobacter agglomerans* and *Enterobacter amnigenus* were able to grow in the soil containing Aze as the sole nitrogen source.[20] It has been used as a L-proline analogue for a variety of studies on protein processing and protein structures since 1960s.[21] Interestingly, Aze is able to inhibit growth of Japanese *encephalitis* virus.[22] 3-Hydroxy-Aze **2.51** is an azetidine analogue of dihydroxyl proline **2.49** (Figure 2.15A), a naturally occurring non-proteinogenic amino acid found in plants and microorganisms.[23] The mono-hydroxylated proline analogue **2.50** is abundant in various proteins and plays crucial role to maintain the structure and stability of collagen.[24] (Figure 2.15A) The essential roles of the hydroxylation of L-proline by 2-oxoglutarate (2-OG)-dependent prolyhydroxylases in collagen stabilization, hypoxia sensing and translational regulation has been explored.[25]

3-Hydroxy-Aze has only been prepared by enzymatic oxidation of Aze by proline hydroxylase.[26]

trans,*trans*-3-Hydroxy-4-hydroxymethyl-Aze **2.24L**, its enantiomer **2.24D** (Figure 2.15A) and their amides are the first synthetic free 3-hydroxy-Aze analogues.[7] Although these two amino acids do not show any inhibition of any glycosidases, **2.24L/D** and their analogues might be useful tools in peptidomimetic studies. More interestingly, Aze-containing dipeptides were reported in recent studies as Human cytomegalovirus inhibitors[27] and as modulators of autoimmunity.[2c] However, as illustrated in Figure 2.15B, the ease of aldol ring fragmentation in azetidine acids **2.24L/D** and amides **2.23L/D** precludes their use in peptidomimetics.

2.2 Aim

This chapter describes the design and syntheses of 3-fluoro and 3-azido azetidines as potential inhibitors of glycosidases and stable building blocks for novel peptidomimetics (Figure 2.16). The replacement of 3-hydroxyl group by fluoride or azido groups is expected to stabilize the molecule by avoiding the aldol fragmentation of the β-hydroxy carbonyl moiety (Figure 2.15B). Introduction of fluorine into the ring might bring unexpected properties to the molecule because of the special features of fluorine, e.g. strong electron withdrawing effect, gauche effect and potential hydrogen bonding. Fluorination has been a common strategy in the development of novel drugs.[28] Although replacement of a hydroxyl group by fluoride in iminosugars normally leads to reduction of glycosidase inhibition, a recent report showed that introduction of fluorine in australine increased glycosidase inhibition.[29] Also, the 5-fluoro-4-*epi*-isofagomine showed potent inhibition against D-galactosidase and is a potent pharmaceutical chaperon for Fabry's disease.[30] In spite of the growing interests on Aze and fluorinated iminosugars, 3-fluoro-Aze has

only been synthesized by a direct photofluorination in 1970.[31] Reports on 3-fluoro-Aze in peptidomimitic studies are few.

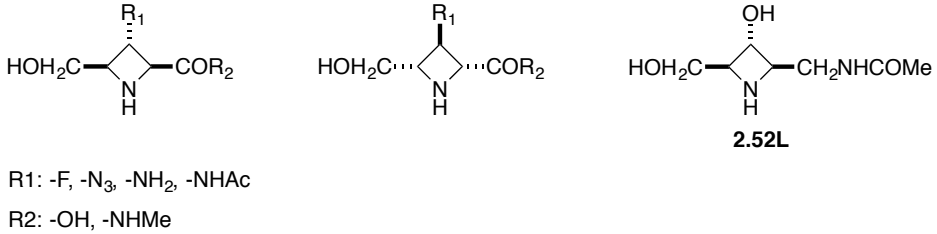


Figure 2.16 Synthetic targets (1)

Introduction of the 3-azido group to azetidine ring would allow for the formation of 3-acetamido azetidine iminosugar for biological tests and 3-amino azetidines as peptide scaffolds (Figure 2.16). Acetamido azetidine **2.52L**, a novel isostere of **2.23L**, is another synthetic target. The 3-hydroxyl group is maintained in **2.52L** and the molecule is more stable than **2.23L** due to lack of β-OH carbonyl moiety. Additionally, as shown in Figure 2.17, ADMDP **2.54**, the amide analogue of DMDP **2.53**, is a good inhibitor of β-*N*-acetyl-hexosaminidase (IC_{50} 0.16 μM).[32] Its analogue **2.55** has been studied as a drug candidate for the treatment of osteoarthritis.[33] Acetamido azetidine **2.52L** and its enantiomer **2.52D** can be considered as the ring contraction analogue of ADMDP **2.54**.

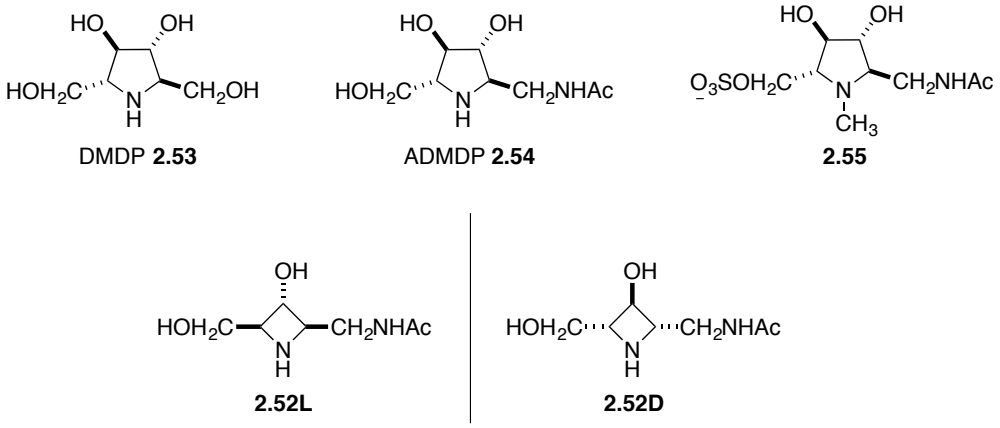


Figure 2.17 Synthetic targets (2)

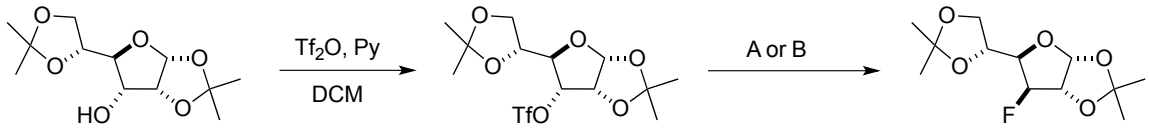
2.3 Results and discussion

2.3.1 Synthesis

The synthesis will be discussed as the following sections: i) synthesis of 3-fluoro-azetidine and *trans*,*trans*-3,4-difluoro-proline derivatives; ii) synthesis of 3-azido-azetidine derivatives; iii) synthesis of 3-hydroxy-azetidine derivatives; iv) synthesis of short oligomers of azetidine amino acids.

2.3.1.1 Synthesis of 3-fluoro-azetidine and 3,4-difluoro-proline derivatives

Fluorine was introduced into the carbohydrate at the first stage of synthesis. After triflation of 1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose **2.56** (Scheme 2.4), which was made from D-glucose by reported procedures,[7] fluorine was introduced with suitable reagents to give 3-fluoroglucose **2.58** *via* a S_N2 mechanism. *tetra*-*N*-Butylammonium fluoride (TBAF) gave a 44% yield. A better yield (86%) was achieved by the treatment of cesium fluoride in *tert*-butanol.[34]



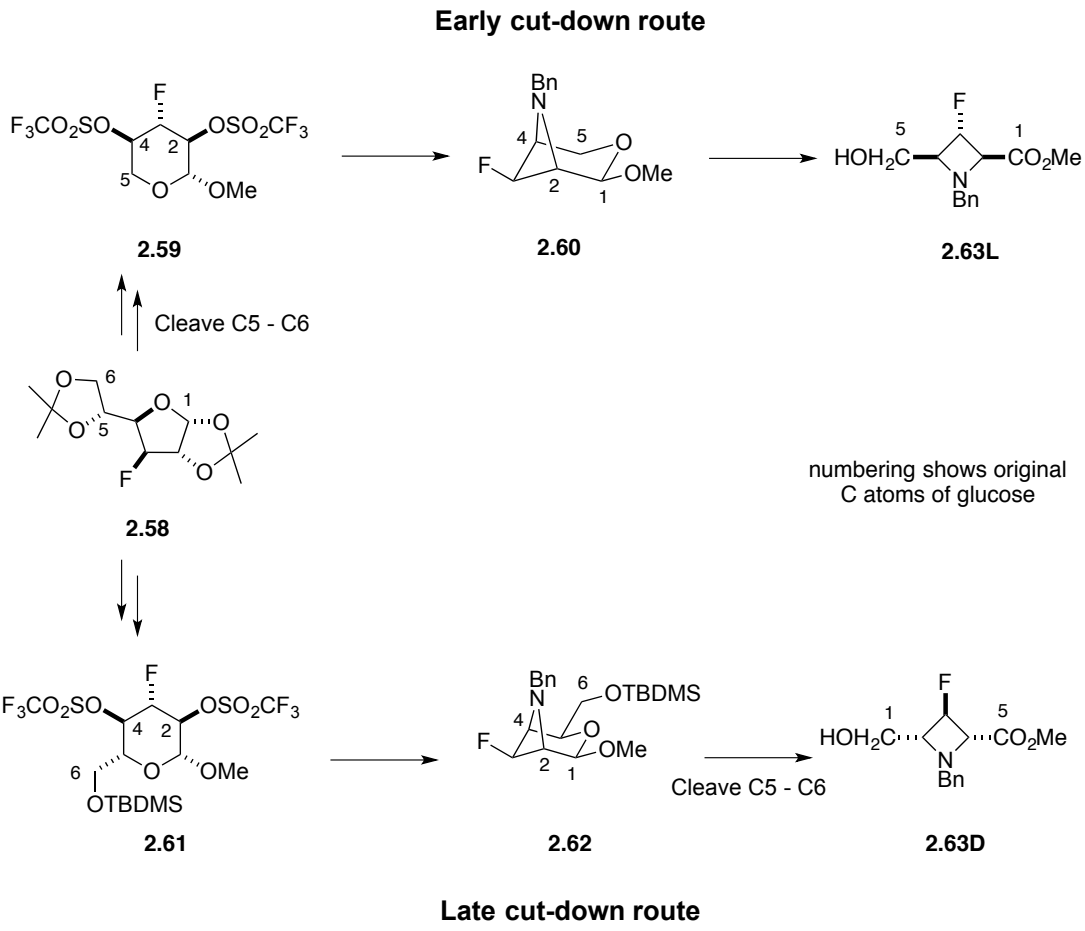
2.56 **2.57** **2.58**

	Reagent	Solvent	T (°C)	Time (h)	Yield (%)
A	TBAF	DCM	-20 to RT	48	44
B	CsF	*tert*-BuOH	80	26	86

Scheme 2.4 Conditions for 3-fluorination of **2.56**

As shown in Scheme 2.5, treatment of 1,3-di-*O*-triflates derived from carbohydrates with benzylamine followed by the opening of a bicyclic intermediate provides a strategy for efficiently accessing a variety of azetidines.[7] The 3-fluoroglucose **2.58** was transformed to

bicyclic azetidines **2.60** and **2.62** in high yields by the cleavage of C5 - C6 bond at either the late stage or early stage of the synthesis. Further manipulation gave the enantiomers **2.63L** and **2.63D** that were subjected to further syntheses.

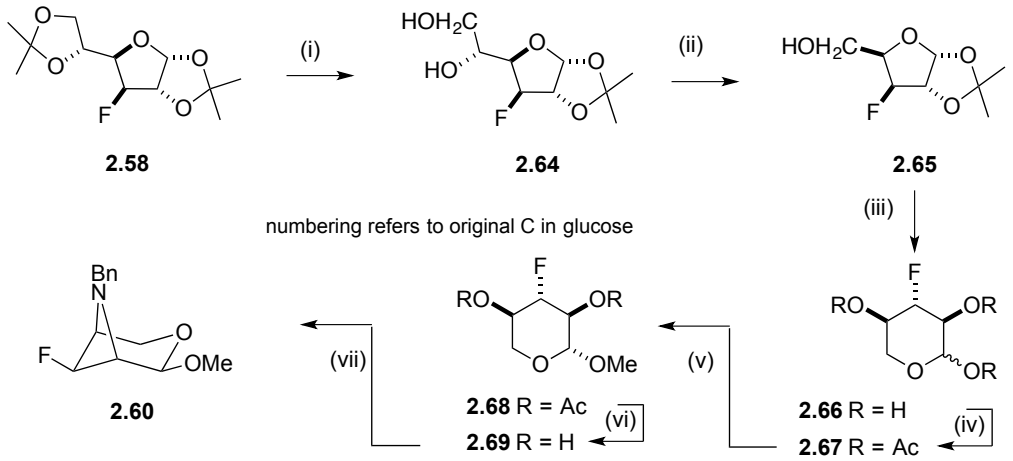


Scheme 2.5 Synthesis strategy

i) Early cut-down route

As shown in (Scheme 2.5), access to azetidine **2.63L** required the cleavage of C5 – C6 bond at the first stage of the synthesis. Selective deprotection of **2.58** was achieved under mild acidic conditions to give monoacetonide **2.64** (Scheme 2.6). Periodate cleavage of **2.64** followed by reduction of the resulting aldehyde with sodium borohydride afforded **2.65** in high yield (92%). Hydrolysis of **2.65** by DOWEX® 50WX8-200 resin (H^+ form) in water gave an anomeric mixture of 3-fluoroxylose **2.66**, in an α/β ratio of 3:2. After acetylation, the triacetate **2.67** was treated

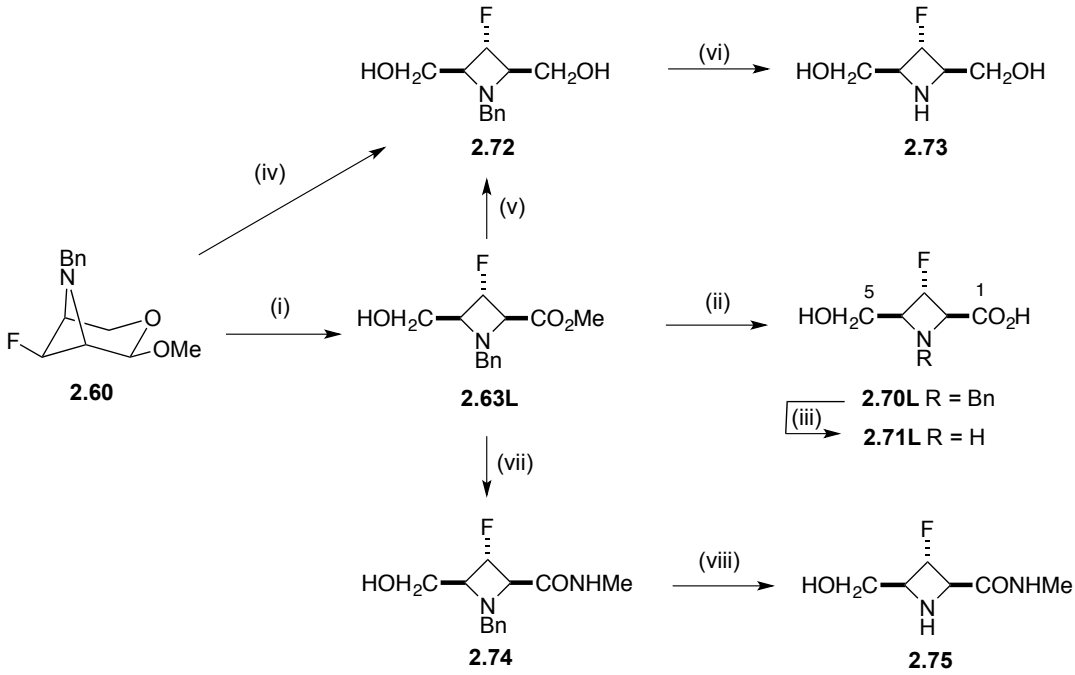
with HBr in acetic acid to give the anomeric bromides that were then transformed to β-methyl xylopyranoside **2.68** under Koenigs-Knorr condition[35] in a yield of 60%. After removal of acetyl protecting groups, esterification of diol **2.69** with triflic anhydride in the presence of pyridine gave a ditriflate that was treated with benzylamine in acetonitrile to yield bicyclic azetidine **2.60** (97% from **2.69**, 41% from **2.58**).



(i) MeOH, 1% H_2SO_4 (aq.), 100%; (ii) $NaIO_4$, water, 1,4-dioxane, then $NaBH_4$, 92%; (iii) DOWEX® 50WX8-200, water/1,4-dioxane, 88%; (iv) Ac_2O, pyridine, 89%; (v) HBr, AcOH; then Ag_2CO_3, MeOH, 60%; (vi) MeONa, MeOH, 97%; (vii) $(CF_3SO_2)_2O$, pyridine, DCM; then $BnNH_2$, MeCN, 97% (2 steps)

Scheme 2.6 Synthesis of bicyclic azetidine **2.24**

Hydrolysis of **2.60** with hydrochloric acid in aqueous 1,4-dioxane gave the corresponding lactol that was difficult to purify. However, treatment of the crude lactol with iodine and potassium carbonate in methanol allowed the efficient oxidation of the crude lactol to azetidine methyl ester **2.63L** (74%, 2 steps) as a key building block for accessing different 3-fluoro-azetidine targets (Scheme 2.7)



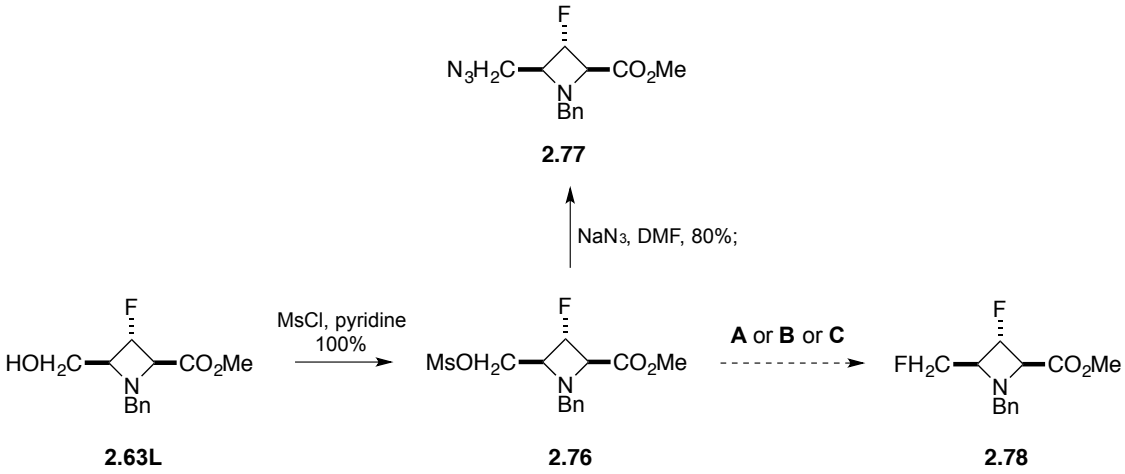
(i) HCl (2M, aq.)/1,4-dioxane (5:1); then I_2, K_2CO_3, MeOH, 74% (2 steps); (ii) K_2CO_3, water, 1,4-dioxane, 43%; (iii) Pd/C, H_2, water, 1,4-dioxane, 82%; (iv) HCl (2M, aq.)/1,4-dioxane (5:1); then $NaBH_4$, MeOH, 40%; (v) $NaBH_4$, MeOH, 90%; (vi) Pd/C, H_2, water, 1,4-dioxane, 100%; (vii) $MeNH_2$, $CaCl_2$, MeOH, 74%; (viii) Pd/C, H_2, HCl, water, 1,4-dioxane, 90%

Scheme 2.7 Synthesis of azetidine targets

N-Benzyl acid **2.70L** (43% from **2.63L**) was obtained by treatment with potassium carbonate in aqueous 1,4-dioxane. Purification was achieved by passing the crude product through a short column of DOWEX® 50WX8-200 resin. The hydrogenolysis of **2.70L** catalyzed by palladium on charcoal under a hydrogen atmosphere gave the free azetidine carboxylic acid **2.71L** (26% from **2.60**) (Scheme 2.7).

The *N*-benzyl-*meso*-diol **2.72** was synthesized by two approaches. It was initially formed from the reduction of the crude lactol derived from bicyclic azetidine **2.60** with sodium borohydride in methanol in a yield of 40%. In comparison, reduction of ester **2.63L** with sodium borohydride gave **2.72** in a better yield (90% from **2.63L**, 67% from **2.60**). Subsequent removal of benzyl protecting group gave free *meso*-diol **2.73** (40% from **2.60**) as a novel 3-fluoroazetidine

iminosugar. Reaction of ester **2.63L** with methylamine in methanol in the presence of calcium chloride formed the methylamide **2.74** (74%). Removal of the benzyl group by hydrogenolysis in aqueous acid yielded the hydrochloride salt of amide **2.75** (49% from **2.60**) as a 3-fluoro analogue of **2.24L**. Unlike 3-OH azetidine amide **2.24L**, the amide **2.75** and acid **2.71L** were stable to a range of pH conditions and no ring fragmentation was observed. This allowed the further synthesis of peptides with those building blocks (Scheme 2.8).



	Reagent (eq)	Solvent	Temp (°C)	Time (h)	Yield (%)
A	TBAF (1.1)	THF	50	8	0%[a]
B	CsF (3)	DMF	50	24	0%[a]
C	CsF (5.6)	*tert*-BuOH	60 to 80	20	28%[b]

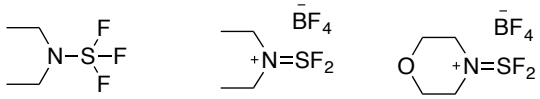
[a]: Crude NMR showed no sign of **2.78** while **2.63L** was detected;

[b]: Yield after flash column chromatography but still with impurities;

Scheme 2.8 Attempts of synthesis of 5-azido and 5-fluoro azetidines

The mesylate **2.76**, prepared by the treatment of ester **2.63L** with mesyl chloride in pyridine in a yield of 100%, allowed the formation of more building blocks. Azide was introduced by the treatment of the mesylate **2.76** with sodium azide in DMF to form 3-fluoro-5-azido-azetidine **2.77** (80%). Attempts of introducing fluorine to C5 from mesylate **2.76** by nucleophilic substitution were found to be problematic. TBAF and cesium fluoride were tested under a range of conditions. Only a low yield of **2.78** (28% with impurities) was observed by the treatment of

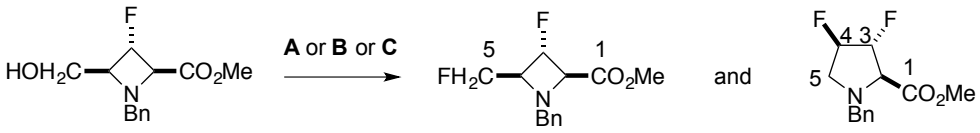
2.76 with cesium fluoride at 80 °C (Scheme 2.8).



DAST XtalFluor-E XtalFluor-M

Figure 2.18 DAST and XtalFluor slats

Recent reports[28d, 36] showed two fluorination reagents, XtalFluor-E and XtalFluor-M gave efficient deoxyfluorination of a range of substrates under mild conditions (Figure 2.18). The reaction mechanism is similar to that of diethylaminosulfur trifluoride (DAST). Initially, methyl ester **2.63L** was treated with XtalFluor-E and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DCM from -78 °C to room temperature. Surprisingly, the reaction gave two difluoro products: 3,5-difluoro azetidine **2.78** (8%) and *trans*,*trans*-3,4-difluoro proline methyl ester **2.79** (22%). A range of reaction conditions was tested to improve selectivity and yields (Scheme 2.9).[28d, 36] The yield of difluoro azetidine **2.78** remained low (trace - 8%). In contrast, the yield of difluoro pyrrolidine **2.79** was improved to 84% by using Xtalfluor-M together with triethylamine and triethylamine trihydrofluoride (TEA•3HF) in DCM. The configuration of **2.79** was confirmed by the following Nuclear Magnetic Resonance (NMR) evidence: H-3 and H-4 showed strong coupling constants with fluorine ($J_{3,F}$ 50.1 Hz and $J_{4,F}$ 50.7 Hz); ^{1}H-^{1}H Nuclear Overhauser Effect Spectroscopy (nOe) and ^{19}F-^{1}H nOe analysis indicated the 3,4-*trans*-difluoro configuration (Figure 2.19, Figure 2.20).



2.63L **2.78** **2.79**

	Reagent[a]	Additive 1 (eq)	Additive 2 (eq)	T (°C)	Time (h)	Yield (**2.78**)	Yield (**2.79**)
A	XtalFluor-E	DBU (1.5)	*N/A*	-78 to RT	18	8%	22%
B	XtalFluor-M	TEA•3HF (2)	TEA (1)	-78 to RT	3	trace	43%[b]
C	XtalFluor-M	TEA•3HF (2)	TEA (1)	-78 to RT	18	trace	84%

[a]: All reactions were performed with **1.5 equivalent** of XtalFluor reagent in **DCM;**

[b]: 47% of **2.63L** was recovered after reaction;

Scheme 2.9 Attempts of deoxofluorination with XtalFluor salts

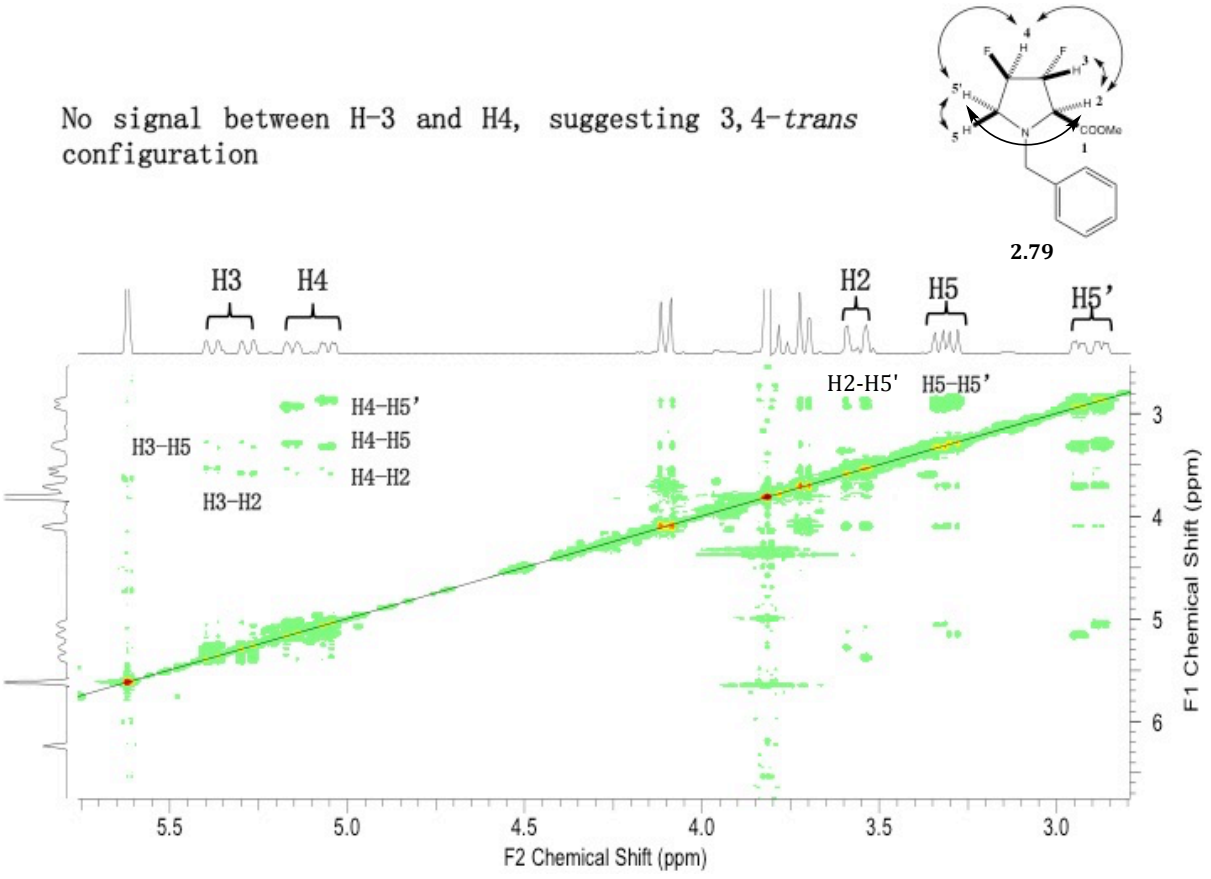


Figure 2.19 ^{1}H-^{1}H Nuclear Overhauser Effect Spectroscopy (nOe) of **2.79**

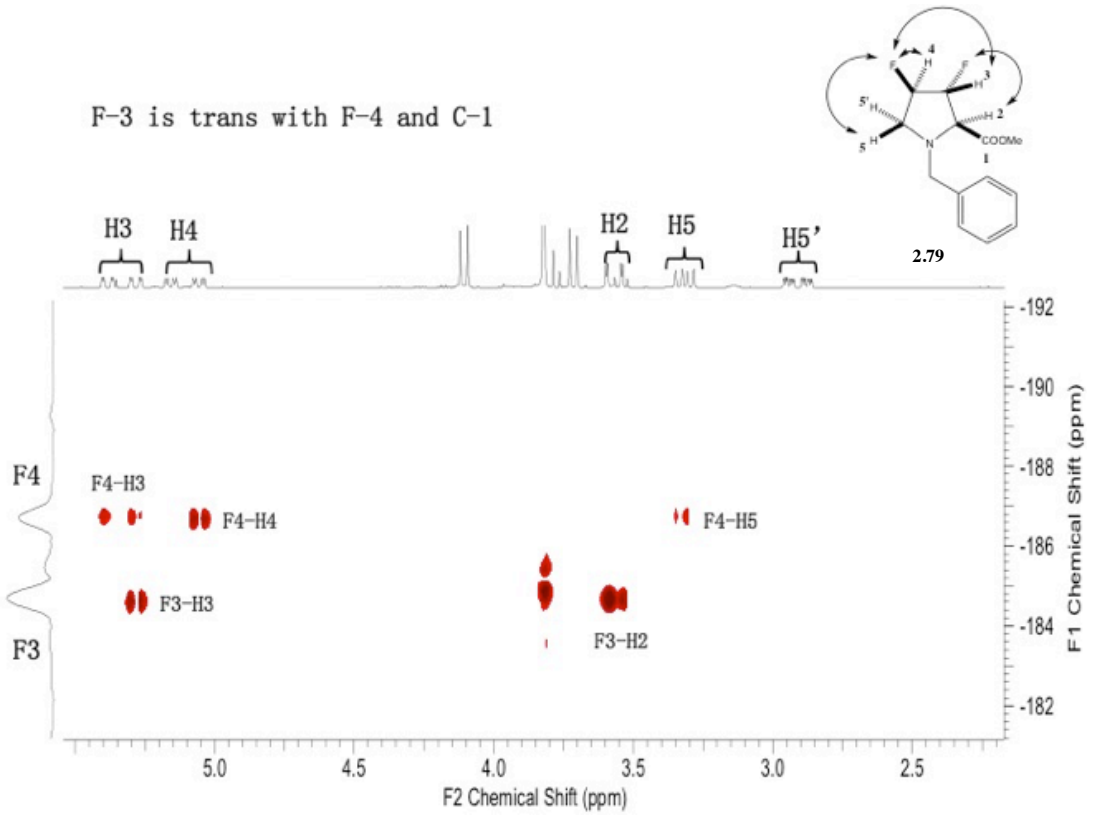
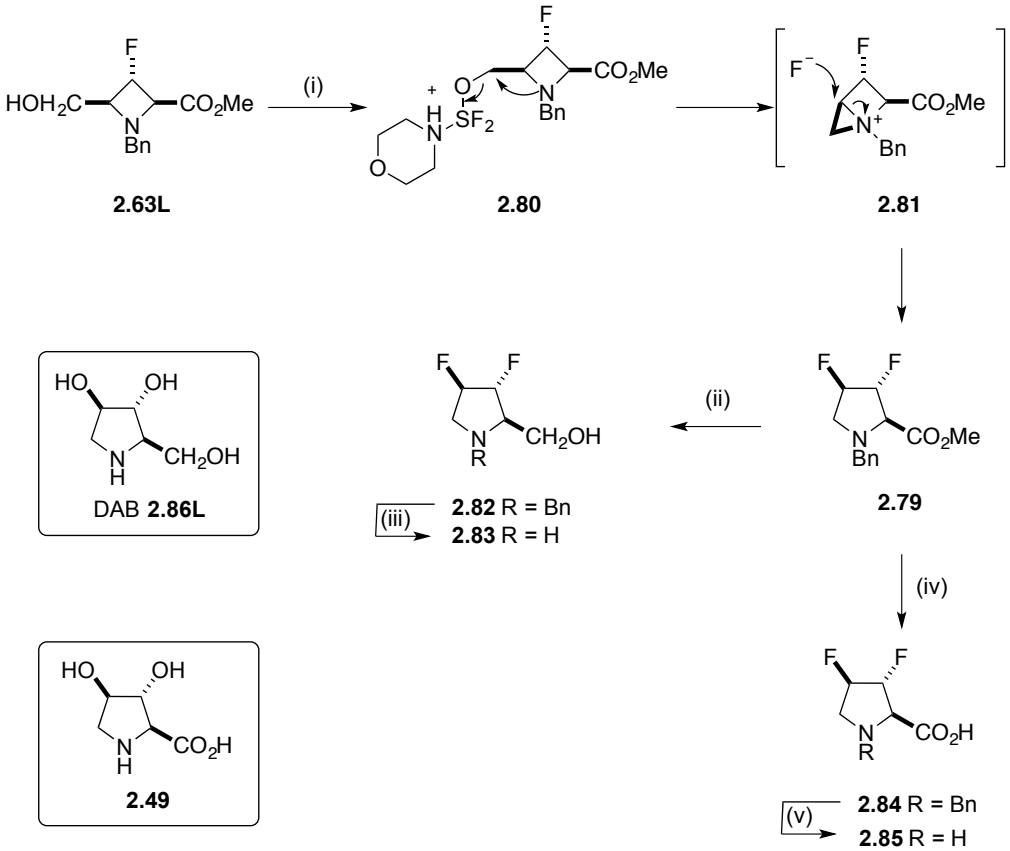


Figure 2.20 ^{19}F-^{1}H Nuclear Overhauser Effect Spectroscopy (nOe) of **2.79**

A mechanism of the formation of **2.79** was proposed (Scheme 2.10): neighboring group participation by the ring nitrogen on intermediate **2.80** gave the bicyclic aziridinium ion **2.81**. Fluoride was then introduced into the ring, with ring expansion, to stereospecifically afford the *trans*-difluoro pyrrolidine **2.79**. The regioselectivity can be explained by the ring expansion leading to less strained pyrrolidine ring.

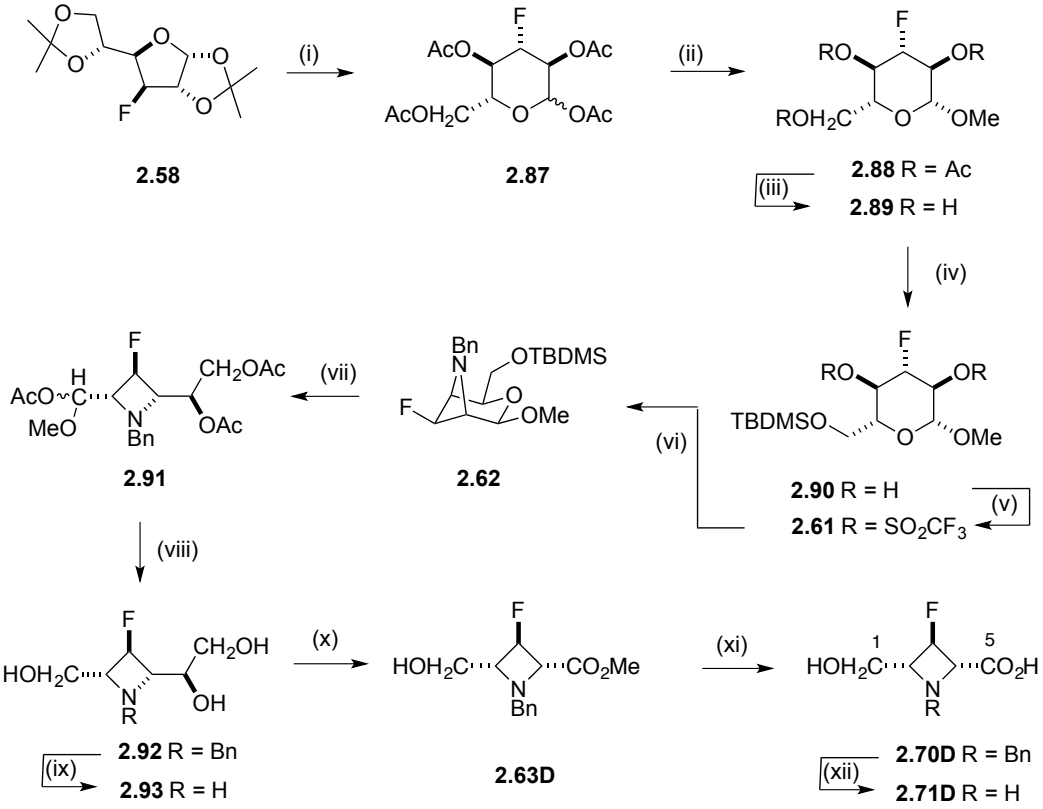


(i) XtalFluor-M, Et_3N, $Et_3N{\bullet}3HF$, DCM, 84%; (ii) $LiAlH_4$, THF, 66%; (iii) Pd/C, H_2, water, 1,4-dioxane, 82%; (iv) NaOH, water, 1,4-dioxane, 64%; (v) Pd/C, H_2, water, 1,4-dioxane, 86%

Scheme 2.10 Synthesis of difluoro proline derivatives

A difluoro pyrrolidine iminosugar **2.83**, the difluoro derivative of DAB **2.86L**, was obtained by the reduction of **2.79** by lithium aluminium hydride to give **2.82**. Subsequent hydrogenolytic removal of the *N*-benzyl group from **2.82** afforded **2.83** (45% from **2.63L**). The hydrolysis of **2.79** with aqueous sodium hydroxide gave *N*-benzyl L-proline **2.84** (64%) that was subsequently hydrogenated to form a difluoro proline **2.85** (46% from **2.63L**). It is worth noting that this is the first synthesis of *trans*,*trans*-difluoroproline **2.85**, a difluorinated analogue of dihydroxyproline **2.49** (Scheme 2.10).

ii) Late cut-down route



(i) DOWEX® 50WX8-200, then Ac_2O, pyridine, 97% (ii) HBr, AcOH, then Ag_2CO_3, MeOH, 87%; (iii) NaOMe, MeOH, 96%; (iv) TBDMSCl, imidazole, 98%; (v) $(CF_3SO_2)_2O$, pyridine; (vi) $BnNH_2$, DIPEA, MeCN, 84%; (vii) $Et_2O{\bullet}BF_3$, Ac_2O, 100%; (viii) DIBALH, toluene, then $NaBH_4$, MeOH, 97%; (ix) Pd/C, H_2, water, 1,4-dioxane, 88%; (x) $NaIO_4$, water, 1,4-dioxane, then I_2, K_2CO_3, MeOH, 69%; (xi) K_2CO_3, water, 1,4-dioxane, 57%; (xii) Pd/C, H_2, water, 1,4-dioxane, 45%

Scheme 2.11 Late cut-down route

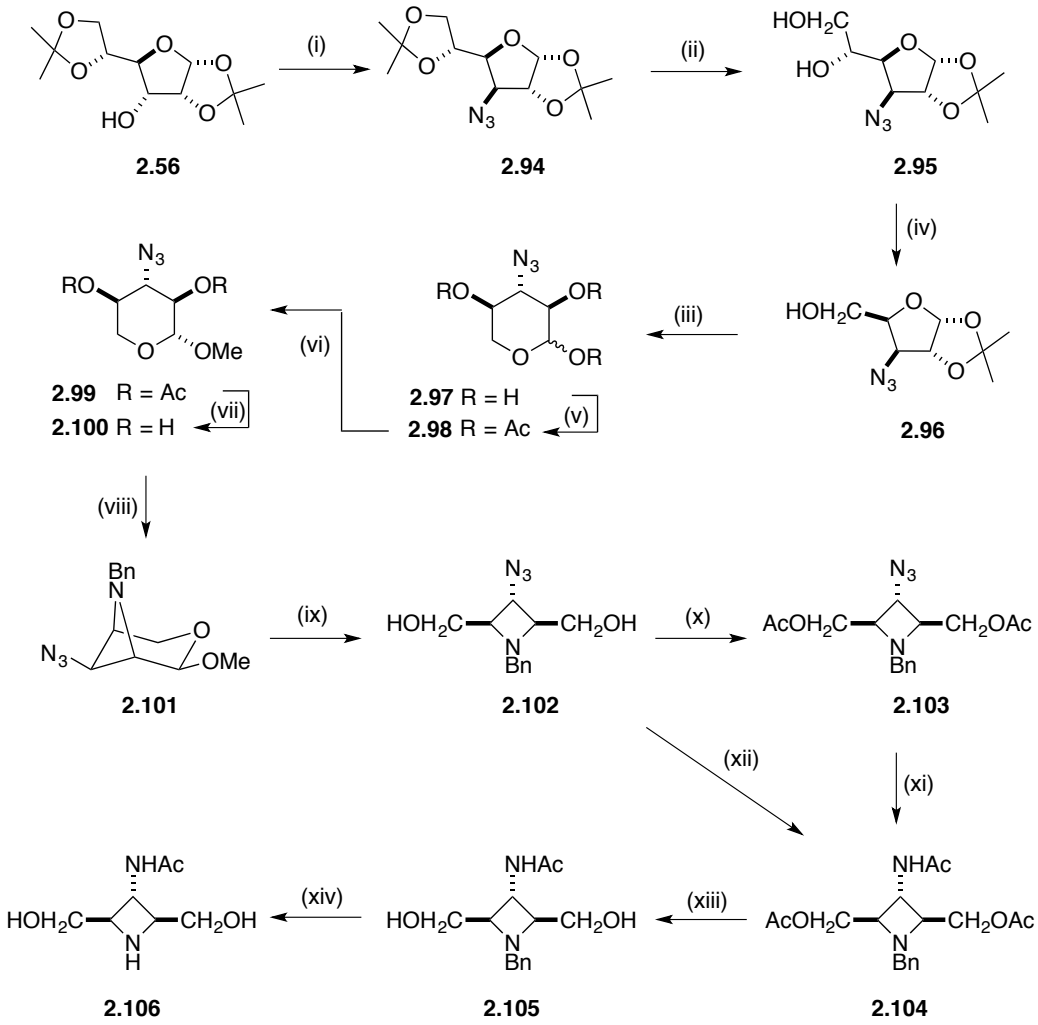
This route was developed in collaboration with Tom Vermaas and Dr Sarah Jenkinson (Scheme 2.11). First, anomeric tetraacetate **2.87** (α/β, 1:1) was formed by the acetylation of 3-fluoroglucose from the hydrolysis of **2.58** in a yield of 97% (2 steps). A Koenigs-Knorr reaction then gave β-methyl pyranoside **2.88** (87%, 2 steps). After removal of acetyl groups, the primary hydroxyl group in the triol **2.89** was selectively protected to form the silyl ether **2.90** (98%). Triflation and subsequent treatment of the resulting ditriflate with benzylamine in the presence of *N*,*N*-diisopropylethylamine (DIPEA) gave **2.62** (84%). The hydrolysis of **2.62** under a wide range of conditions gave a crude product that was directly reduced with sodium

borohydride. But only traces of triol **2.92** were detected. However, the acetolysis of **2.62** with acetic anhydride in the presence of boron trifluoride efficiently produced an epimeric mixture of the triacetate **2.91** (100%). Treatment of **2.91** with DIBALH followed by sodium borohydride gave triol **2.92** in a yield of 97% (2 steps). Subsequent hydrogenolysis gave the free triol **2.93** (59% from **2.58**).

Periodate cleavage of the C5 - C6 bond of **2.92** give a crude aldehyde that was oxidized to ester **2.63D** by iodine with potassium carbonate in a yield of 69% (2 steps). After sequential hydrolysis and hydrogenation gave amino acid **2.71D** (11% from **2.58**) as a novel fluorinated peptide scaffold.

2.3.1.2 Synthesis of 3-azido-azetidine derivatives

The same strategy used for the fluoro azetidines allowed access to novel azido azetidine derivatives (Scheme 2.12). These compounds provide a set of NHAc azetidines, which could be studied for hexosaminidase inhibition.



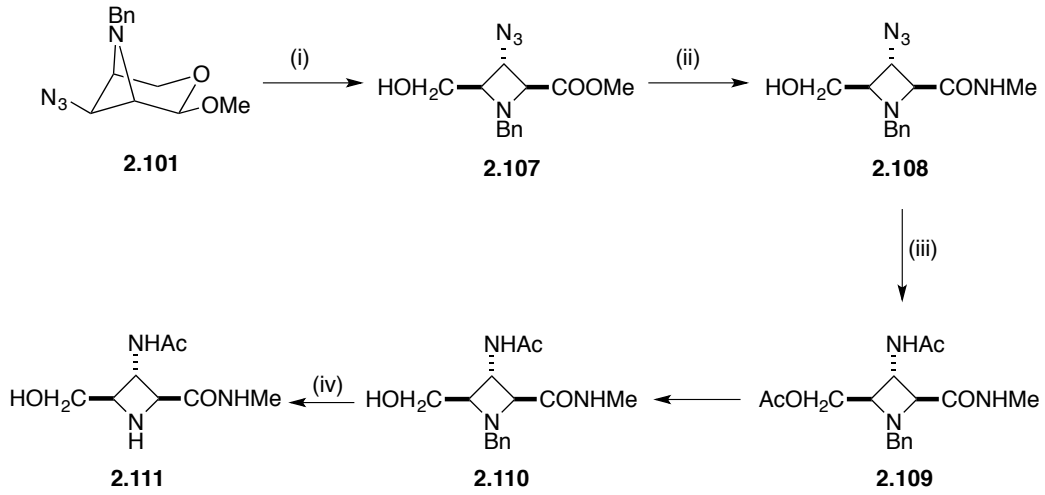
(i) Tf_2O/pyridine then NaN_3, 78% (2 steps); (ii) CH_3COOH/water 7:3, 60%; (iii) $NaIO_4$ then $NaBH_4$, 96% (2 steps); (iv) DOWEX® 50WX8-200, 100%; (v) Ac_2O/pyridine, 98%; (vi) $BiBr_3$, TMSBr, then Ag_2CO_3, $CaSO_4$, 74%; (vii) NaOMe, 100%; (viii) Tf_2O/pyridine then $BnNH_2$, 71%; (ix) HCl (2M, aq.)/1,4-dioxane (5:1) then $NaBH_4$; (x) Ac_2O/pyridine, 76% (3 steps from **2.101**); (xi) Zinc powder, $CuSO_4$, THF/AcOH/Ac_2O, 100%; (xii) Zinc powder, $CuSO_4$, THF/AcOH/Ac_2O, 24% (3 steps from **2.101**); (xiii) MeONa, MeOH, 79%; (xiv) Pd/C, H_2, water, 73%;

Scheme 2.12 Synthesis of 3-acetamido azetidines (1)

3-Azidoglucose **2.94** was effectively synthesized by the treatment of the 3-*O*-triflic ester of 1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose **2.56** with sodium azide in DMF in a yield of 78% (2 steps). Then the established procedures gave the xylopyranose triacetate **2.98** in high yield (56%, 4 steps) as an anomeric mixture (α/β, 10:3). The transformation of **2.98** to anomeric bromides under established conditions (HBr, acetic acid) was not effective and gave only poor conversion to β-methyl pyranoside **2.99** (30%, 2 steps) by Koenigs-Knorr reaction. A higher

yield of **2.99** was obtained using an alternative bromination procedure[37]: **2.98** was reacted with bismuth (III) bromide in the presence of bromotrimethylsilane (TMSBr) to afford the crude bromide which was then stirred with silver carbonate in methanol to afford β-methyl azidopyranoside **2.99** in a yield of 74% (2 steps).

The diol **2.100**, formed by the basic hydrolysis of **2.99,** was efficiently transformed to bicyclic azetidine **2.101** by the established strategy in a yield of (71%, 2 steps) (Scheme 2.13). Acidic hydrolysis of **2.101** followed by the reduction of the lactol with sodium borohydride gave the crude diol **2.102**. Purification of **2.102** with DOWEX® 50WX8-200 resin column or flash column chromatography was not effective. Reductive acetylation of crude azide **2.102** with zinc powder in the presence of copper sulphate (sat., aq.) in a mixture of THF/acetic acid/acetic anhydride afforded triacetate **2.104** in a low yield of 24% (3 steps from **2.101**). Alternately, acetylation of crude **2.102** leaded to the diacetate **2.103** which was easily purified in a yield of 76% (3 steps from **2.101**). Subsequent reductive acetylation by zinc powder gave **2.104** in quantitative yield. Removal of the *O*-acetate and *N*-benzyl groups afforded the 3-acetamidoazetidine diol **2.106** (58%, 2 steps).



(i) HCl (2M, aq.)/1,4-dioxane (5:1), then I_2, K_2CO_3, 85% (2 steps); (ii) $MeNH_2$, $CaCl_2$, 80%; (iii) Zinc powder, $CuSO_4$, THF/AcOH/Ac_2O, then MeONa, 85% (2 steps); (iv) Pd/C, H_2, water, 72%

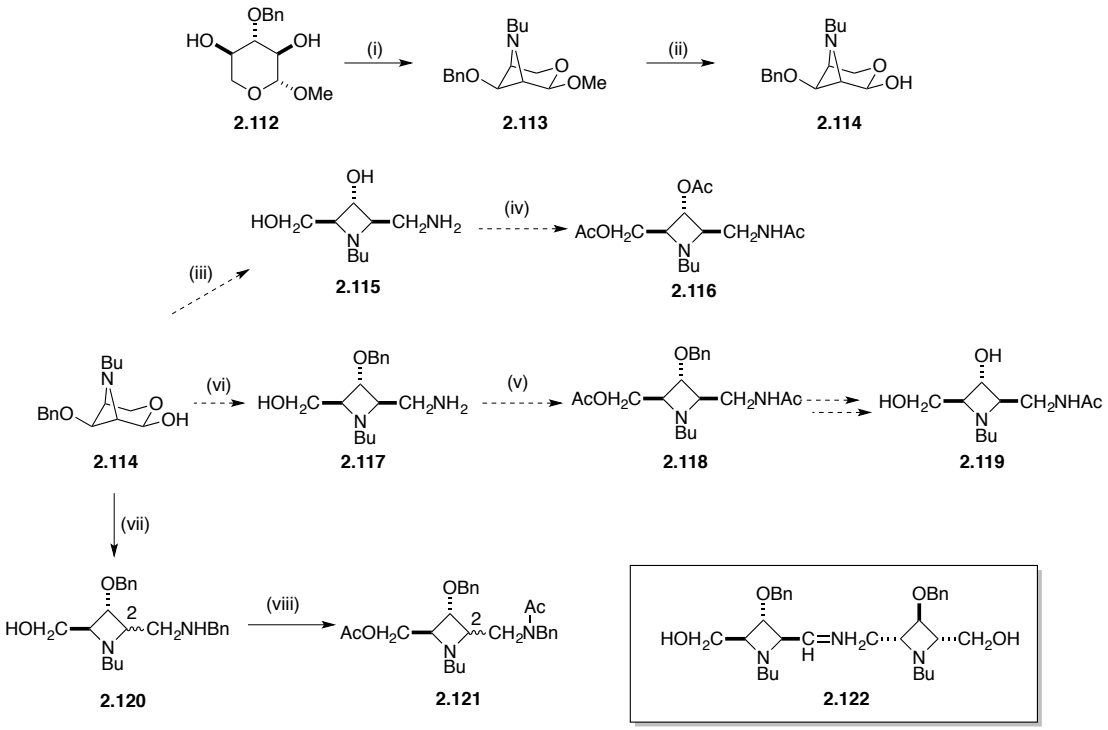
Scheme 2.13 Synthesis of 3-acetamido azetidines (2)

The amide azetidine **2.111** was also synthesized from bicyclic **2.101** (Scheme 2.13). After the hydrolysis of bicyclic azetidine **2.101** in aqueous acid, oxidation of the lactol by iodine in methanol gave the methyl ester **2.107** as one precursor of peptide building blocks (85%, 2 steps). Treatment of methylamine and calcium chloride with ester **2.107** gave methyl amide **2.108** (80%) which was subjected to reductive acetylation to **2.109** with zinc powder and copper sulfate in THF/acetic acid/acetic anhydride. Subsequent removal of *O*-acetyl group and *N*-benzyl group gave the diamide **2.111** (61% from **2.108**).

2.3.1.3 Synthesis of 3-hydroxy azetidine acetamides

The *O*-benzyl protected pyranoside **2.112** (Scheme 2.14) was the starting material and is readily available on large scale.[7] *N*-Butyl bicyclic azetidine **2.113** (98%, 2 steps) was synthesized from the triflation of **2.112** followed by the treatment with butylamine. Initial attempts to synthesize the exocyclic acetamide **2.52L** from lactol **2.114** by reductive amination were found to be problematic. The reductive aminations of **2.114** with ammonium chloride under hydrogen

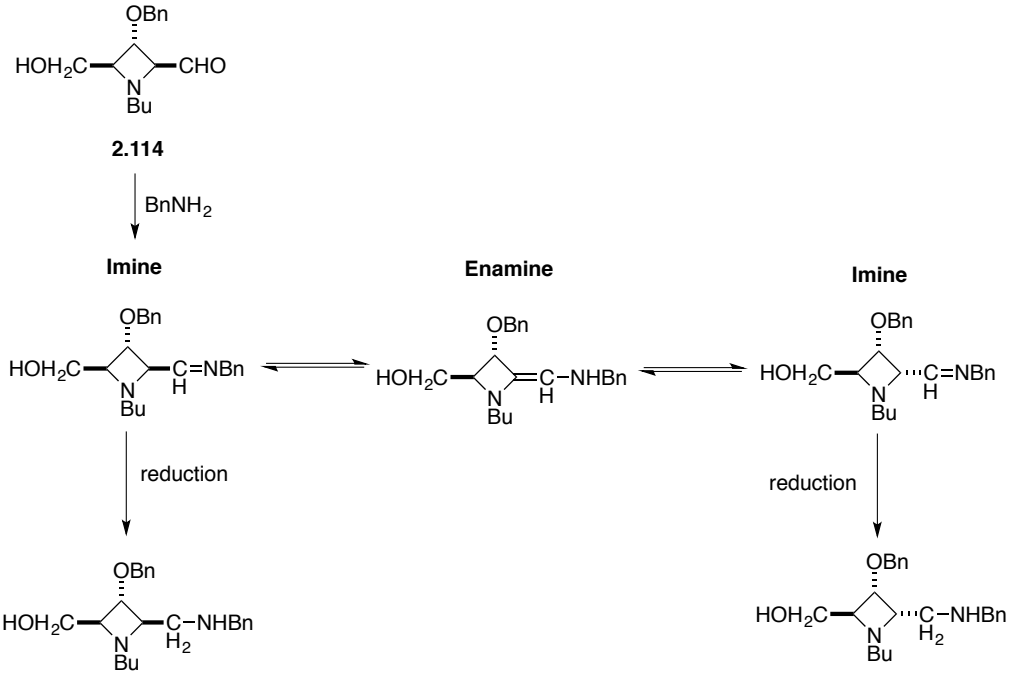
atmosphere catalyzed by palladium on charcoal or ammonium formate in the presence of sodium cyanoborohydride were expected to give primary amine **2.115** or **2.117**. However, after acetylation, none of them gave any desired product **2.116** or **2.118**. Interestingly, low-resolution mass spectrometry (LRMS) suggested that the formation of imine **2.122** derived from the primary amine and lactol. This indirect evidence indicated that the *in situ* dimerisation might be the reason of a complex reaction mixture. To solve this problem, benzylamine was used with sodium cyanoborohydride in a weak acidic condition to react with lactol **2.114**. The secondary amine **2.120**, which was less likely to dimerize, was treated with acetic anhydride/pyridine to give a crude residue of diacetate **2.121** (35%, 3 steps) but epimerization at C-2 was observed according to NMR spectra, possibly due to an imine-enamine tautomerism occurring in the reductive amination step (Scheme 2.15).



(i) Tf_2O/pyridine then $BuNH_2$, 65 °C, 98% (2 steps); (ii) HCl (2M, aq.)/1,4-dioxane (5:1), 40 °C; (iii) NH_4Cl, HCl, Pd/C, H_2, 1,4-dioxane/water, 2 days; (iv) Ac_2O/pyridine; (v) CH_3COONH_4, $NaCNBH_3$, EtOH/AcOH 100:1, 7h; (vi)

Ac_2O/pyridine; (vii) $BnNH_2$, $NaCNBH_3$, EtOH/AcOH 100:1, 7h; (viii) Ac_2O/pyridine, 35% (3 steps)

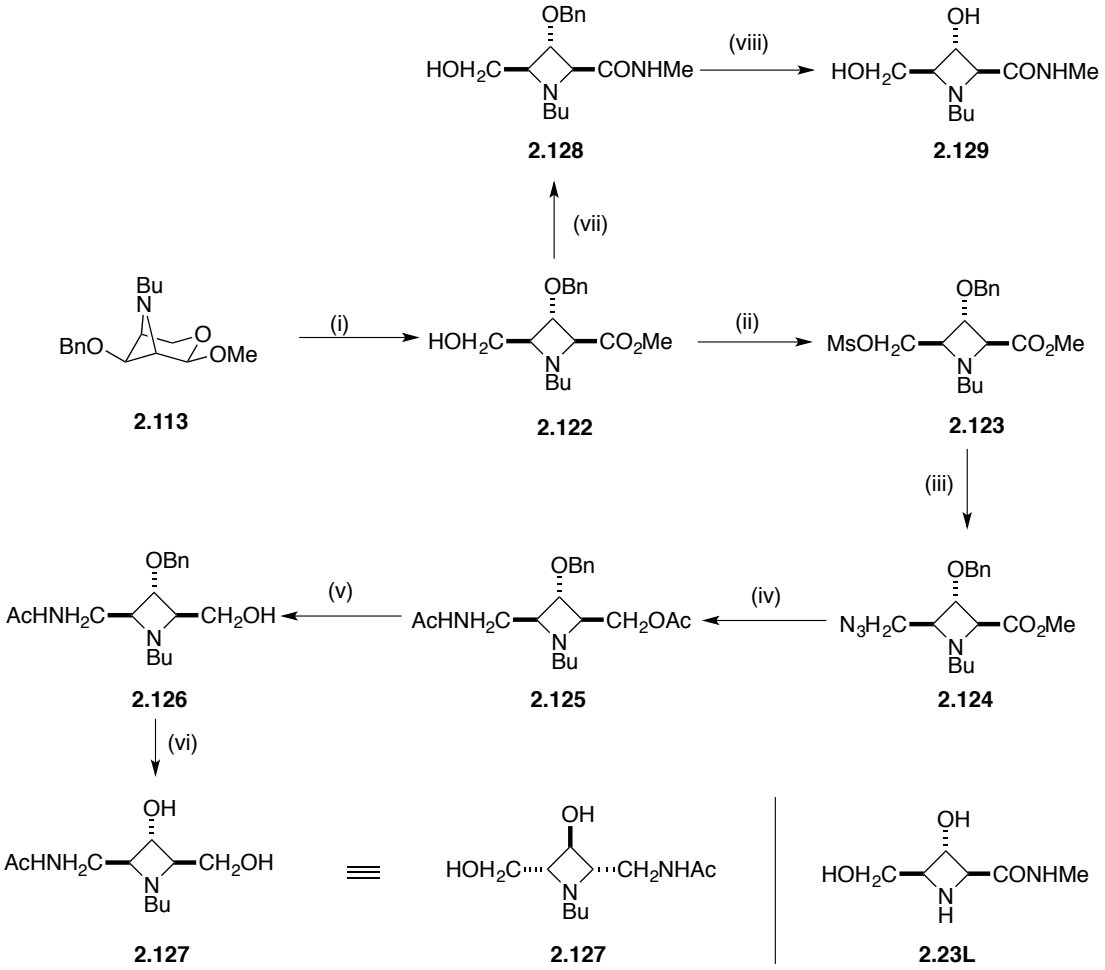
Scheme 2.14 Attempted reductive amination of **2.78**



Scheme 2.15 Proposed mechanism of epimerization in the reaction of **2.114** with benzylamine

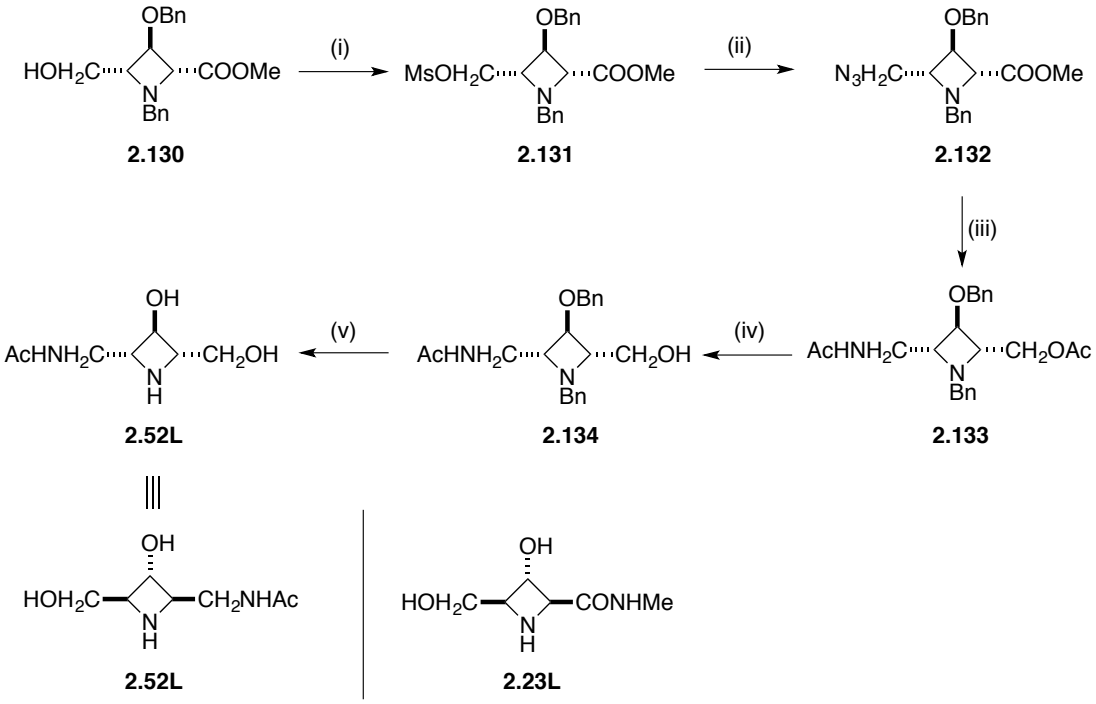
An alternative strategy was employed (Scheme 2.16). Azetidine ester **2.122** was made from the oxidation of the corresponding lactol of **2.113** by iodine in methanol in a yield of 54% (2 steps). **2.128**, an *N*-butyl derivative of **2.23L**, was obtained by reaction of ester **2.122** with methylamine in the presence of calcium chloride. Subsequent hydrogenolysis catalyzed by palladium on charcoal gave **2.129** (51% from **2.113**). By analogy with the 3-hydroxyamide **2.23L**, **2.129** was stable in neutral and acidic conditions but quickly decomposed when pH > 8 by retroaldol ring opening of the azetidine. Ester **2.122** was mesylated to afford **2.123** (100%), which on the treatment with sodium azide in DMF gave the azido ester **2.124**. Reaction of **2.124** with lithium aluminium hydride followed by acetylation formed diacetate **2.125** (92%, 2 steps).

Subsequent hydrolysis in basic conditions gave **2.126**. Hydrogenolysis of the benzyl group in **2.126** gave the acetamido azetidine **2.127** (18% from **2.113**) with the opposite stereochemistry to the potent hexosaminidase inhibitor **2.23L**. To access the desired stereochemistry, previously synthesized D-ribonate **2.130** was used.[7] An identical strategy was performed to access target **2.52L** (21% from **2.130**, Scheme 2.17).



(i) 1,4-dioxane/water (5:1), then I_2, K_2CO_3, MeOH, 54% (2 steps); (ii) MsCl, pyridine, 100%; (iii) NaN_3, DMF, 86%; (iv) $LiAlH_4$, THF, -78 °C, then Ac_2O/pyridine, 92%; (v) MeONa, MeOH, 60 °C, 42%; (vi) Pd/C, H_2, 98%; (vii) $MeNH_2$, $CaCl_2$, MeOH, 51%; (viii) Pd/C, H_2, 100%;

Scheme 2.16 Synthesis of azetidine amide **2.128**

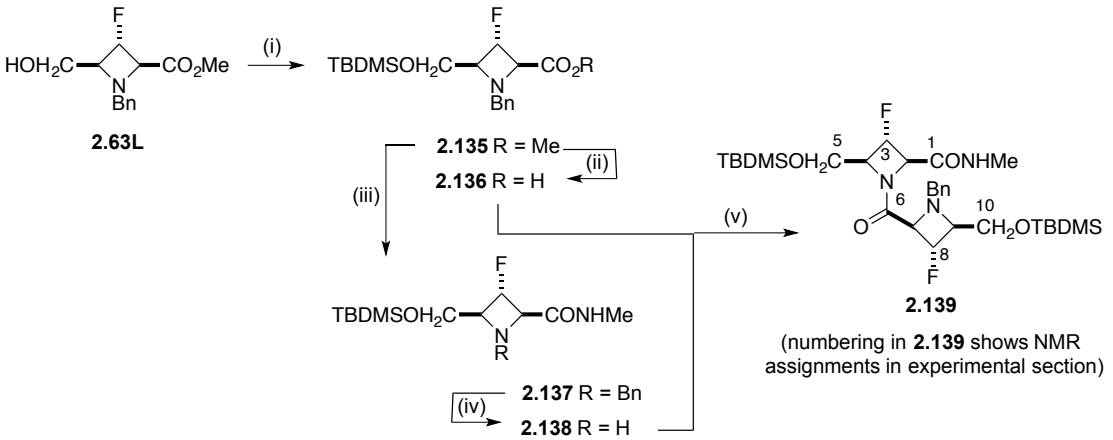


(i) MsCl, pyridine, 88%; (ii) NaN_3, DMF, 78%; (iii) $LiAlH_4$, THF, -78 °C, then Ac_2O, Pyridine, 79%; (iv) MeONa, MeOH, 60 °C, 38%; (v) Pd/C, H_2, HCl, water, 100%;

Scheme 2.17 Synthesis of amide azetidine **2.52L**

2.3.1.4 Synthesis of short oligomers of azetidine amino acids

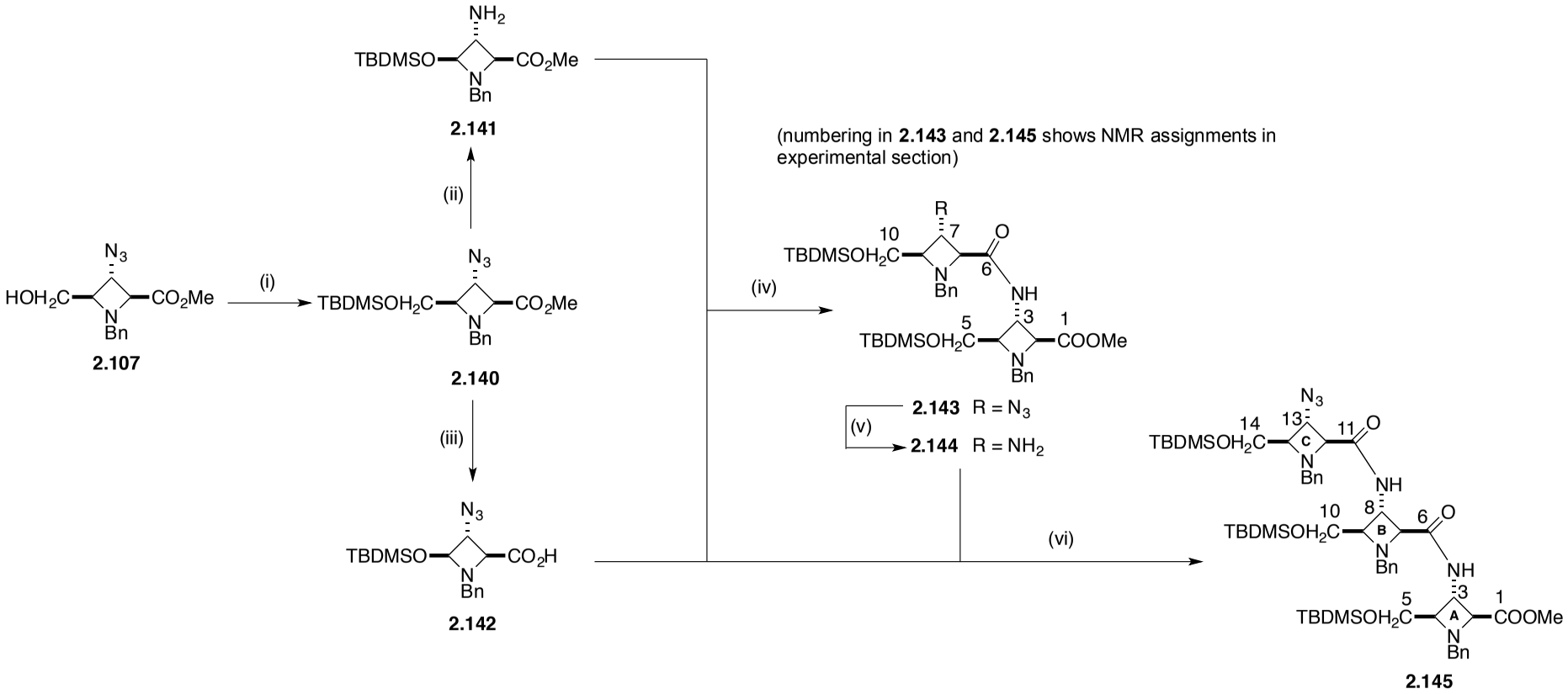
The replacement of 3-hydroxy group in azetidine **2.23L** with fluoride or azido/acetamido groups avoided the instability of β-hydroxy-carbonyl moiety to retroaldol fragmentation. Two dipeptides and one tripeptide were synthesized to show the potential application of these stable azetidine building blocks as novel peptidomimetics.



(i) TBDMSCl, imidazole, DMF, 95%; (ii) K_2CO_3, water/1,4-dioxane, 1:1; (iii) $MeNH_2$, $CaCl_2$, MeOH, 100%; (iv) Pd/C, H_2, water/1,4-dioxane, 1:2; (v) **2.136**, **2.138**, HBTU, DMF, 45% from **2.135**.

Scheme 2.18 Synthesis of dipeptide **2.139**

As shown in Scheme 2.18, the protection of primary hydroxyl in **2.63L** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole gave **2.135** in a yield of 95%. Hydrolysis of **2.135** with potassium carbonate in aqueous 1,4-dioxane afforded free acid **2.136** without purification. Another portion of ester **2.135** was treated with methylamine and calcium chloride to access the corresponding amide **2.137** (100%). After removal of the *N*-benzyl group by hydrogenolysis, the crude amine **2.138** and acid **2.136** were treated with *N*,*N*,*N*',*N*'-tetramethyl-O-(1H-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU) in DMF to yield the stable dipeptide **2.139** (45% from **2.135**).



(i) TBDMSCl, imidazole, DMF, 96%; (ii) Pd/C, H_2, water/1,4-dioxane, 1:1; (iii) K_2CO_3, water/1,4-dioxane, 1:1; (iv) **2.141**, **2.142**, HBTU, DMF, 78% from **2.140**; (v) Pd/C, H_2, water; (iv) **2.144**, **2.142**, HBTU, DMF, 72% from **2.143**;

Scheme 2.19 Synthesis of dipeptide **2.143** and tripeptide **2.145**

3-Azido azetidine **2.107** is a suitable scaffold for the incorporation of a β-amino acid into peptides. Oxetane β-amino acids are known to have a predisposition to induce novel secondary structures in small peptides.[38] **2.107** was protected by TBDMSCl to afford **2.140** (Scheme 2.19). Basic hydrolysis of **2.140** by potassium carbonate gave the free acid **2.142**. Hydrogenation of **2.140** for a short time (30 min) generated the primary amine **2.141** without affecting *N*-benzyl group. Then the coupling of **2.141** and **2.142** by HBTU gave dipeptide **2.143** (78% from **2.140**). After the hydrogenation of dipeptide **2.143** for 3 h, the free amine dipeptide **2.144** was coupled with acid **2.142** to afford tripeptide **2.145** in high yield (72% from **2.143**). Heteronuclear multiple-bond correlation relationship (HMBC) confirmed the ring connections in **2.145** (Appendix 1). Unlike 3-hydroxy azetidine carboxylic acid derivatives, the corresponding amides are not vulnerable to ring fragmentation. Such systems are likely to be useful peptide scaffolds. The conformations of short oligomers **2.139**, **2.143** and **2.145** will be studied in the next stage.

2.3.2 Biological activity

All bioassays were conducted by Atsushi Kato's group (University of Toyama, Japan).

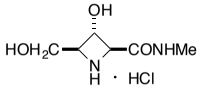
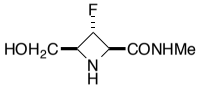
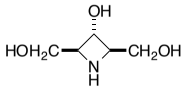
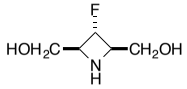
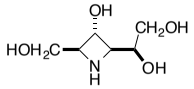
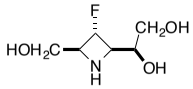
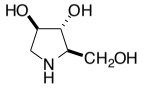
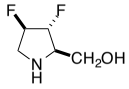
2.3.2.1 Glycosidase inhibition

Fluoro, acetamido and hydroxyl azetidines described in this chapter were tested to compare their inhibition effects against various glycosidases. The procedures have been described in a recent report.[39]

Only a few fluorinated iminosugars have been reported as potent glycosidase inhibitors.[29-30] In

this project, replacement of hydroxyl groups in iminosugars by fluorine group invariably reduced glycosidase inhibition activity (Table 2.1). Whilst 3-hydroxy azetidine amide **2.23L** is a specific inhibitor of β-*N*-acetylglycosaminidases, its 3-fluoro equivalent **2.75** showed no inhibition of any glycosidases. Although *meso*-azetidine triol **2.22**[7] was a potent inhibitor of α,α-trehalase (IC_{50} 30 μM) and a good inhibitor to yeast α-glucosidase (IC_{50} 9.5 μM), no inhibition of any glycosidases was observed in *meso*-3-fluoro azetidine **2.73**. Moreover, neither hexitol **2.147**[7] nor its fluoro equivalent **2.93** were potent glycosidase inhibitors. DAB **2.86D** is a naturally occurring potent glycosidase inhibitor. Unfortunately, its difluoro analogue **2.83** gave negative results for all glycosidases. In addition, all the *N*-benzyl derivatives of these fluoro iminosugars showed no inhibition. As shown in Table 2.2, the 3-acetamidoazetidines (**2.106**, **2.105**, **2.111**, **2.110**) also showed no inhibition of any *N*-acetyl-hexosaminidases. However, **2.52L** and **2.129**, which can be seen as an analogue of amide **2.23L** and DMDP **2.53**, were found to be weak but specific inhibitors of β-*N*-acetylglucosaminidases (Table 2.2).

Table 2.1 Concentration of fluoro iminosugars gaving 50% inhibition of various glycosidases (IC_{50}, μM)

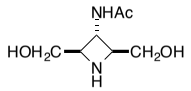
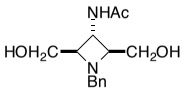
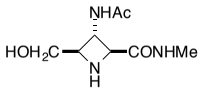
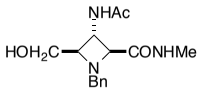
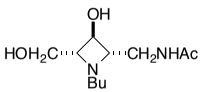
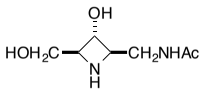
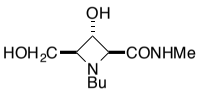
	 **2.23L**	 **2.75**	 **2.22**	 **2.73**	 **2.146**	 **2.93**	 DAB **2.86D**	 **2.83**
α-Mannosidase								
Jack bean	[a]NI [b](0%)	NI (4.7%)	NI (3.6%)	NI (9.2%)	NI (7.2%)	NI (0%)	**320**	NI (3.2%)
α,α-Trehalase								
Porcine kidney	NI (3%)	NI (3.0%)	**30**	NI (48.2%)	NI (0%)	NI (0%)	**61**	NI (3.9%)
Amyloglucosidase								
Aspergillus niger	NI (0%)	NI (0%)	**758**	NI (5.0%)	NI (2.4%)	NI (6.1%)	**362**	NI (2.9%)
Rhizopus Sp	NI (0%)	NI (0%)	**274**	NI (7.6%)	NI (1.1%)	NI (0%)	**160**	NI (17.3%)
β-*N*-Acetylglucosaminidase								
Human placenta	**4.3**	NI (23.9%)	NI (7.4%)	[c]ND	ND	ND	ND	ND
Bovine kidney	**2.7**	NI (20.6%)	NI (8.9%)	NI (9.4%)	**109**	NI (12.2%)	NI (3.4%)	NI (0.8%)
Jack beans	**4.2**	NI (14.8%)	NI (14.8%)	NI (0.5%)	**358**	NI (0.7%)	NI (15.6%)	NI (0%)
HL60	**5.2**	NI (5.1%)	NI (5.1%)	NI (11.2%)	**315**	NI (3.1%)	NI (3.1%)	NI (11.2%)
Aspergillus oryzae	**64**	NI (0%)	NI (0%)	ND	ND	ND	ND	ND
α-*N*-Acetylgalactosaminidase								
Chicken liver	NI (0%)	NI (2.2%)	NI (2.2%)	NI (24.2%)	NI (6.6%)	NI (3.1%)	NI (0%)	NI (22.0%)
β-*N*-Acetylgalactosaminidase								
HL60	**20**	NI (1.8%)	NI (2.1%)	ND	ND	ND	ND	ND
Aspergillus oryzae	**36**	NI (0.8%)	NI (5.8%)	ND	ND	ND	ND	ND

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

[b]( ): Inhibition % at 1000 μM

[c]: Not determined

Table 2.2 Concentration of 3-acetamido and 3-hydroxy iminosugars gaving 50% inhibition of various *N*-Acetylhexosaminidases ($IC_{50,}$ μM)

	 **2.106**	 **2.105**	 **2.111**	 **2.110**	 **2.127**	 **2.52L**	 **2.129**
β-*N*-Acetylglucosaminidase							
Human placenta	NI (11.7%)	NI (26.5%)	NI (6.0%)	NI (22.6%)	NI (27.1%)	**378**	**37**
Bovine kidney	NI (18.6%)	NI (26.5%)	NI (9.0%)	NI (28.2%)	NI (11.0%)	**217**	**28**
Jack beans	NI (9.5%)	NI (0.7%)	NI (2.9%)	NI (15.2%)	NI (19.0%)	**376**	**27**
HL60	NI (16.6%)	NI (28.4%)	NI (3.9%)	NI (14.5%)	NI (30.7%)	**358**	**39**
Aspergillus oryzae	NI (3.9%)	NI (3.9%)	NI (0%)	NI (3.7%)	NI (46.8%)	NI (5.6%)	**156**
α-*N*-Acetylgalactosaminidase							
Chicken liver	NI (0%)	NI (0%)	NI (0%)	NI (15.5%)	NI (4.3%)	NI (13.6%)	NI (0%)
β-*N*-Acetylgalactosaminidase							
HL60	NI (7.7%)	NI (11.6%)	NI (3.9%)	NI (11.0%)	ND	ND	**166**
Aspergillus oryzae	NI (2.3%)	NI (3.8%)	NI (1.3%)	NI (20.4%)	ND	ND	**119**

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

[b]( ): Inhibition % at 1000 μM

2.3.2.2 Inhibition of human cancer cells growth

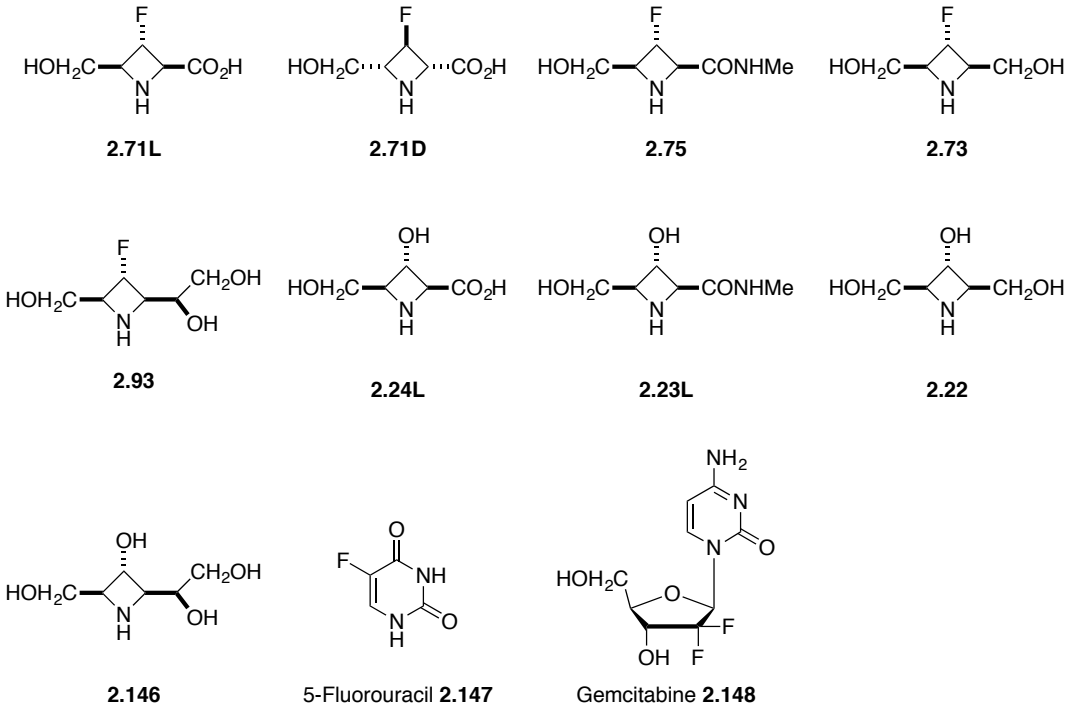


Figure 2.21 Compounds assayed for inhibition of pancreatic cancer growth

Fluoro compounds (**2.71LD**, **2.75**, **2.73**, **2.93**) and their hydroxy equivalents (**2.24L**, **2.23L**, **2.22**, **2.146**) were screened for growth inhibition effects against a human pancreatic carcinoma cell line (PNAC-1) (details of procedures see published paper[40]). Two cancer cell growth inhibitors, 5-fluorouracil **2.147** and gemcitabine **2.148**, were used as positive controls (Figure 2.21). Interestingly, preliminary results showed only the *meso*-azetidine diol **2.73** significantly inhibited cancer cell growth. This result was confirmed with the samples of **2.73** synthesized from two different routes (Scheme 2.7) while its *N*-benzyl analogue **2.72** showed no activity. In contrast, the 3-hydroxy equivalent **2.22** was a potent inhibitor of several glycosidase (Table 2.1) while unable to inhibit cancer cell growth.

In the next stage, the *meso*-azetidine diol **2.73** was tested against different human cancer cell

lines (Table 2.3). Notably, it showed equivalent inhibition effect against PANC-1 (IC_{50} 165.3 ± 9.1 μM) compared to gemicitabine **2.113** (IC_{50} 122.9 ± 66.4 μM). More than that, **2.73** produced a wide inhibition spectrum against human liver carcinoma (Hep G2), human colon adenocarcinoma (SW480) and human breast adenocarcinoma cell line (MCF-7) with IC_{50} of 80.3 ± 6.2 μM, 194.7 ± 1.2 μM and 332.4 ± 50.2 μM respectively. It is worth noting that **2.73** showed no inhibition of any glycosidases (Table 2.1), suggesting it inhibits cancer growth *via* a mechanism other than glycosidase inhibition. Its cytotoxicity to healthy cells and the mechanism of cancer growth inhibition is waiting to be further explored.

Table 2.3 IC_{50} of **2.73**, **2.147**, **2.148** against human cancer cell lines

	IC_{50} (μM)			
	PANC-1	Hep G2	SW480	MCF-7
fluoroazetidine **2.73**	165.3 ± 9.1	80.3 ± 6.2	194.7 ± 1.2	332.4 ± 50.2
5-fluorouracil **2.147**	33.7 ± 11.6	22.5 ± 8.9	9.4 ± 4.1	3.1 ± 0.4
gemcitabine **2.148**	122.9 ± 66.4	2.6 ± 0.1	2.8 ± 0.1	3.1 ± 0.2

2.4 Conclusions

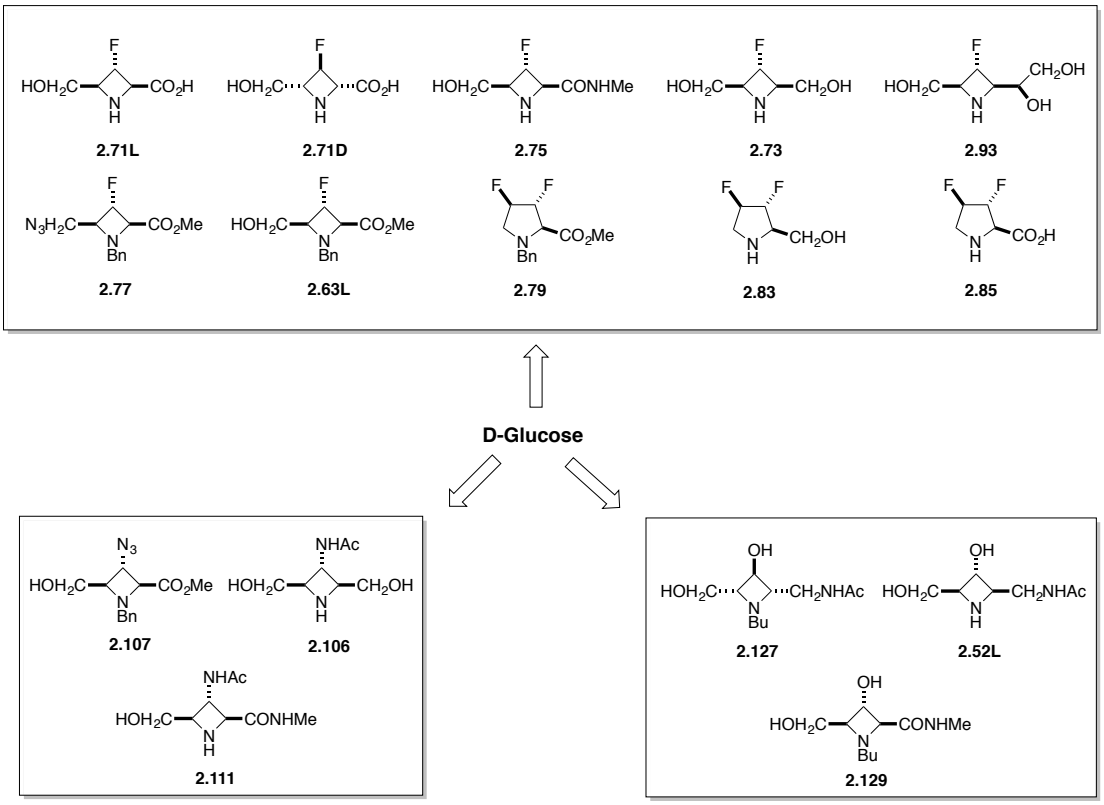


Figure 2.22 Synthesized targets

In this project, effective approaches were developed to access a number of novel 3-fluoro, 3-azido, 3-azetamido and 3-hydroxy azetidines (**2.71LD**, **2.75**, **2.73**, **2.93**, **2.77**, **2.63L**, **2.107**, **2.106**, **2.111**, **2.127**, **2.52L**, **2.129**) from D-glucose (Figure 2.22). A ring expansion reaction with XtalFluor salts enables the effective transformation of fluoro azetidine **2.63L** to difluoro proline methyl ester **2.79** in a yield of 84%. Further manipulation of **2.79** gave targets **2.83** and **2.85**. **2.83** was a difluoro analogue of DAB **2.86D**, a naturally occurring glycosidase inhibitor that was intensively studied; **2.85** was the first synthesized 3,4-difluoro-L-proline. Azetidine amino acids (**2.71LD**), amide **2.75**, methyl esters (**2.77**, **2.63L**, **2.107**) and difluoro proline **2.85** can be useful building blocks in the studies of peptide synthesis, protein conformations and peptidomimetics. To confirm this, three short oligomers of azetidine amino acids (**2.139**, **2.143**,

2.145) have been synthesized.

Glycosidase inhibition assays showed no fluorinated targets were potent inhibitors of any glycosidases. The same results were observed for all 3-acetamidoazetidines. Two 3-hydroxy azetidines **2.52L** and **2.129** could inhibit β-*N*-acetyl-glucosaminidase with IC_{50} 217 – 378 μM and 27 – 156 μM respectively. More interesting results were observed in the study of cancer cells growth inhibition. The screen of fluoro azetidines for the inhibition of human cancer cell lines indicated the *meso*-diol **2.73**, which cannot inhibit any glycosidases, could effectively decrease the growth rate of cancer cells. In further studies, **2.73** was proved to inhibit the growth of various cancer cell lines and its IC_{50} (165.3 ± 9.1) against PANC-1 is comparable with that (122.9 ± 66.4) of gemcitabine **2.147**, a chemotherapy drug against pancreatic cancer. Its cytotoxicity to normal cells and the mechanisms of cancer inhibition is currently under investigation in Atsushi Kato's group (University of Toyama, Japan).

2.5 Experimental

General Experimental

All commercial reagents were used as supplied. Solvents were used as supplied (Analytical or HPLC grade), without prior purification. Thin layer chromatography (TLC) was performed on aluminium sheets coated with 60 F_{254} silica. Plates were visualised using a 0.2% w/v cerium (IV) sulfate and 5% ammonium molybdate solution in 2 M sulfuric acid. Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations of the protected sugars were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm; optical rotations

are quoted in deg•cm^2•g^{-1} at concentrations (*c*) in g•100 mL^{-1}. Infrared spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform spectrophotometer using thin films on a diamond ATR surface (thin film). Only the characteristic peaks are quoted. Low resolution mass spectra (m/z) were recorded on an Agilent 6120 spectrometer and high resolution mass spectra (HRMS m/z) on a Bruker microTOF mass analyzer using electrospray ionization (ESI). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AMX 500 (^{1}H: 500 MHz and ^{13}C: 125.7 MHz) and Bruker AVIII 400 HD nanobay and Bruker DQX 400 spectrometers (^{1}H: 400 MHz and ^{13}C: 100.6 MHz) in the deuterated solvent stated. All chemical shifts (δ) are quoted in ppm and coupling constants (*J*) in Hz. Residual signals from the solvents were used as an internal reference, except in the case of deuterium oxide, where acetonitrile was used as the reference.

(General experimentals are same in all chapters.)

2.5.1 Synthesis of 3-fluoro-azetidine and 3,4-difluoro-proline derivatives

3-Deoxy-1,2;5,6-di-*O*-isopropylidene-3-fluoro-α-D-glucofuranose 2.58

Triflic anhydride (11.0 mL, 38.1 mmol) was added dropwise to a solution of 1,2;5,6-di-*O*-isopropylidene-D-allose **2.56** (5.30 g, 20.3 mmol) and anhydrous pyridine (10 mL, 76.7 mmol) in dichloromethane (25 mL) at -20 °C. After 1.5 h, TLC (cyclohexane/ethyl acetate, 1:1) indicated the consumption of the starting material (R_f 0.30) and the formation of one major product (R_f 0.64). The reaction mixture was diluted with DCM (40 mL) and washed with HCl (2 M, aq. 3 x 40 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed in *vacuo* to give the crude triflate **2.57** (7.5 g) as yellow crystalline solid.

Caesium fluoride (9.10 g, 60.0 mmol) was added in one portion to a solution of the crude triflate

(7.5 g) in *tert*-butanol (30 mL). The reaction mixture was stirred at 80 °C for 26 h until TLC (cyclohexane/ethyl acetate, 2:1) indicated consumption of the triflate (R_f 0.54) and formation of a new product (R_f 0.61). The reaction mixture was diluted with ethyl acetate (50 mL), washed with water/$NaHCO_3$ (sat. aq., 1:1, 50 mL) and brine (50 mL) sequentially and the aqueous layer was back extracted with DCM (3 x 50 mL). The combined organics were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/ethyl acetate, 6:1 to 4:1) to give the title compound **2.58** as clear oil (4.70 g, 89%). HRMS (ESI+ve): found 285.1110 $[M + Na]^+$; $C_{12}H_{19}FNaO_5^+$ requires 285.1109; $[\alpha]_D^{20}$ -19.7 (*c* 1.06, $CHCl_3$), [lit.[41] $[\alpha]_D^{20}$ -37.0 (*c* 1.00, $CHCl_3$)]; ν_{max} (thin film): fingerprint region only; δ_H ($CDCl_3$, 400 MHz): 1.32 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.50 (3H, s, CH_3), 4.03 (1H, dd, H6, $J_{6,5}$ 4.8, J_{gem} 8.8), 4.10 (1H, ddd, H4, $J_{4,3}$ 2.2, $J_{4,5}$ 8.3, $J_{4,F}$ 29.1), 4.12 (1H, dd, H6', $J_{6',5}$ 6.1, J_{gem} 8.8), 4.28 (1H, ddd, H5, $J_{5,6}$ 4.9, $J_{5,6'}$ 6.1, $J_{5,4}$ 8.3), 4.69 (1H, dd, H2, $J_{2,1}$ 3.8, $J_{2,F}$ 10.6), 5.01 (1H, dd, H3, $J_{4,3}$ 2.2, $J_{3,F}$ 49.9), 5.95 (1H, d, H1, $J_{1,2}$ 3.7); δ_C ($CDCl_3$, 100 MHz): 25.1 (CH_3), 26.2 (CH_3), 26.7 (CH_3), 27.0 (CH_3), 67.2 (C6), 72.0 (d, C5, $J_{5,F}$ 7.0), 80.6 (d, C4, $J_{4,F}$ 19.1), 82.5 (d, C2, $J_{2,F}$ 33.4), 93.8 (d, C3, $J_{3,F}$ 184.4), 105.2 (C1), 109.5 ($C(CH_3)_2$), 112.4 ($C(CH_3)_2$); δ_F ($CDCl_3$, 376 MHz): -207.6 (ddd, $J_{F,2}$ 10.8, $J_{F,4}$ 29.2, $J_{F,3}$ 49.8); *m/z* (ESI+ve): 263 ($[M + H]^+$, 100%), 285 ($[M + Na]^+$, 72%).

3-Deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose 2.64

A solution of the diacetonide **2.58** (4.20 g, 16.0 mmol) in methanol (20 mL) and 1% aqueous sulphuric acid (20 mL) was stirred at room tempterature for 18 h after which TLC (ethyl acetate) indicated that the disappearance of starting material (R_f 0.88) and the formation of a product (R_f 0.65). The reaction mixture was neutralized with triethylamine and the solvent was removed in

vacuo to give a residue that was purified by column chromatography (cyclohexane/ethyl acetate, 3:1 to 0:1) to form the monoacetonide **2.64** (3.55 g, 100%).

HRMS *m/z* (ESI+ve): found 245.0794 $[M + Na]^+$, $C_9H_{15}FNaO_5^+$ requires 245.0796; $[\alpha]_D^{20}$ -18.5 (*c* 0.80, $CHCl_3$); ν_{max} (thin film): 3411 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.33 (3H, s, CH_3), 1.50 (3H, s, CH_3), 2.68 (2H, s, OH), 3.75 (1H, ddd, H6, *J* 0.5, $J_{6,5}$ 5.1, J_{gem} 11.5), 3.86 (1H, dd, H6', $J_{6',5}$ 3.2, J_{gem} 11.5) 3.96 (1H, ddd, H5, $J_{5,6'}$ 3.3, $J_{5,6}$ 5.3, $J_{5,4}$ 8.7), 4.16 (1H, ddd, H4, $J_{4,3}$ 2.2, $J_{4,5}$ 8.8, $J_{4,F}$ 29.3), 4.70 (1H, dd, H2, $J_{2,1}$ 3.9, $J_{2,F}$ 10.8), 5.09 (1H, dd, H3, $J_{3,4}$ 2.2, $J_{3,F}$ 49.9), 5.96 (1H, d, H1, $J_{1,2}$ 3.7); δ_C ($CDCl_3$, 100 MHz): 26.2 (CH_3), 26.6 (CH_3), 64.1 (C6), 68.3 (d, C5, $J_{5,F}$ 6.4), 79.7 (d, C4, $J_{4,F}$ 19.1), 82.4 (d, C2, $J_{2,F}$ 31.8), 94.1 (d, C3, $J_{3,F}$ 182.8), 105.1 (C1), 112.4 ($C(CH_3)_2$); δ_F ($CDCl_3$, 376 MHz): -208.0 (ddd, $J_{F,2}$ 10.8, $J_{F,4}$ 29.2, $J_{F,3}$ 49.8); *m/z* (ESI+ve): 245 ($[M+Na]^+$, 100%).

3-Deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-xylofuranose 2.65

Sodium periodate (14.5 g, 67.6 mmol) was added in portions to a solution of diol **2.64** (12.5 g, 56.3 mmol) in 1,4-dioxane/water (2:1, 60 mL). The reaction mixture was stirred at room temperature for 3 h until TLC (cyclohexane/ethyl acetate, 1:1) showed the consumption of starting material (R_f 0.15) and the formation of a new major product (R_f 0.31) after which time ethanol (50 mL) was added and stirred for a further 20 min. The white solid formed was removed by filtration and sodium borohydride (2.13 g, 56.3 mmol) was added to the stirred reaction mixture. After 2 h, the formation of the desired product and the consumption of the intermediate aldehyde were confirmed by mass spectrometry ($[M + MeOH + Na^+]$ 245). The reaction mixture was adjusted to pH 7 by addition of acetic acid. The mixture was filtered and concentrated under reduced pressure to give a residue that was purified by column

chromatography (cyclohexane/ethyl acetate, 6:1 to 1:1) to afford the title compound **2.65** (9.90 g, 92%). HRMS *m/z* (ESI+ve): found 215.0686 [M + Na]$^+$; $C_8H_{13}FNaO_4^+$ requires 215.0690; $[\alpha]_D^{20}$ -25.1 (*c* 0.95, $CHCl_3$), [lit.[42] $[\alpha]_D^{20}$ -17.1 (*c* 1.06, DCM)]; ν_{max} (thin film): 3437 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.32 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.98 (1H, s, OH), 3.88 (1H, dd, H5, $J_{5,4}$ 5.5, J_{gem} 11.7), 3.93 (1H, ddd, H5', J 1.2, $J_{5',4}$ 6.6, J_{gem} 11.7), 4.35 (1H, dddd, H4, $J_{4,3}$ 2.3, $J_{4,5}$ 5.5, $J_{4,5'}$ 6.6, $J_{4,F}$ 30.2), 4.70 (1H, dd, H2, $J_{2,1}$ 3.9, $J_{2,F}$ 11.2), 4.97 (1H, dd, H3, $J_{3,4}$ 2.4, $J_{3,F}$ 50.4), 5.99 (1H, d, H1, $J_{1,2}$ 3.7); δ_C ($CDCl_3$, 100 MHz): 26.2 (CH_3), 26.6 (CH_3), 59.8 (d, C5, $J_{5,F}$ 9.5), 80.2 (d, C4, $J_{4,F}$ 19.1), 82.8 (d, C2, $J_{2,F}$ 33.4), 94.1 (d, C3, $J_{3,F}$ 184.4), 104.8 (C1), 112.3 ($C(CH_3)_2$); δ_F($CDCl_3$, 376 MHz): -208.7 (ddd, $J_{F,2}$ 11.4, $J_{F,4}$ 30.2, $J_{F,3}$ 50.4); *m/z* (ESI+ve): 215 ([M+Na]$^+$, 100%).

3-Deoxy-3-fluoro-D-xylose 2.66

DOWEX® 50WX8-200 (2.00 g) was added to a solution of monoacetonide **2.65** (9.90 g, 51.6 mmol) in 1,4-dioxane/water (1:1, 60 mL). The reaction mixture was stirred at 80 °C for 18 h, after which TLC analysis (ethyl acetate) indicated the disappearance of starting material (R_f 0.74) and the formation of a single product (R_f 0.29). The reaction mixture was filtered and the solvent removed *in vacuo* to give a residue that was purified by column chromatography (cyclohexane/ethyl acetate, 4:1 to 10% methanol in ethyl acetate) to give the unprotected xylose **2.66** (6.90 g, 88%) as a white solid in a 3:2, α:β ratio.

HRMS *m/z* (ESI+ve): found 175.0383 [M + Na]$^+$; $C_5H_9FNaO_4^+$ requires 175.0377; m.p. 106 - 108 °C, [lit.[43] m.p. 127 °C]; ν_{max} (thin film): 3284 (br, OH); δ_H (CD_3OD, 400 MHz): 3.17 - 3.23 (1H, ddd, H5β, J 1.2, J 10.8, J_{gem} 11.6), 3.28 - 3.36 (1H, m, H2β), 3.51 - 3.61 (2H, m, H2α, H5α), 3.65 - 3.78 (3H, m, H4α, H4β, H5'α), 3.84 (1H, dt, H5'β, $J_{5',4}$ 6.1, J_{gem} 11.5), 4.16 (1H, dt, H3β, $J_{3,2} = J_{3,4}$ 8.8,

$J_{3,F}$ 53.1), 4.42 (1H, d, H1β, $J_{1,2}$ 7.8), 4.44 (1H, dt, H3α, $J_{3,2}$ = $J_{3,4}$ 8.4, $J_{3,F}$ 54.4), 5.07 (1H, t, H1α, $J_{1,2}$ = $J_{1,F}$ 3.7); δ_C (CD_3OD, 100 MHz): 61.8 (d, C5α, $J_{5,F}$ 8.0), 65.7 (d, C5β, $J_{5,F}$ 9.5), 69.6 (d, C4α, $J_{4,F}$ 19.1), 69.9 (d, C4β, $J_{4,F}$ 17.5), 72.2 (d, C2α, $J_{2,F}$ 15.9), 74.5 (d, C2β, $J_{2,F}$ 17.5), 94.4 (d, C1α, $J_{1,F}$ 11.1) 96.5 (d, C3α, $J_{3,F}$ 179.6) 98.2 (d, C3β, $J_{3,F}$ 182.8) 98.4 (d, C1β, $J_{1,F}$ 12.7); δ_F (CD_3OD, 376 MHz): -195.8 (ddt, Fβ, *J* 6.7, $J_{F,2}$ = $J_{F,4}$ 13.6, $J_{F,3}$ 52.8), -201.3 – -201.5 (m, Fα); *m/z* (ESI+ve): 175 ($[M + Na]^+$, 100%).

3-Deoxy-3-fluoro-1,2,4-tri-*O*-acetyl-D-xylopyranose 2.67

A solution of xylose **2.66** (6.90 g, 45.4 mmol) in acetic anhydride/pyridine (1:1, 60 mL) was stirred at room temperature for 14 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the consumption of the starting material (R_f 0.00) and the formation of a single major product (R_f 0.71). The solvent was concentrated under reduced pressure, the residue dissolved in ethyl acetate (60 mL) and washed sequentially with HCl (2 M, aq., 2 x 60 mL), $NaHCO_3$ (sat. aq., 60 mL) and brine (60 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to afford a residue which was purified by column chromatography (cyclohexane/ethyl acetate, 7:1 to 5:1) to give triacetate xylopyranose **2.67** (11.3 g, 89%) as a white solid in an 8:5, α:β ratio of anomers.

HRMS *m/z* (ESI+ve): found 301.0696 $[M + Na]^+$, $C_{11}H_{15}FNaO_7^+$ requires 301.0696; m.p. 44 – 48 °C; ν_{max} (thin film): 1751 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 2.09 (3H, s, CH_3), 2.11 (12H, s, 4 x CH_3), 2.15 (3H, s, CH_3), 3.46 (1H, dd, H5β, $J_{5,4}$ 7.8, J_{gem} 12.2), 3.61 (1H, dd, H5α, $J_{5,4}$ 10.8, J_{gem} 11.2), 3.97 (1H, ddd, H5'α, *J* 4.6, *J* 6.3, J_{gem} 11.1), 4.18 (1H, dt, H5'β, $J_{5',4}$ = *J* 4.7, J_{gem} 12.2), 4.63 (1H, dt, H3β, $J_{3,2}$ = $J_{3,4}$ 7.7, $J_{3,F}$ 49.9), 4.82 (1H, dt, H3α, $J_{3,2}$ = $J_{3,4}$ 9.3, $J_{3,F}$ 53.3), 5.02-5.18 (4H, m, H2α, H2β, H4α, H4β),

5.69 (1H, d, H1β, $J_{1,2}$ 6.6), 6.27 (1H, t, H1α, $J_{1,2} = J_{1,F}$ 3.7); δ_C ($CDCl_3$,100 MHz): 20.5, 20.6, 20.7 (x 3), 20.8 (CH_3), 60.1 (d, C5α, $J_{5,F}$ 6.4), 61.8 (d, C5β, $J_{5,F}$ 5.6), 68.2 (d, C4β, $J_{4,F}$ 21.5), 68.8 (d, C4α, $J_{4,F}$ 17.5), 69.2 (d, C2β, $J_{2,F}$ 21.5), 69.6 (d, C2α, $J_{2,F}$ 17.5), 88.5 (d, C3α, $J_{3,F}$ 190.0), 89.1 (d, C3β, $J_{3,F}$ 188.4), 89.6 (d, C1α, $J_{1,F}$ 9.5), 91.6 (d, C1β, $J_{1,F}$ 8.7), 169.7, 169.1 (x2), 169.6, 169.7 (C=O); δ_F ($CDCl_3$, 376 MHz) -194.9 (ddt, Fβ, $J_{F,5}$ 4.6, $J_{F,2} = J_{F,4}$ 12.6, $J_{F,3}$ 50.4), -199.0 (dddt, Fα, $J_{F,1}$ 3.4, $J_{F,5}$ 4.6, $J_{F,2} = J_{F,4}$ 12.6, $J_{F,3}$ 53.1); *m/z* (ESI+ve): 301 ([M + Na]$^+$, 100%).

Methyl 3-deoxy-2,4-di-*O*-acetyl-3-fluoro-β-D-xylopyranoside 2.68

Hydrobromic acid (33% wt. in acetic acid, 24.3 mL, 98.9 mmol) was added dropwise to a solution of triacetate **2.67** (5.50 g, 19.8 mmol) in acetic acid and DCM (7:3, 50 mL) and the reaction was stirred at 5 °C for 5 h until TLC (cyclohexane/ethyl acetate, 1:1) showed the disappearance of starting material (R_f 0.63) and the formation of a major product (R_f 0.77). The reaction mixture was diluted with DCM (20 mL), washed successively with ice water (50 mL), cold $NaHCO_3$ (sat. aq., 50 mL) and ice water (50 mL). The solvent was concentrated *in vacuo* to afford the crude bromide as an orange solid.

Silver carbonate (9.30 g, 33.4 mmol) was added to a solution of the crude bromide in methanol (90 mL) and the reaction mixture was stirred in the dark at rt for 15 h until TLC (cyclohexane/ethyl acetate, 1:1) showed the formation of major product (R_f 0.47) and no remaining starting material. After filtration, the solvent was removed *in vacuo* to afford a residue that was purified by column chromatography (cyclohexane/ethyl acetate, 8:1 to 5:1) to afford the pure methyl diacetate **2.68** (3.00 g, 60%) as a white solid. HRMS *m/z* (ESI+ve): found 273.0747 [M + Na]$^+$; $C_{10}H_{15}FNaO_6^+$ requires 273.0745; m.p. 78 – 80 °C; $[\alpha]_D^{20}$ -71.3 (*c* 0.85, $CHCl_3$);

[lit.[44] m.p. 80 - 81 °C; $[\alpha]_D^{20}$ -73 (*c* 1.00, $CHCl_3$)]; ν_{max} (thin film): 1749 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 2.10 (3H, s, $COC\underline{H}_3$), 2.12 (3H, s, $COC\underline{H}_3$), 3.31 (1H, dd, H5, $J_{5,4}$ 8.3, J_{gem} 12.0), 3.46 (3H, s, OCH_3), 4.15 (1H, dt, H5', $J_{5',4} = J_{5',F}$ 4.9, J_{gem} 12.0), 4.36 (1H, d, H1, $J_{1,2}$ 6.4), 4.57 (1H, dt, H3, $J_{3,2} = J_{3,4}$ 7.9, $J_{3,F}$ 50.5), 4.99 - 5.08 (2H, m, H2, H4); δ_C ($CDCl_3$, 100 MHz): 20.7 (2 x $CO\underline{C}H_3$), 56.5 (OCH_3), 60.8 (d, C5, $J_{5,F}$ 6.4), 68.9 (d, C4, $J_{4,F}$ 20.7), 70.4 (d, C2, $J_{2,F}$ 20.7), 89.7 (d, C3, $J_{3,F}$ 188.4), 101.0 (d, C1, $J_{1,F}$ 8.0), 169.2 (C=O), 169.7 (C=O); δ_F ($CDCl_3$, 376 MHz): -194.7 (ddt, $J_{F,5'}$ 5.0, $J_{F,2} = J_{F,4}$ 12.9, $J_{F,3}$ 50.4); *m/z* (ESI+ve): 273 ($[M + Na]^+$, 100%).

Methyl 3-deoxy-3-fluoro-β-D-xylopyranoside 2.69

Sodium methoxide (177 mg, 3.3 mmol) was added to a solution of diacetate **2.68** (8.20 g, 32.8 mmol) in methanol (100 mL). The reaction mixture was stirred at 40 °C for 15 h when TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the disappearance of starting material (R_f 0.47) and the formation of a product (R_f 0.19). The solvent was concentrated *in vacuo* and the residue purified by column chromatography (cyclohexane/ethyl acetate, 1:1 to 0:1) to obtain the title compound **2.69** (5.27 g, 97%) as a white solid.

HRMS *m/z* (ESI+ve): found 189.0527 $[M + Na]^+$; $C_6H_{11}FNaO_4^+$ requires 189.0534; m.p. 100 – 102 °C; $[\alpha]_D^{20}$ -61.8 (*c* 0.43, MeOH); [lit.[44] mp. 102 – 104 °C; $[\alpha]_D^{20}$ -56.0 (*c* 1.00, MeOH)]; ν_{max} (thin film): 3395 (br, OH), 1749 (s, C=O); δ_H (CD_3CN, 400 MHz): 3.15 (1H, ddd, H5, $J_{5,F}$ 1.2, $J_{5,4}$ 10.2, J_{gem} 11.6), 3.35 (1H, dddd, H2, $J_{2,OH}$ 4.2, $J_{2,1}$ 7.5, $J_{2,3}$ 8.7, $J_{2,F}$ 14.2), 3.43 (3H, s, CH_3), 3.54 (1H, d, OH, *J* 4.8), 3.58 (1H, d, OH, *J* 4.8), 3.72 (1H, ddddd, H4, $J_{4,OH}$ 5.1, $J_{4,5'}$ 5.6, $J_{4,3}$ 8.3, $J_{4,5}$ 10.2, $J_{4,F}$ 13.4), 3.84 (1H, dt, H5', $J_{5',4} = J_{5',F}$ 5.9, J_{gem} 11.6), 4.11 (1H, dd, H1, *J* 1.0 $J_{1,2}$ 7.6), 4.17 (1H, dt, H3, $J_{3,2} = J_{3,4}$ 8.6, $J_{3,F}$ 52.8); δ_C (CD_3CN, 100 MHz): 57.3 (CH_3), 65.2 (d, C5, $J_{5,F}$ 8.8), 69.0 (d, C4, $J_{4,F}$ 17.5), 72.7 (d, C2,

$J_{2,F}$ 18.3), 97.7 (d, C3, $J_{3,F}$ 182.0), 104.9 (d, C1, $J_{1,F}$ 11.1); δ_F (CD_3CN, 376 MHz): -194.8 (ddt, $J_{F,5'}$ 5.7, $J_{F,2} = J_{F,4}$ 13.7, $J_{F,3}$ 52.6); *m/z* (ESI+ve): 189.1 ([M + Na]$^+$, 100%).

Methyl *N*-benzyl-3-fluoro-2,4-imino-2,3,4-trideoxy-β-L-ribo-pyranoside 2.60

Triflic anhydride (14.0 mL, 125.2 mmol) was added dropwise to a solution of diol **2.69** (5.20 g, 31.3 mmol) and pyridine (11.0 mL, 187.8 mmol) in DCM (50 mL) at -20 °C. The reaction mixture was stirred between -20 and -10 °C for 2 h, after which TLC (cyclohexane/ethyl acetate, 1:1) showed the consumption of starting material (R_f 0.12) and the formation of one major product (R_f 0.65). The reaction mixture was diluted with DCM (30 mL) and washed with HCl (2 M, aq., 2 x 70 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to afford the crude triflate (13.3 g) as an orange solid.

Benzylamine (13.0 mL, 156.5 mmol) was added to a solution of crude triflate (13.3 g) in acetonitrile (80 mL) and the reaction mixture was stirred at 65 – 70 °C for 2 h until TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the consumption of the starting material (R_f 0.65) and the formation of a single product (R_f 0.67). The solvent was concentrated *in vacuo* and the residue was purified by column chromatography (cyclohexane/ethyl acetate, 7:1 to 2:1) to obtain ribopyranoside **2.60** (7.20 g, 97%) as a light yellow oil.

HRMS *m/z* (ESI+ve): found 238.1239 [M + H]$^+$; $C_{13}H_{17}FNO_2^+$ requires 238.1238; $[\alpha]_D^{20}$ -13.7 (*c* 1.13, $CHCl_3$); ν_{max} (thin film): fingerprint region only; δ_H ($CDCl_3$, 400 MHz): 3.42 (3H, s, OC$\underline{H}_3$), 3.64 (1H, ddt, H4, $J_{4,5} = J_{4,5'}$ 1.7, $J_{4,2}$ 4.5, $J_{4,F}$ 11.0), 3.73 (1H, ddd, H2, *J* 1.3, $J_{2,4}$ 4.5, $J_{2,F}$ 11.6), 3.75 (1H, br-d, H5, J_{gem} 11.0), 4.09 (2H, s, C$\underline{H}_2$Ph), 4.33 (1H, ddd, H5', *J* 1.3, *J* 5.1, J_{gem} 10.9), 4.67 (1H, t, H1, *J* 1.2), 4.93 (1H, d, H3, $J_{3,F}$ 58.9), 7.15 - 7.37 (5H, m, Ar); δ_C ($CDCl_3$, 100 MHz): 51.3 ($\underline{C}H_2$Ph), 55.9

(OCH_3), 61.5 (d, C5, $J_{5,F}$ 5.6), 64.5 (d, C4, $J_{4,F}$ 18.3), 67.4 (d, C2, $J_{2,F}$ 18.3), 93.3 (d, C3, $J_{3,F}$ 210.6), 100.2 (d, C1, $J_{1,F}$ 8.0), 126.7, 128.2, 128.3, 138.9 (Ar); δ_F ($CDCl_3$, 376 MHz): -206.5 (br-d, $J_{F,3}$ 59.3) *m/z* (ESI+ve): 238.2 ($[M+H]^+$, 100%), 260 ($[M + Na]^+$, 2%).

Methyl *N*-benzyl-3-fluoro-2,4-imino-2,3,4-trideoxy-L-ribonate 2.63L

Ribopyranoside **2.60** (200 mg, 0.84 mmol) was dissolved in 2 M aq. HCl/1,4-dioxane (5:1, 2 mL). The reaction mixture was stirred at 40 °C for 21 h. After the consumption of starting material and the formation of aldehyde was confirmed by mass spectrometry ($[M + MeOH + Na]^+$ 278), the solvent was removed *in vacuo* to give a black solid. A solution of the black solid and potassium carbonate (349 mg, 2.53 mmol) was stirred at 0 °C under nitrogen atmosphere. Iodine (271 mg, 1.09 mmol), which was pre-dissolved in anhydrous methanol (4 mL) by sonication, was added dropwise into the reaction mixture at 0 °C, then the mixture was stirred under 5 °C for 2 h until the completion of reaction was confirmed by mass spectrum. Sodium sulfite (sat. aq., 5 mL) was poured into the reaction mixture and distilled water (40 mL) was added to dissolve the precipitate. The aqueous layer was extracted with ethyl acetate (4 x 50 mL), and the combined organic layers were dried ($MgSO_4$), filtrated and concentrated in *vacuo* to afford the crude product which was further purified by column chromatography (cyclohexane/ethyl acetate, 5:1 to 1:1) to yield the title compound **2.63L** as a brown oil (157 mg, 74%).

Large scale: Ribopyranoside **2.60** (1.565 g, 6.6 mmol) was dissolved in 2 M aq. HCl/1,4-dioxane (5:1, 120 mL). The reaction mixture was stirred at 40 °C for 21 h. After the consumption of starting material and the formation of aldehyde was confirmed by mass spectrometry ([M +

MeOH + Na]$^+$ 278), solvent was removed *in vacuo* to give a black solid. A solution of the black solid and potassium carbonate (2.74 g, 19.8 mmol) was stirred at 0 °C under nitrogen atmosphere in MeOH (60 mL). Iodine (2.18g, 8.6 mmol), which was pre-dissolved in anhydrous methanol (60 mL) by sonication, was added dropwise into the reaction mixture at 0 °C. Then the mixture was stirred at 0 °C for 2 h until the completion of reaction was confirmed by mass spectrum. Sodium sulfite (sat. aq., 30 mL) was poured into the reaction mixture and distilled water (100 mL) was added to dissolve the precipitate. The aqueous layer was extracted with ethyl acetate (4 x 100 mL), and the combined organic layers were dried ($MgSO_4$), filtrated and concentrated in *vacuo* to afford the crude product which was further purified by column chromatography (cyclohexane/ethyl acetate, 5:1 to 1:1) to yield the title compound **2.63L** as a brown oil (920 mg, 55%).

HRMS *m/z* (ESI+ve): found 276.1014 [M + Na]$^+$; $C_{13}H_{16}FNNaO_3^+$ requires 276.1006; $[\alpha]_D^{20}$ -43.8 (*c* 0.54, $CHCl_3$); ν_{max} (thin film): 3439 (br, OH), 1740 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 2.35 (1H, s, OH), 3.17 (1H, dd, H5, $J_{5,4}$ 2.7, J_{gem} 12.1), 3.37 (1H, ddddd, H4, *J* 0.6, *J* 2.2, *J* 2.7, *J* 4.9, *J* 21.7), 3.44 (1H, dd, H5', $J_{5',4}$ 2.1, J_{gem} 12.1), 3.70 (3H, s, OCH_3), 3.73 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.5), 3.78 (1H, ddd, H2, *J* 0.6, $J_{2,3}$ 4.8, $J_{2,F}$ 22.2), 3.99 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.5), 5.05 (1H, dt, H3, $J_{3,2}$ = $J_{3,4}$ 4.9, $J_{3,F}$ 55.9), 7.26 - 7.36 (5H, m, Ar); δ_C ($CDCl_3$, 100 MHz): 52.1 (OCH_3), 60.1 (d, C5, $J_{5,F}$ 4.0), 60.5 ($\underline{C}H_2$Ph), 68.0 (d, C2, $J_{2,F}$ 21.5), 69.7 (d, C4, $J_{4,F}$ 20.7), 84.4 (d, C3, $J_{3,F}$ 213.8), 128.0, 128.6, 129.3, 135.9 (Ar), 170.2 (d, C1, *J* 5.6); δ_F ($CDCl_3$, 376 MHz): -181.5 (dt, $J_{F,2}$ = $J_{F,4}$ 22.0 $J_{F,3}$ 55.7; *m/z* (ESI+ve): 254.2 ([M + H]$^+$, 100%), 276.1 ([M + Na]$^+$, 22%).

Methyl *N*-benzyl-3-fluoro-2,4-imino-2,3,4-trideoxy-L-ribonamide 2.74

Methylamine (0.3 mL, 2.6 mmol, in absolute ethanol) was added to a solution of methyl ester **2.63L** (32 mg, 0.13 mmol) and calcium chloride (14 mg, 0.13 mmol) in anhydrous methanol (1.5 mL). The reaction mixture was stirred at 40 °C for 2 h until the completion of reaction was confirmed by mass spectrometry ($[M+H]^+$ 253). The reaction mixture was poured onto ethyl acetate (50 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to yield the title compound **2.74** as a yellow oil which was used without further purification (24 mg, 74%).

HRMS *m/z* (ESI+ve): found 275.1166 $[M + Na]^+$; $C_{13}H_{17}FN_2NaO_2^+$ requires 275.1166; $[\alpha]_D^{20}$ -16.5 (*c* 1.22, $CHCl_3$); ν_{max} (thin film): 3337 (br, OH, NH), 1654 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 2.62 (3H, dt, C<u>H</u>$_3$, *J* 1.2, $J_{CH3,NH}$ 4.9), 3.36 - 3.46 (1H, m, H4), 3.40 (1H, dd, H5, $J_{5,4}$ 2.8, J_{gem} 11.9), 3.53 (1H, dd, H5', $J_{5,4}$ 3.1, J_{gem} 12.1), 3.71 (1H, ddt, H2, *J* 1.6, $J_{2,3}$ 4.9, $J_{2,F}$ 23.2), 3.75 (1H, d, C<u>H</u>$_2$Ph, J_{gem} 12.0), 3.80 (1H, d, C<u>H</u>$_2$Ph, J_{gem} 12.2), 4.81 (1H, dt, H3, $J_{3,2} = J_{3,4}$ 4.6, $J_{3,F}$ 56.2), 6.70 (1H, br-s, NH), 7.26 - 7.35 (5H, m, ArH); δ_C ($CDCl_3$, 100 MHz): 25.6 (<u>C</u>H$_3$), 60.8 (d, C5, $J_{5,F}$ 4.0), 70.3 (d, C4, $J_{2,F}$ 19.1), 61.6 (<u>C</u>H$_2$Ph), 70.5 (d, C2, $J_{4,F}$ 19.1), 85.8 (C3, d, $J_{3,F}$ 216.2), 128.2, 128.8, 129.3, 136.2 (Ar), 170.1 (C1); δ_F ($CDCl_3$, 376 MHz): -177.1 (dt, $J_{F,2} = J_{F,4}$ 23.4 $J_{F,3}$ 56.3); *m/z* (ESI+ve): 253 ($[M + H]^+$, 100%), 275 ($[M + Na]^+$, 25%).

Methyl 3-fluoro-2,4-imino-2,3,4-trideoxy-L-ribonamide 2.75

10% Palladium on charcoal (10 % wt., 5 mg) was added to a solution of protected riboamide **2.74** (24 mg, 0.095 mmol) in 1,4-dioxane/water (1:2, 3 mL). The reaction mixture was flushed with argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 3 h at room temperature under hydrogen until the completion of reaction was confirmed by mass spectrometry ($[M+H]^+$ 163). After filtration, the solvent was removed *in vacuo* to afford the title

compound **2.75** as light yellow oil that was used without further purification (14 mg, 90%).

HRMS *m/z* (ESI+ve): found 185.0696 $[M + Na]^+$; $C_6H_{11}FN_2NaO_2^+$ requires 185.0697; $[\alpha]_D^{20}$ -61.5 (*c* 0.69, MeOH); ν_{max} (thin film): 3275 (br, m, O-H, N-H), 1659 (s, C=O); δ_H (CD_3OD, 400 MHz): 2.81 (3H, s, C<u>H</u>$_3$), 3.74 (2H, s, H5), 4.28 (1H, br-d, H4, $J_{4,F}$ 16.8), 4.65 (1H, br-d, H2, $J_{2,F}$ 17.3), 5.10 (1H, br-d, H3, $J_{3,F}$ 55.5); δ_C (CD_3OD, 100 MHz): 26.0 (<u>C</u>H$_3$), 63.4 (d, C5, $J_{5,F}$ 4.0), 64.1 (d, C2, $J_{2,F}$ 16.0), 64.3 (d, C4, $J_{4,F}$ 16.0), 90.6 (d, C3, $J_{3,F}$ 214.0), 174.2 (C1); δ_F (CD_3OD, 376 MHz): -177.0 (dt, $J_{F,2} = J_{F,4}$ 19.8 $J_{F,3}$ 54.3); *m/z* (ESI+ve): 163 ($[M + H]^+$, 100%), 185 ($[M + Na]^+$, 16%).

_N_-Benzyl-3-fluoro-2,4-imino-2,3,4-trideoxy-L-ribonic acid 2.70L

Potassium carbonate (29 mg, 0.21 mmol) was added to a solution of methyl ester **2.63L** (40 mg, 0.16 mmol) in 1,4-dioxane/water (1:2, 3 mL). The reaction mixture was stirred at 40 °C for 18 h until the completion of reaction was confirmed by mass spectrometry. HCl (2 M, aq., 0.4 mL) was added to adjust the mixture to pH 4. The solvent was removed *in vacuo* to obtain the crude acid that was then loaded in 1,4-dioxane/water (1:2) onto a short column of DOWEX® 50WX8-200 (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After washing again with water and 1,4-dioxane and water (1:2), the pure product was released with aqueous ammonia (2 M). The solvent was then removed *in vacuo* to yield the title compound **2.70L** as a light yellow glass (22 mg, 43%).

HRMS *m/z* (ESI+ve): found 240.1020 $[M + H]^+$; $C_{12}H_{15}FNO_3^+$ requires 240.1030; $[\alpha]_D^{20}$ -13.5 (*c* 1.07, water); ν_{max} (thin film): 3070 (br, OH), 1630 (s, C=O); δ_H (Py-d_5, 400 MHz): 3.63 (1H, dq, H4, $J_{4,3} = J_{4,5}$ 4.5, $J_{4,F}$ 22.1), 3.73 (1H, dd, H5, $J_{5,4}$ 4.6, J_{gem} 11.7), 3.77 (1H, dd, H5', $J_{5',4}$ 4.0, J_{gem} 11.6), 3.95 (1H, d, C<u>H</u>$_2$Ph, J_{gem} 13.0), 4.15 (1H, dd, H2, $J_{2,3}$ 5.0, $J_{2,F}$ 23.3), 4.35 (1H, d, C<u>H</u>$_2$Ph, J_{gem} 13.0), 5.62

(1H, dt, H3, $J_{3,2}$ = $J_{3,4}$ 4.9, $J_{3,F}$ 57.0), 7.22 - 7.64 (5H, m, ArH), 7.87 (2H, br-s, OH); δ_C (Py-d_5, 100 MHz): 61.4 ($\underline{C}H_2Ph$), 62.4 (d, C5, $J_{5,F}$ 3.2), 69.6 (d, C2, $J_{2,F}$ 18.3), 70.7 (d, C4, $J_{4,F}$ 18.3), 88.0 (d, C3, $J_{3,F}$ 210.6), 128.1, 129.0, 130.6, 137.8 (Ar), 174.0 (C1); δ_F (Py-d_5, 376 MHz): -178.5 (dt, $J_{F,2}$ = $J_{F,4}$ 22.8, $J_{F,3}$ 56.8); *m/z* (ESI+ve): 240 ($[M + H]^+$, 100%), 262 ($[M + Na]^+$, 25%).

3-Fluoro-2,4-imino-2,3,4-trideoxy-L-ribonic acid [(2*R*,3*S*,4*S*)-3-fluoro-4-(hydroxy methyl)azetidine-2-carboxylic acid] 2.71L

10% Palladium on charcoal (10% wt., 5 mg) was added to a solution of *N*-benzyl protected ribonic acid **2.70L** (21 mg, 0.09 mmol) in 1,4-dioxane/water (2 mL, 1:2). The reaction was flushed with nitrogen, argon and hydrogen gas sequentially and stirred vigorously for 15 h at rt under hydrogen until mass spectrometry showed the completion of reaction. After filtration, the solvent was removed *in vacuo* to obtain a residue that was purified on a short column of DOWEX® 50WX8-200 (as illustrated above). The solvent was removed *in vacuo* to afford the title compound **2.71L** as a light yellow glass (11 mg, 82%).

HRMS *m/z* (ESI-ve): found 148.0409 $[M - H]^-$; $C_5H_7FNO_3^-$ requires 148.0415; $[\alpha]_D^{20}$ -30.6 (*c* 0.50, water); ν_{max} (thin film): 3233 (br, OH), 1630 (s, C=O); δ_H (D_2O, 400 MHz): 3.91 (1H, dd, H5, $J_{5,4}$ 3.7, J_{gem} 13.2), 3.97 (1H, dd, H5', $J_{5',4}$ 3.9, J_{gem} 13.2), 4.66 (1H, dq, H4, $J_{4,3}$ = $J_{4,5}$ = $J_{4,5'}$ 4.2, $J_{4,F}$ 19.3), 4.92 (1H, dd, H2, $J_{2,3}$ 4.8, $J_{2,F}$ 21.3), 5.31 (1H, dt, H3, $J_{3,2}$ = $J_{3,4}$ 4.8, $J_{3,F}$ 56.1); δ_C (D_2O, 100 MHz): 58.0 (d, C5, $J_{5,F}$ 4.0), 63.8 (d, C2, $J_{2,F}$ 24.6), 64.9 (d, C4, $J_{4,F}$ 26.2), 87.9 (d, C3, $J_{3,F}$ 210.6), 170.4 (C1); δ_F (D_2O, 376 MHz): -178.3 (dt, $J_{F,2}$ = $J_{F,4}$ 20.4, $J_{F,3}$ 56.1); *m/z* (ESI-ve): 148 ($[M - H]^+$, 100%).

***N*-Benzyl-3-fluoro-2,4-imino-2,3,4-trideoxy-*meso*-ribitol 2.72**

Method 1:

The bicyclic azetidine **2.60** (100 mg, 0.42 mmol) was dissolved in 2 M aq. HCl/1,4-dioxane (5:1, 1 mL). The reaction mixture was stirred at 40 °C for 23 h after which the consumption of starting material and the formation of aldehyde was confirmed by mass spectrometry ($[M + MeOH + Na]^+$ 278). The solvent was removed in *vacuo* to give a black glass.

Sodium borohydride (196 mg, 2.52 mmol) was added to a solution of the black residue in methanol (4 mL). After stirring at room temperature for 2 h, mass spectrometry ($[M + Na]^+$ 248) showed the completion of the reaction. The solvent was concentrated *in vacuo* to obtain a residue which was purified on a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral) to yield the desired diol **2.72** as a brown oil (39 mg, 40% over 2 steps).

Method 2:

Sodium borohydride (15 mg, 0.39 mmol) was added to a solution of methyl ester **2.60L** (100 mg, 0.39 mmol) in methanol (2 mL) at 0°C. After stirring at rt for 3 h, mass spectrometry (m/z 248) showed the completion of reaction. The solvent was removed *in vacuo* to obtain a residue that was purified on a short column of DOWEX® 50WX8-200 (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral) to yield the desired diol **2.72** as a brown oil (79 mg, 90%)

HRMS *m/z* (ESI+ve): found 248.1057 $[M + Na]^+$; $C_{12}H_{16}FNNaO_2^+$ requires 248.1057; ν_{max} (thin film): 3355 (br, OH); δ_H ($CDCl_3$, 400 MHz): 2.81 (2H, br-s, OH x 2), 3.25 (2H, ddt, H2(4), $J_{2,1} = J_{2,1'}$ 3.2, $J_{2,3}$ 5.1, $J_{2,F}$ 22.8), 3.36 (2H, dd, H1(5), $J_{1,2}$ 3.2, J_{gem} 11.7), 3.52 (2H, dd, H1' (5'), $J_{1',2}$ 2.8, J_{gem} 11.9), 3.78 (2H, s, C$\underline{H}_2$Ph), 4.93 (1H, dt, H3, $J_{3,2} = J_{3,4}$ 4.7, $J_{3,F}$ 56.8), 7.27-7.36 (5H, m, ArH); δ_C

($CDCl_3$, 100 MHz): 60.8 (d, C1(5), *J* 4.8), 61.2 ($\underline{C}H_2Ph$), 70.3 (d, C2(4), *J* 19.9), 83.9 (d, C3, *J* 207.4), 127.9, 128.6, 129.1 (ArCH), 137.0 (ArC); δ_F ($CDCl_3$, 376 MHz): -184.2 (dt, $J_{F,2} = J_{F,4}$ 22.9, $J_{F,3}$ 56.1); *m/z* (ESI+ve): 248 ($[M + Na]^+$, 100%).

3-Fluoro-2,4-imino-2,3,4-trideoxy-*meso*-ribitol 2.73

10% Palladium on charcoal (10% wt., 5 mg) was added to a solution of *N*-benzyl protected ribonic acid **2.72** (20 mg, 0.09 mmol) in 1,4-dioxane/water (1:2). The reaction was flushed with nitrogen, argon and hydrogen gas sequentially and stirred vigorously for 5 h at rt under hydrogen until mass spectrometry showed the completion of reaction ($[M + H]^+$ 136). After filtration, the solvent was concentrated *in vacuo* to obtain a residue which was purified by a short column of DOWEX® 50WX8-200 (as described above). The solvent was concentrated *in vacuo* to afford the title compound **2.73** as a light yellow oil (12 mg, 100%).

HRMS *m/z* (ESI+ve): found 158.0590 $[M + Na]^+$; $C_5H_{10}FNNaO_2^+$ requires 158.0588; ν_{max} (thin film): 3330 (br, OH); δ_H (D_2O, 400 MHz): 3.66 (4H, d, H1(5), $J_{1,2}$ 5.7), 3.90 (2H, dq, H2(4), $J_{2,1} = J_{2,3} = J$ 5.8, $J_{2,F}$ 21.4), 4.80 (1H, dt, H3, $J_{3,2} = J_{3,4}$ 5.7, $J_{3,F}$ 56.0); δ_C (D_2O, 100 MHz): 62.3 (d, C2(4), $J_{2,F}$ 20.0), 62.8 (d, C1(5), $J_{1,F}$ 3.8), 89.3 (d, C3, $J_{3,F}$ 209.8); δ_F (D_2O, 376 MHz): -174.8 (dt, $J_{F,2} = J_{F,4}$ 21.8, $J_{F,3}$ 56.4); *m/z* (ESI+ve): 136 ($[M + H]^+$, 100%).

Methyl *N*-benzyl-3-fluoro-2,4-imino-5-*O*-mesyl-2,3,4-trideoxy-L-ribonate 2.76

Mesyl chloride (0.05 mL, 0.60 mmol) was added to a solution of methyl ester **2.63L** (100 mg, 0.40 mmol) in pyridine (3 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h when TLC (cyclohexane/ethyl acetate, 1:1) showed the consumption of starting material (R_f 0.38) and the

formation of product (R_f 0.46). The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (cyclohexane/ethyl acetate, 5:1 to 2:1) to give the product **2.76** as a yellow oil (133 g, 100%).

HRMS *m/z* (ESI+ve): found 354.0777 $[M + Na]^+$; $C_{14}H_{18}FNNaO_5S^+$ requires 354.0782; $[\alpha]_D^{20}$ -22.1 (*c* 0.57, $CHCl_3$); ν_{max} (thin film): 1744 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 2.97 (3H, s, CH_3), 3.44 (1H, dq, H4, $J_{4,3} = J_{4,5} = J_{4,5}$ 4.6, $J_{4,F}$ 20.7), 3.66 (3H, s, CH_3), 3.73 (1H, dd, H2, $J_{2,3}$ 5.1, $J_{2,F}$ 21.3), 3.79 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.7), 3.93 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.5), 3.97 (1H, dd, H5, $J_{5,4}$ 4.2, J_{gem} 11.3), 4.10 (1H, dd, H5', $J_{5',4}$ 4.5, J_{gem} 11.6), 4.95 (1H, dt, H3, $J_{3,2} = J_{3,4}$ 5.0, $J_{3,F}$ 55.3), 7.25 - 7.36 (5H, m, ArH); δ_C ($CDCl_3$, 100 MHz): 37.6 (SO_2CH_3), 52.1 (OCH_3), 60.5 ($\underline{C}H_2$Ph), 66.4 (d, C4, $J_{4,F}$ 21.5), 67.7 (d, C2, $J_{2,F}$ 21.5), 67.9 (d, C5, $J_{5,F}$ 4.0), 84.5 (d, C3, $J_{3,F}$ 217.0), 128.0, 128.5, 129.6 (ArCH), 135.3 (ArC), 169.8 (C1, d, *J* 4.8); δ_F ($CDCl_3$, 376 MHz): -179.6 (dt, $J_{F,2} = J_{F,4}$ 20.9, $J_{F,3}$ 55.3); *m/z* (ESI+ve): 332 ($[M + H]^+$, 100%).

Methyl *N*-benzyl-5-azido-3-fluoro-2,4-imino-2,3,4,5-tetradeoxy-L-ribonate 2.77

Sodium azide (13 mg, 0.20 mmol) was added to a solution of mesylate **2.76** (50 mg, 0.15 mmol) in DMF (2 mL) and the reaction mixture was stirred at 60 °C for 26 h. After this time TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the disappearance of the starting material (R_f 0.48) and the formation of a single product (R_f 0.78). The reaction mixture was diluted with ethyl acetate (20 mL) and washed with 1:1 water/brine (sat., aq.) (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate, 7:1 to 5:1) to yield the title compound **2.77** as a light yellow oil (32 mg, 80%)

HRMS *m/z* (ESI+ve): found 301.1072 $[M + Na]^+$; $C_{13}H_{15}FN_4NaO_2^+$ requires 301.1071; $[\alpha]_D^{20}$ -61.7

(*c* 1.62, $CHCl_3$); ν_{max} (thin film): 2102 (s, N_3), 1743 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 3.05 (1H, dd, H5, $J_{5,4}$ 4.4, J_{gem} 13.2), 3.10 (1H, dd, H5', $J_{5',4}$ 4.4, J_{gem} 13.2), 3.33 (1H, ddq, H4, J 0.7, $J_{4,5} = J_{4,5'} = J_{4,3}$ 4.7, $J_{4,F}$ 21.2), 3.70 (3H, s, CH_3), 3.70 (1H, ddd, H2, J 0.7, $J_{2,3}$ 5.0, $J_{2,F}$ 21.5), 3.75 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.5), 4.00 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.7), 4.96 (1H, dt, H3, $J_{3,2}$=$J_{3,4}$ 5.0, $J_{3,F}$ 55.7), 7.30 - 7.35 (5H, m, ArH); δ_C ($CDCl_3$, 100 MHz): 51.5 (d, C5, $J_{5,F}$ 4.0), 52.1 (CH_3), 60.9 ($\underline{C}H_2$Ph), 67.4 (d, C4, $J_{4,F}$ 20.7), 68.0 (d, C2, $J_{2,F}$ 21.5), 85.3 (d, C3, $J_{3,F}$ 215.4), 127.9, 128.5, 129.7, 135.6 (Ar), 170.0 (d, C1, $J_{1,F}$ 5.6); δ_F ($CDCl_3$, 376 MHz): -179.7 (dt, $J_{F,2} = J_{F,4}$ 21.5, $J_{F,3}$ 55.7); *m/z* (ESI+ve): 301 ($[M + Na]^+$, 100%).

Methyl *N*-benzyl-3,5-difluoro-2,4-imino-2,3,4,5-tetradeoxy-L-ribonate 2.78

Method 1:

Cesium fluoride (100 mg, 0.65 mmol) was added to a solution of mesylate **2.76** (60 mg, 0.18 mmol) in *tert*-butanol (3 mL) at 60 °C. After stirring at 60 °C for 17 h, TLC showed the formation of one major product (R_f 0.55) and some remaining **2.76** (R_f 0.16). More caesium fluoride (55 mg, 0.36 mmol) was added and the mixture was stirred for a further 3 h at 80 °C. After removing solvent in vacuo, the mixture was diluted with brine (10 mL) and washed with DCM (3 x 10 mL). The organic phases were combined, dried ($MgSO_4$) and solvent was removed *in vacuo* to yield a crude that was purified by flash column chromatography (cyclohexane/ethyl acetate, 7:1 to 5:1) to obtain **2.78** with impurities (12.9 mg, 28%).

Method 2:

XtalFluor-E (66.9 mg, 0.30 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 44.7 mg, 0.30 mmol) were added into a solution of **2.63L** (50 mg, 0.20 mmol) in DCM (3 mL) at -78 °C. After stirring at -78 °C for 30 min, the mixture was stirred for a further 18 h until TLC

(cyclohexane/ethyl acetate, 1:1) showed the formation of one major products (R_f 0.81), one minor product (R_f 0.67) and some remaining starting material (R_f 0.45). TLC remained same after the addition of more XtalFluor-E (66.9 mg, 0.30 mmol) and DBU (44.7 mg, 0.30 mmol) with stirring for 3 h. The mixture was diluted with water (5 mL) and washed with DCM (3 x 5 mL). The organic phases were combined, dried ($MgSO_4$) and solvent was removed *in vacuo* to yield a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate, 7:1 to 5:1) to obtain **2.79** (11 mg, 22%) as a yellow oil and **2.78** (4 mg, 8%) as a yellow oil. NMR data showed **2.78** was not pure enough for full characterization. The data of **2.79** is given in the following experimental.

NMR and LRMS data of **2.78**: δ_H ($CDCl_3$, 400 MHz): 3.40 (1H, tq, H4, $J_{4,3}$ = $J_{4,5}$ = $J_{4,5'}$ 5.1, $J_{4,3F}$ = $J_{4,5F}$ 20.4), 3.50 (1H, s, CH_3), 3.71 (1H, dd, H2, $J_{2,3}$ 5.1, $J_{2,3F}$ 21.2), 3.77 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.8), 4.01 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.8), 4.15 (1H, ddd, H5, $J_{5,4}$ 4.5, $J_{5,5'}$ 10.5, $J_{5,5F}$ 24.5), 4.26 (1H, ddd, H5', $J_{5',4}$ 4.5, $J_{5,5'}$ 10.5, $J_{5,5F}$ 24.5), 4.97 (1H, dt, H3, $J_{3,2}$ = $J_{3,4}$ 5.1, $J_{3,3F}$ 55.8); δ_F ($CDCl_3$, 376 MHz): -184.5 - -184.7 (m, 3F), -186.5 - -186.8 (m, 5F); *m/z* (ESI+ve): 256 ($[M + H]^+$, 100%)

_N_-Benzyl-3_R_,4_R_-difluoro-L-proline methyl ester 2.79

A solution of azetidine methyl ester **2.63L** (289 mg, 0.79 mmol) in DCM (2 mL) was added dropwise to a solution of XtalFluor-M (289 mg, 1.19 mmol) and TEA•3HF (0.26 mL, 1.19 mmol) in DCM (2 mL) at -78 °C. After stirring for 1 h, the mixture was stirred at room temperature for 18 h until TLC (cyclohexane/ethyl acetate, 2:1) showed the disappearance of starting material (R_f 0.16) and the formation of major product (R_f 0.56). The mixture was diluted with half saturated $NaHCO_3$ (10 mL) and stirred for a further 20 min, before extraction with DCM (3 x 20

mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure to obtain a crude residue which was purified by flash column chromatography (cyclohexane/ethyl acetate, 7:1 to 6:1) to yield the desired product **2.79** as a yellow oil (169 mg, 84%).

HRMS *m/z* (ESI+ve): found 278.0958 $[M + Na]^+$; $C_{13}H_{15}F_2NNaO_2^+$ requires 278.0963; $[\alpha]_D^{20}$ -64.2 (*c* 0.31, $CHCl_3$); ν_{max} (thin film): 1746 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 2.85 (1H, dddd, H5, $J_{5,3F}$ 2.0, $J_{5,4}$ 5.0, J_{gem} 11.7, $J_{5,4F}$ 31.9), 3.26 (1H, ddt, H5', $J_{5',3} = J_{5',4}$ 1.4, J_{gem} 11.6, $J_{5',4F}$ 20.9), 3.51 (1H, dd, H2, $J_{2,3}$ 3.7, $J_{2,3F}$ 26.9), 3.66 (1H, d, C$\underline{H}_2$Ph, J_{gem} 13.1), 3.76 (3H, s, OCH_3), 4.05 (1H, d, C$\underline{H}_2$Ph, J_{gem} 13.2), 5.05 (1H, dddt, H4, $J_{4,3} = J_{4,5'}$ 1.3, $J_{4,5}$ 4.9, $J_{4,3F}$ 14.9, $J_{4,4F}$ 50.7), 5.27 (1H, dddt, H3, $J_{3,4} = J_{3,5'}$ 1.3, $J_{3,2}$ 3.7, $J_{3,4F}$ 16.6, $J_{3,3F}$ 50.1), 7.25-7.36 (5H, m, ArH); δ_C ($CDCl_3$,100 MHz): 52.4 (OCH_3), 56.2 (dd, C5, $J_{5,3F}$ 2.4, $J_{5,4F}$ 23.1), 57.5 ($\underline{C}H_2$Ph), 69.8 (dd, C2, $J_{2,3F}$ 0.8, $J_{2,4F}$ 26.2), 97.9 (dd, C4, $J_{4,3F}$ 29.0, $J_{4,4F}$ 182.4), 97.9 (dd, C3, $J_{3,4F}$ 32.7, $J_{3,3F}$ 186.4), 127.5, 128.4, 128.9, 136.7 (Ar), 170.4 (d, C1, $J_{1,F}$ 8.8); δ_F ($CDCl_3$, 376 MHz): -184.6 (ddddd, 3F, $J_{3F,5}$ 2.3, $J_{3F,4F}$ 8.0, $J_{3F,4}$ 14.9, $J_{3F,2}$ 26.3, $J_{3F,3}$ 50.4), -186.6 (ddddd, 4F, $J_{4F,3F}$ 7.9, $J_{4F,3}$ 16.4, $J_{4F,5'}$ 20.9, $J_{4F,5}$ 32.0, $J_{4F,4}$ 50.7); *m/z* (ESI+ve): 256 ($[M + H]^+$, 100%).

N-Benzyl-2*R*,3*R*-difluoro-2,4-imino-1,2,3,4-tetradeoxyl-D-arabinofuranose 2.82

Lithium aluminum hydride (1 M in THF) (0.24 mL, 0.24 mmol) was added dropwise to a solution of difluoroproline methyl ester **2.79** (30 mg, 0.120 mmol) in anhydrous THF at -78 °C. The reaction mixture was stirred for 1 h until TLC analysis (cyclohexane/ethyl acetate, 2:1) showed the consumption of the starting material (R_f 0.67) and formation of a major product (R_f 0.37). The reaction was quenched with NH_4Cl (sat. aq.) to pH 6 and extracted with ethyl acetate (2 x 15 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield pure

alcohol **2.82** as a clear oil which was used without further purification (18 mg, 66%).

HRMS *m/z* (ESI+ve): found 250.1008 $[M + Na]^+$; $C_{12}H_{15}F_2NNaO^+$ requires 250.1014; $[\alpha]_D^{20}$ -70.6 (*c* 0.90, MeOH); ν_{max} (thin film): 3348 (br, w, O-H); δ_H (CD_3OD, 400 MHz): 2.72 (1H, dddd, H1, $J_{1,3F}$ 3.0, $J_{1,2}$ 4.6, J_{gem} 12.1, $J_{1,2F}$ 36.7), 2.74-2.85 (1H, m, H4), 3.09 (1H, ddq, H1', *J* 1.0, J_{gem} 12.1, $J_{1',2F}$ 20.5), 3.48 (1H, d, C$\underline{H}_2$Ph, J_{gem} 13.2), 3.64 (1H, ddt, H5, *J* = *J* 0.7, $J_{5,4}$ 6.1, J_{gem} 11.5), 3.67 (1H, ddt, H5', *J* 1.1, $J_{5',4}$ 4.9, J_{gem} 11.6), 4.05 (1H, d, C$\underline{H}_2$Ph, J_{gem} 13.1), 4.91-5.11 (2H, m, H3, H2), 7.23-7.36 (5H, m, ArH); δ_C (CD_3OD,100 MHz): 58.3 (dd, C1, $J_{1,3F}$ 2.4, $J_{1,2F}$ 22.3), 59.5 ($\underline{C}H_2$Ph), 61.6 (d, C5, $J_{5,4F}$ 6.4), 71.3 (dd, C4, *J* 1.6, $J_{4,3F}$ 22.3), 95.3 (dd, C2, $J_{2,3F}$ 29.4, $J_{2,2F}$ 179.6), 98.6 (dd, C3, $J_{3,2F}$ 28.6, $J_{3,3F}$ 177.2), 128.4, 129.4, 130.1, 139.2 (Ar); δ_F (CD_3OD, 376 MHz): -187.7 (ddddd, 3F, $J_{3F,1}$ 3.4, $J_{3F,2F}$ 6.9, $J_{3F,2}$ 13.7, $J_{3F,4}$ 28.6, $J_{3F,3}$ 50.1), -188.1 (ddddd, 2F, $J_{2F,3F}$ 6.9, $J_{2F,3}$ 17.2, $J_{2F,1'}$ 20.6, $J_{2F,1}$ 36.6, $J_{2F,2}$ 50.4); *m/z* (ESI+ve): 228 ($[M + H]^+$, 100%).

2*R*,3*R*-Difluoro-2,4-imino-1,2,3,4-tetradeoxy-D-arabinofuranose 2.83

10% Palladium on charcoal **2.82** (10% wt., 5 mg) was added to a solution of *N*-benzyl-difluoro alcohol (15 mg, 0.066 mmol) in 1,4-dioxane/water (1:1, 2 mL). The reaction was flushed with argon and hydrogen gas sequentially and stirred vigorously for 2 h at rt under hydrogen when mass spectrometry showed the completion of reaction ($[M + H]^+$ 138). After filtration, the solvent was removed *in vacuo* to afford the title compound **2.83** as a clear oil (7 mg, 82%).

HRMS *m/z* (ESI+ve): found 138.0725 $[M + H]^+$; $C_5H_{10}F_2NO^+$ requires 138.0725; $[\alpha]_D^{20}$ +4.3 (*c* 0.37, MeOH); ν_{max} (thin film): 3321 (br, m, O-H); δ_H (D_2O, 400 MHz) free base: 3.18 (1H, dddd, H1, $J_{1,3F}$ 2.2, $J_{1,2}$ 3.9, J_{gem} 13.9, $J_{1,2F}$ 36.7), 3.24 (1H, dd, H1', J_{gem} 13.9, $J_{1',2F}$ 23.3), 3.33 (1H, ddt, H4, $J_{4,3}$ 3.5, $J_{4,5}$ = $J_{4,5'}$ 6.5, $J_{4,3F}$ 27.5), 3.69 (1H, dd, H5, $J_{5,4}$ 6.5, J_{gem} 11.6), 3.73 (1H, ddt, H5', $J_{5',3}$ = $J_{5',F}$ 1.0, $J_{5',4}$ 6.0,

J_{gem} 11.7), 5.04 (1H, dddt, H3, $J_{3,2} = J_{3,5'}$ 1.0, $J_{3,4}$ 3.5, $J_{3,2F}$ 16.4, $J_{3,3F}$ 50.1), 5.27 (1H, dddt, H2, $J_{2,1'} = J_{2,3}$ 1.0, $J_{2,1}$ 4.1, $J_{2,3F}$ 12.5, $J_{2,2F}$ 50.0); δ_C (D_2O, 100 MHz): 50.5 (dd, C1, $J_{1,3F}$ 2.4, $J_{1,2F}$ 23.4), 61.3 (d, C5, $J_{5,F}$ 7.6), 64.9 (d, C4, $J_{4,3F}$ 23.8), 96.8 (dd, C2, $J_{2,3F}$ 30.5, $J_{2,2F}$ 174.5), 97.8 (dd, C3, $J_{3,2F}$ 29.5, $J_{3,3F}$ 177.3); δ_F (D_2O, 376 MHz): -188.5 (ddddd, 3F, $J_{3F,1}$ 2.3, $J_{3F,2F}$ 6.9, $J_{3F,2}$ 12.6, $J_{3F,4}$ 27.5, $J_{3F,3}$ 50.3), -188.9 (ddddd, 2F, $J_{3F,2F}$ 6.9, $J_{2F,3}$ 16.0, $J_{2F,1'}$ 22.7, $J_{2F,1}$ 35.2, $J_{2F,2}$ 50.4); *m/z* (ESI+ve): 138 ($[M + H]^+$, 100%).

_N_-Benzyl-3_R_,4_R_-difluoro-L-proline 2.84

Sodium hydroxide (1 M, aq., 0.24 mL, 0.24 mmol) was added to a solution of difluoroproline methyl ester **2.79** (28 mg, 0.11 mmol) in 1,4-dioxane/water (1:1, 2 mL). After stirring at 40 °C for 4 h, mass spectrometry showed the formation of product and disappearance of starting material. Then solvent was removed *in vacuo* to afford a residue which was loaded onto a Serdolit® CG 400 resin column (the resin was pre-stirred in sodium hydroxide (1 M, aq.) for 15 min, and then flushed with water until the eluent was neutral). After washing with 1,4-dioxane and water, acetic acid (2 M, aq.) was used to release the product. The solvent was removed *in vacuo* to give the pure product **2.84** as light yellow glass (17 mg, 64%).

HRMS *m/z* (ESI+ve): found 264.0805 $[M + Na]^+$; $C_{12}H_{13}F_2NNaO_2^+$ requires 264.0807; $[\alpha]_D^{20}$ -11.7 (*c* 0.16, MeOH), ν_{max} (thin film): 3030 (br, OH), 1677 (s, C=O); δ_H (D_2O, 400 MHz): 3.96 (1H, ddt, H5, $J_{5,4} = J_{5,3F}$ 3.1, J_{gem} 14.0, $J_{5,4F}$ 40.0), 4.21 (1H, dd, H5', J_{gem} 13.9, $J_{5',4F}$ 17.3), 4.56 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.9), 4.70 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.9), 4.73 (1H, d, H2, $J_{2,F}$ 26.6), 5.48 - 5.63 (2H, m, H3, H4), 7.49 - 7.59 (5H, m, Ar); δ_C (D_2O,100 MHz): 58.3 (d, C5, $J_{5,4F}$ 21.0), 60.7 ($\underline{C}H_2$Ph), 72.0 (d, C2, $J_{2,3F}$ 23.8), 91.8 (dd, C3/C4, *J* 33.4, *J* 186.0), 95.0 (dd, C3/C4, *J* 31.5, *J* 179.3), 129.4, 129.7 (ArCH), 131.1 (ArC), 131.5 (ArCH), 167.5 (d, C1, $J_{1,F}$ 9.5); δ_F (D_2O, 376 MHz): -182.0 (ddddd, 3F, $J_{3F,5}$ 2.9, $J_{3F,4F}$ 6.3,

$J_{3F,4}$ 14.3, $J_{3F,2}$ 26.9, $J_{3F,3}$ 48.2), -193.2 (ddddd, 4F, $J_{4F,3F}$ 8.0, $J_{4F,3}$ 14.9, $J_{4F,5'}$ 17.2, $J_{4F,5}$ 39.7, $J_{4F,4}$ 47.4); *m/z* (ESI+ve): 242 ($[M + H]^+$, 100%).

3*R*,4*R*-Difluoro-L-proline 2.85

10% Palladium on charcoal (10% wt., 5 mg) was added to a solution of *N*-benzyl-difluoro proline **2.84** (17 mg, 0.07 mmol) in 1,4-dioxane/water (1:1, 2 mL). The reaction was flushed with argon and hydrogen sequentially and stirred vigorously for 4 h at rt under hydrogen until mass spectrometry showed the completion of reaction (*m/z* (ESI-ve): 150 $[M-H]^-$). After filtration, the solvent was removed *in vacuo* to afford the title compound **2.85** as a light yellow glass (10 mg, 86%).

HRMS *m/z* (ESI-ve): found 150.0367 $[M - H]^-$; $C_5H_6F_2NO_2^-$ requires 150.0372; $[\alpha]_D^{20}$ -4.6 (*c* 0.19, MeOH); ν_{max} (thin film): 3023 (br, OH), 1636 (s, C=O); δ_H (D_2O, 400 MHz): 3.82 (1H, ddd, H5, $J_{5,4}$ 3.1, J_{gem} 14.5, $J_{5,4F}$ 39.1), 3.96 (1H, dd, H5', J_{gem} 14.2, $J_{5',4F}$ 21.8), 4.61 (1H, d, H2, $J_{2,F}$ 24.9), 5.42 - 5.54 (1H, m, H4), 5.58 (1H, dd, H3, $J_{3,4}$ 6.3, $J_{4,F}$ 47.9); δ_C (D_2O, 100 MHz): 50.3 (d, C5, $J_{5,4F}$ 22.9), 66.5 (d, C2, $J_{2,3F}$ 20.0), 92.2 (dd, C4, $J_{4,3F}$ 32.4, $J_{4,4F}$ 177.4), 95.5 (dd, C3, $J_{3,4F}$ 32.9, $J_{3,3F}$ 182.6), 168.7 (d, C1, $J_{1,F}$ 12.4); δ_F (D_2O, 376 MHz, ^{1}H-decoupled): -185.8 (d, *J* 15.6), -190.5 (d, *J* 15.6); *m/z* (ESI-ve): 150 ($[M - H]^-$, 100%).

2.5.2 Synthesis of 3-azido-azetidine derivatives

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 2.94

Triflic anhydride (10.2 mL, 59.0 mmol) was added dropwise to a stirred solution of **2.56** (11.8 g, 45.4 mmol) and pyridine (0.3 mL) in DCM (40 mL) at -30 °C. The mixture was stirred at -30 °C

for 2 h until TLC analysis (cyclohexane/ethyl acetate, 2:1) indicated the complete conversion of starting material (R_f 0.40) to product (R_f 0.70). The reaction mixture was diluted with DCM (10 mL), washed successively with HCl (2M, aq., 10 mL) and sodium bicarbonate (sat., aq., 50 mL), dried and concentrated *in vacuo* to afford the crude triflate. Sodium azide (3.9 g, 60.5 mmol) was added in a solution of the crude triflate in DMF (30 mL) at room temperature and stirred overnight. TLC analysis (cyclohexane/ethyl acetate, 2:1) showed one major product formed (R_f 0.75). Reaction mixture was diluted with ethyl acetate (30 mL), washed with half saturated brine (2 × 30 mL). Aqueous layers were combined and extracted with ethyl acetate (2 × 20 mL). The organic extracts were combined, dried, and concentrated *in vacuo*. Flash column chromatography (cyclohexane/ethyl acetate 10:1) gave the azide **2.94** as a colorless oil. Proton NMR signals showed the existence of small amount of impurities. Column chromatography in less polar solvent (toluene/acetone, 100:1) afforded the pure product (10.1 g, 78%) as a colorless syrup.

HRMS (ESI+ve): found 308.1214 [M + Na^+]; $C_{12}H_{19}N_3NaO_5^+$ requires 308.1217; $[\alpha]_D^{20}$ -47.5 (*c* 1.2, CH_3Cl); [lit.[45] $[\alpha]_D^{22}$ -41.8 (*c* 4.3, $CHCl_3$)]; ν_{max} (thin film): 2105 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.33 - 1.58 (12H, 4 x s, CH_3); 3.99 (1H, dd, H6, $J_{6,5}$ 4.8, J_{gem} 8.6), 4.09 - 4.11 (2H, m, H3 H4), 4.15 (1H, dd, H6', $J_{6',5}$ 6.0, J_{gem} 8.8), 4.25 (1H, m, H5), 4.64(1H, d, H2, $J_{2,1}$ 3.5), 5.87 (1H, d, H1, $J_{1,2}$ 3.5); δ_C ($CDCl_3$, 100 MHz): 25.1 (CH_3), 26.2 (CH_3), 26.6 (CH_3), 26.9 (CH_3), 66.3 (C4), 67.6 (C6), 73.0 (C5), 80.4 (C3), 83.4 (C2), 105.0 (C1), 109.6 ($\underline{C}(CH_3)_2$), 112.3 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 308 ([M + Na^+], 100%).

3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose 2.95

The diacetonide **2.94** (10.1 g, 35.3 mmol) was dissolved in 7:3 acetic acid/water (30 mL) and stirred for 40 h at room temperature. TLC analysis (cyclohexane/ethyl acetate, 2:1) indicated the formation of a major product (R_f 0.35) and the complete consumption of starting material (R_f 0.75). The reaction mixture was concentrated in *vacuo* and purified by column chromatography (cyclohexane/ethyl acetate 2:1) to afford the monoacetonide **2.95** (5.2 g, 60%) as a white solid. HRMS (ESI+ve): found 268.0903 [M + Na^+]; $C_9H_{15}N_3NaO_5^+$ requires 268.0904; m.p.: 80 - 82 °C [lit.[46] m.p. 83 - 84 °C]; $[\alpha]_D^{20}$ -32.7 (*c* 0.47 in CH_3Cl); ν_{max} (thin film): 3396 (br, OH), 2106 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.34 - 1.52 (6H, 2 × s, CH_3); 3.76 (1H, dd, H6, $J_{6,5}$ 5.0, J_{gem} 11.1), 3.90 (1H, dd, H6', $J_{6',5}$ 3.3, J_{gem} 11.4), 3.95 (1H, m, H5), 4.17 (1H, d, H3, $J_{3,4}$ 3.0), 4.20 (1H, dd, H4, $J_{4,3}$ 3.0, $J_{4,5}$ 8.1), 4.66 (1H, d, H2, $J_{2,1}$ 3.7), 5.89 (1H, d, H1, $J_{1,2}$ 3.7); δ_C ($CDCl_3$, 100 MHz): 26.2 (CH_3), 26.6 (CH_3), 64.2 (C6), 66.4 (C3), 69.6 (C5), 78.9 (C4), 83.2 (C2), 104.9 (C1), 112.3 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 268 ([M + Na^+], 59%), 513 ([2M+Na^+], 100%);

3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose 2.96

Sodium periodate (5.4 g, 25.4 mmol) was added to the diol **2.95** (5.2 g, 21.2 mmol) in water (30 mL). The reaction mixture was stirred at room temperature for 4 h when TLC analysis (cyclohexane/ethyl acetate, 2:1) showed the complete conversion of starting material (R_f 0.25) to a single product (R_f 0.50). Ethanol (20 mL) was added to the mixture and a white suspension was formed after 20 min stirring. The reaction mixture was filtered and the filter pad washed with ethanol (25 mL). $NaBH_4$ (801 mg, 21.2 mmol) was added portionwise to the filtrate and the mixture was stirred again for 1.5 h. Analysis by mass spectrometry indicated the completion of the reaction ([M + $Na]^+$ 238). Acetic acid was added to adjust the solution to pH 7. The mixture

was then concentrated *in vacuo* and co-evaporated with ethanol to afford a yellow solid that was purified by flash column chromatography (cyclohexane/ethyl acetate 3:1) to afford the azide **2.96** as a white crystalline solid (4.38 g, 96%).

HRMS (ESI+ve): found 238.0796 [M + Na^+]; $C_8H_{13}N_3NaO_4^+$ requires 238.0798; m.p. 68 - 70°C; $[\alpha]_D^{20}$ -57.8 (*c* 0.38 in CH_3Cl); [lit.[47] $[\alpha]_D^{26}$ -49.6 (*c* 1.0, EtOH)]; ν_{max} (thin film): 3448 (br, OH), 2103 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.27 - 1.45 (6H, 2 × s, CH_3); 3.78 (1H, dd, H5, $J_{5,4}$ 6.1, J_{gem} 11.5), 3.86 (1H, dd, H5', $J_{5',4}$ 6.1, J_{gem} 11.5), 3.96(1H, d, H3, $J_{3,4}$ 3.4), 4.30 (1H, dt, H4, $J_{4,3}$ 3.4, $J_{4,5}$ = $J_{4,5'}$ 6.1), 4.62 (1H, d, H2, $J_{2,1}$ 3.9), 5.87 (1H, d, H1, $J_{1,2}$ 3.9); δ_C ($CDCl_3$, 100 MHz): 26.3 (CH_3), 26.6 (CH_3), 60.9 (C5), 66.1 (C3), 79.4 (C4), 83.6 (C2), 104.7 (C1), 112.3 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 238 ([M + Na^+], 100%).

3-Azido-3-deoxy-D-xylopyranose 2.97

DOWEX® 50WX8-200 (1.00 g) was added to a solution of monoacetonide **2.96** (4.38 g, 20.4 mmol) in 1,4-dioxane/water (1:1, 40 mL). The reaction mixture was stirred at 80 °C for 20 h after which TLC analysis (cyclohexane/ethyl acetate, 2:1) indicated the disappearance of starting material (R_f 0.50) and the formation of a single product (R_f 0.10). The reaction mixture was filtered and the solvent removed *in vacuo* to give a residue that was purified by column chromatography (cyclohexane/ethyl acetate, 1:1 to 10% methanol in ethyl acetate) to give a mixture of pyranoside. **2.97** (3.57 g, 100%) as a white solid.

HRMS (ESI+ve): found 198.0479 [M + Na^+]; $C_{12}H_{19}N_3NaO_5^+$ requires 198.0485; m.p. 72 – 74 °C; ν_{max} (thin film): 3336 (br, OH), 2107 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 3.38 (1H, dd, H5β, $J_{5,4}$ 9.0, J_{gem} 11.9), 3.55 (1H, t, H5α, J_{gem} = $J_{5,4}$ 10.8), 3.67 (1H, t, H3β, $J_{3,2}$ = $J_{3,4}$ 9.4), 3.87 (1H, dd, H5α', $J_{5',4}$ 5.9,

J_{gem} 11.2), 3.92 (1H, d, H3α, $J_{3,2}$ 10.2), 4.09 (1H, dd, H5β', $J_{5',4}$ 5.1, J_{gem} 11.9), 4.78 - 4.82 (3H, m, H2α, H4α, H4β), 4.89 (1H, dd, H2β, $J_{2,1}$ 7.4, $J_{2,3}$ 9.6), 5.57 (1H, d, H1β, $J_{1,2}$ 7.3), 6.16 - 6.17(1H, d, H1α, $J_{1,2}$ 3.7); δ_C ($CDCl_3$, 100 MHz): 92.4 (C1β), 88.7 (C1α), 69.1, 69.2, 69.8, 70.0 (C2α, C4α, C2β, C4β), 63.8 (C5β), 62.9 (C3β), 60.6 (C5α), 60.4 (C3α); *m/z* (ESI+ve): 198 ([M + Na^+], 100%,).

3-Azido-3-deoxy-1,2,4-tri-*O*-acetyl-D-xylopyranose 2.98

A solution of the azide **2.97** (3.78 g, 21.6 mmol) in acetic anhydride/pyridine (1:1, 20 mL) was stirred at room temperature overnight. TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the complete conversion of starting material (R_f 0.05) to product (R_f 0.90). Removal of the solvent *in vacuo* gave a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate, 4:1) to afford an α:β mixture of the anomers **2.98** (6.38 g, 98%)in a ratio of 10:3 as indicated by NMR.

HRMS (ESI+ve): found 324.0804 [M + Na^+]; $C_{11}H_{15}N_3NaO_7^+$ requires 324.0802; m.p. 46 – 48 °C; ν_{max} (thin film): 2106 (s, N_3), 1752 (s, C=O); β anomer: δ_H ($CDCl_3$, 400 MHz): 3.44 (1H, dd, H5', $J_{5',4}$ 9.1 J_{gem} 11.9); 3.74(1H, t, H3, $J_{3,2}$ = $J_{3,4}$ 9.3); 4.14 (1H, dd, H5, $J_{5,4}$ 5.0 J_{gem} 11.9); 4.83 (1H, m, H4); 4.96 (1H, dd, H2, $J_{2,1}$ 7.3 $J_{2,3}$ 9.6); 5.64 (1H, d, H1, $J_{1,2}$ 7.3); δ_C ($CDCl_3$, 100 MHz): 62.8 (C3), 63.7 (C5), 69.2 (C4), 69.7 (C2), 92.3 (C1), 170.4 (C=O), 170.8 (C=O), 171.2 (C=O); *m/z* (ESI+ve): 324 ([M + Na^+], 100%).

Methyl 3-azido-3-deoxy-2,4-di-*O*-acetyl-β-D-xylopyranoside 2.99

Method 1:

HBr (33% in acetic acid, 1.23 mL, 7.1 mmol) was added dropwise to a solution of triacetate **2.98**

(789 mg, 2.62 mmol) in acetic acid/DCM (7:3, 20 mL) at 10 °C. The reaction mixture was stirred under 5 °C for 4 h when TLC analysis (cyclohexane/ethyl acetate 2:1) showed complete conversion of starting material (R_f 0.53) to a product (R_f 0.62). The reaction mixture was then diluted with DCM (20 mL) and poured into ice-water (40 mL). The organic layer was firstly washed with cold sodium bicarbonate (sat., aq., 2 × 40 mL) and then with ice-water (20 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford crude bromide. The crude bromide was dissolved in anhydrous methanol (10 mL) in a foil-wrapped flask. Silver carbonate was added and reaction stirred at room temperature overnight. TLC (cyclohexane/ethyl acetate, 2:1) showed the complete conversion of starting material (R_f 0.62) to product (R_f 0.26). The reaction mixture was then filtered through celite and the filter-pad washed with DCM (10 mL) and finally concentrated to dryness in *vacuo*. Flash chromatography (cyclohexane/ethyl acetate, 4:1) gave the β-pyranoside **2.99** as a colorless oil (230 mg, 30%).

Method 2:

$BiBr_3$ (475 mg, 1.06 mmol) and Me_3SiBr (11.2 mL, 84.4 mmol) were added portionwise to a stirred solution of triacetate compound **2.98** (6.38 g, 21.1 mmol) in DCM (30 mL). The reaction mixture was stirred at room temperature for 4 h until TLC analysis (cyclohexane/ethyl acetate, 2:1) showed the complete conversion of starting material (R_f 0.53) to product (R_f 0.62). Reaction mixture was poured into cold sodium bicarbonate (sat., aq., 30 mL) and extracted twice with DCM (2 x 30 mL). The organics were combined, dried ($MgSO_4$) and concentrated *in vacuo*. Crude bromide was dissolved in DCM/MeOH (1:1, 30 mL) in a foil-wrapped flask. Silver carbonate (9.79 g, 42.2 mmol) and $CaSO_4$ (5.74 g, 42.2 mmol) were added. The reaction was stirred at room temperature for 1 hour and mixture filtered through Celite and washed with DCM (30 mL).

Column chromatography (cyclohexane/ethyl acetate, 6:1) afforded the β-pyranoside **2.99** (4.25 g, 74%) as a colorless oil.

HRMS (ESI+ve): found 296.0848 [M + Na^+]; $C_{10}H_{15}N_3NaO_6^+$ requires 296.0853; $[\alpha]_D^{20}$ -65.0 (*c*, 0.72 CH_3Cl); ν_{max} (thin film): 2105 (s, N_3), 1743 (C=O); δ_H ($CDCl_3$, 400 MHz): 2.12-2.14 (6H, 2 × s, CH_3), 3.31 (1H, dd, H5, $J_{5,4}$ 9.1 J_{gem} 11.9), 3.39 (3H, s, OCH_3), 3.67 (1H, t, H3, $J_{3,2} = J_{3,4}$ 9.3), 4.14 (1H, dd, H5', $J_{5',4}$ 5.3 J_{gem} 11.9), 4.81-4.85 (2H, m, H2, H4); δ_C ($CDCl_3$, 100 MHz): 20.7 (2 x CH_3), 56.6 (OCH_3), 62.8 (C5), 63.0 (C3), 69.6 (C4), 70.7 (C2), 101.8 (C1), 169.3 (C=O), 169.7 (C=O); *m/z* (ESI+ve): 296 ([M + Na^+], 70%), 569 ([2M + Na^+], 100%);

Methyl 3-azido-3-deoxy-β-D-xylopyranoside 2.99

Sodium methoxide (84.2 mg, 1.56 mmol) was added to a solution of diacetate **2.98** (4.25 g, 15.6 mmol) in anhydrous methanol (40 mL) at 40 °C and stirred for 15 h until TLC (cyclohexane/ethyl acetate, 1:3) analysis indicated the complete conversion of starting material (R_f 0.2) to product (R_f 0.4). The reaction mixture was neutralized with 2M HCl, diluted with ethyl acetate (40 mL), washed with water (2 × 40 mL) and the combined aqueous layers extracted with ethyl acetate (2 × 40 mL). The combined organics were washed with brine (2 × 40 mL) and dried ($MgSO_4$), filtered and concentrated *in vacuo* to obtain a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate, 3:1) yielding the azide **2.99** (2.95 g, 100%) as a pale yellow solid.

HRMS (ESI+ve): found 212.0638 [M + Na]$^+$; $C_6H_9N_3NaO_3^+$ requires 212.0642; $[\alpha]_D^{20}$ -31.1 (*c*, 0.55 CH_3Cl); ν_{max} (thin film): 2106 (s, N_3); m.p. 48 - 50°C; δ_H (MeOD, 400 MHz): 3.14 (1H, dd, H2, $J_{2,1}$ 7.6, $J_{2,3}$ 10.2), 3.23 (1H, dd, H3, $J_{3,2}$ 10.2, $J_{3,4}$ 9.3), 3.26 (1H, dd, H5', $J_{5',4}$ 7.8, J_{gem} 11.4), 3.41 (3H, s,

OCH_3), 3.46 (1H, m, H4), 3.88 (1H, dd, H5, $J_{5,4}$ 5.3 J_{gem} 11.4), 4.15 (1H, d, H1, $J_{1,2}$ 7.6); δ_C (MeOD, 100 MHz): 58.0 (CH_3), 66.7 (C4), 69.0 (C5), 70.0 (C3), 72.7 (C2), 105.0 (C1); *m/z* (ESI+ve): 212 (100%, $[M + Na]^+$);

Methyl 3-azido-*N*-benzyl-2,3,4-trideoxy-2,4-imino-β-L-riboside 2.101

Triflic anhydride (7.1 mL, 63.2 mmol) was added dropwise to a stirred solution of diol **2.99** (3.0 g, 15.8 mmol) and pyridine (5.6 mL, 94.8 mmol) in anhydrous DCM (30 mL). The mixture was stirred at -30 °C for 2 huntil TLC (cyclohexane/ethyl acetate, 2:1) analysis indicated the disappearance of starting material (R_f 0.1). Then the mixture was diluted with DCM (10 mL) and successively washed with HCl (aq., 2M., 2 × 40 mL) and sodium bicarbonate (sat., aq., 40 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford the crude ditriflate **2.100** that was used without further purification. Benzylamine (6.6 ml, 79.0 mmol) was added to a solution of crude triflate in acetonitrile (30 mL). The reaction mixture was stirred at 70 °C with condenser attached for 2 h until mass spectrometry analysis showed the formation of desired product ($[M + H]^+$ 261). Then the reaction mixture was cooled and concentrated *in vacuo* to give a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate, 7:1) to afford the bicyclic azetidine **2.101** as a yellow oil (2.90 g, 71% (2 steps)).

HRMS (ESI+ve): found 261.1344 $[M + H]^+$; $C_{13}H_{17}N_4O_2^+$ requires 261.1346; $[\alpha]_D^{20}$ -47.4 (*c* 0.72, CH_3Cl); ν_{max} (thin film): 2099 (s, N_3); δ_H (CD_3CN, 400 MHz): 3.32 (3H, s, CH_3); 3.41 (1H, s, H3); 3.52 (2H, m, H4, H2); 3.69 (1H, dd, H5, $J_{5,4}$ 1.2, $J_{5,5'}$ 11.2); 3.93 (1H, d, C$\underline{H}_2$Ph, J_{gem} 14.2); 4.02 (1H, d, C$\underline{H}_2$Ph, J_{gem} 14.2); 4.26 (1H, dd, H5', $J_{5',4}$ 1.5, $J_{5',5}$ 11.0); 4.61 (1H, s, H1); 7.12 - 7.30 (5H, m, Ar);

δ_C(CD_3CN, 100 MHz): 51.1 ($\underline{C}H_2Ph$), 55.6 (CH_3), 61.0 (C3), 61.1 (C5), 65.3 (C4), 67.4 (C2), 100.3 (C1), 126.8, 128.3, 128.4, 138.8 (Ar); *m/z* (ESI+ve): 261 (100%, $[M + H]^+$).

3-Azido-*N*-benzyl-2,4-imino-2,3,4-trideoxy-*meso*-ribitol 2.102

A solution of the bicyclic azetidine **2.101** (200 mg, 0.76 mmol) in 2 M aq. HCl/1,4-dioxane (5:1, 6 mL) was stirred at 40 °C for 18 h after which the consumption of starting material and the formation of product was confirmed by mass spectrometry ($[M + MeOH + Na]^+$ 301). The solvent was removed in *vacuo* to give a residue that was dissolved in methanol (4 mL) and sodium borohydride (115 mg, 3.04 mmol) was added. The reaction mixture was stirred at room temperature for 2 h when mass spectrometry showed the completion of reaction ($[M + Na]^+$ 271). The solvent was concentrated *in vacuo* to obtain a polar residue (320 mg). Purification with a short column of DOWEX® 50WX8-200 or flash column chromatography was not successful. The crude diol **2.102** was used for the following steps without further purification.

3-Azido-*N*-benzyl-1,5-di-*O*-acetyl-2,4-imino-2,3,4-trideoxy-*meso*-ribitol 2.103

A solution of the diol **2.102** (320 mg) in acetic anhydride/pyridine (1:1, 4 mL) was stirred at room temperature for 16 h when TLC (cyclohexane/ethyl acetate, 1:1) indicated the formation of the only product (R_f 0.72). The mixture was concentrated *in vacuo* and the residue purified by flash column chromatography (cyclohexane/ethyl acetate/triethylamine, 6:1:0.01) to obtain the diacetate **2.103** (191 mg, 76% 3 steps from **2.101**) as a light yellow oil.

HRMS *m/z* (ESI+ve): found 355.1372 $[M + Na]^+$; $C_{16}H_{20}N_4NaO_4^+$ requires 355.1377; ν_{max} (thin film): 2104 (s, N_3), 1742 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 2.03 (6H, s, 2 x CH_3), 3.20 (2H, m, H2, H4),

3.67 (1H, t, H3, $J_{3,2}$ = $J_{3,4}$ 6.1), 3.71 (2H, br-s, CH_2Ph), 3.82 (2H, dd, H1, H5, $J_{1(5),2(4)}$ 4.29, J_{gem} 11.4), 4.03 (2H, dd, H1', H5'), $J_{1'(5'),2'(4')}$ 4.29, J_{gem} 11.4), 7.25 – 7.34 (5H, m, Ar); δ_C ($CDCl_3$,100 MHz): 20.8 (2 x CH_3), 56.7 (C1, C5), 61.0 ($\underline{C}H_2Ph$), 64.4 (C3), 67.2 (C2, C4), 128.5, 129.2, 136.6 (Ar), 170.7 (2 x C=O); *m/z* (ESI+ve): 355 ($[M + Na]^+$, 100%).

3-Acetamido-*N*-benzyl-1,5-di-*O*-acetyl-2,4-imino-2,3,4-trideoxy-*meso*-ribitol 2.104

Method 1 (reductive acetylation of **2.102**):

Zinc powder (494 mg, 7.6 mmol) and copper sulfate (sat, aq., 0.95 mL) were added to a solution of crude diol **2.102** (from **2.101** (100 mg, 0.38 mmol)) in THF/acetic acid/acetic anhydride (3:2:1, 6 mL). The mixture was stirred at room temperature for 1 hour until mass spectrometry showed the formation of desired product ($[M + Na]^+$ 371). Then the mixture was filtered with Celite and solvent was removed *in vacuo* to give a residue that was purified by flash column chromatography (ethyl acetate/methanol/triethylamine, 10:1:0.01) to give the protected amide compound **2.104** (31 mg, 24%, 3 steps from **2.101**) as a light yellow solid.

Method 2 (reductive acetylation of **2.103**):

Zinc powder (743 mg, 11.4 mmol) and copper sulfate (sat, aq., 1.42 mL) were added to a solution of the diacetate **2.103** (190 mg, 0.57 mmol) in THF/acetic acid/acetic anhydride (3:2:1, 6 mL). The mixture was stirred at room temperature for 1 hour until mass spectrometry showed the formation of desired product ($[M + Na]^+$ 371). Then the mixture was filtered with celite and solvent was removed *in vacuo* to give the title compound **2.104** (202 mg, 100%) as a light yellow solid without further purification.

HRMS *m/z* (ESI+ve): found 371.1576 $[M + Na]^+$; $C_{18}H_{24}N_2NaO_5^+$ requires 371.1577; ν_{max} (thin

film): 1741 (s, $COCH_3$), 1658 (s, NHCO); δ_H ($CDCl_3$, 400 MHz): 1.94 (3H, s, NHCOC<u>H</u>$_3$), 1.98 (6H, s, 2 x OCOC<u>H</u>$_3$), 3.15 (2H, ddd, H2 and H4, $J_{2(4),1'(5')}$ 4.29, $J_{2(4),1(5)}$ 5.6, $J_{2(4),3}$ 7.1), 3.73 (2H, s, C<u>H</u>$_2$Ph), 3.90 (2H, dd, H1 and H5, $J_{1(5),2(4)}$ 5.6, J_{gem} 11.9), 4.12 (2H, dd, H1' and H5', $J_{1'(5'),2(4)}$ 4.3, J_{gem} 11.9), 5.89 (1H, br-s, NH), 7.25-7.33 (5H, m, Ar); δ_C($CDCl_3$,100 MHz): 20.8 (2 x OCO<u>C</u>H$_3$), 23.1 (NHCO<u>C</u>H$_3$), 45.9 (C3), 60.8 (CH_2Ph), 65.4 (C1, C5), 67.6 (C2, C4), 127.5, 128.4, 129.3, 137.2 (Ar), 169.7 (NH<u>C</u>O), 170.9 (2 x O<u>C</u>O); *m/z* (ESI+ve): 349 ($[M + H]^+$, 100%).

3-Acetamido-*N*-benzyl-2,4-imino-2,3,4-trideoxy-*meso*-ribitol 2.105

Sodium methoxide (3.2 mg, 0.06 mmol) was added to a solution of **2.104** (200 mg, 0.57 mmol) in methanol (5 mL). The mixture was stirred at 60 °C for 16 h until mass spectrometry indicated the formation of product ($[M + H]^+$ 265). After removal of the solvent *in vacuo*, the residue was dissolved in ethanol (5 mL) and passed through a glass microfiber filter paper and then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). The ion exchange column was then washed with water, 1,4-dioxane and then water; the pure product was then eluted with aqueous ammonia (2 M). Removal of solvent *in vacuo* gave the *meso*-diol **2.105** (120 mg, 79%) as a light yellow glass.

HRMS *m/z* (ESI+ve): found 265.1559 $[M + H]^+$; $C_{14}H_{21}N_2O_3^+$ requires 265.1547; ν_{max} (thin film): 1633 (s, NHCO); δ_H (CD_3OD, 400 MHz): 1.92 (3H, s, NHCOC<u>H</u>$_3$), 3.08 (2H, br-dt, H2, H4, $J_{2(4),1'(5')}$ = $J_{2(4),1(5)}$ 4.7, $J_{2(4),3}$ 6.9), 3.33 (2H, dd, H1, H5, $J_{1(5),2(4)}$ 4.1, J_{gem} 11.7), 3.37 (2H, dd, H1', H5', $J_{1'(5'),2(4)}$ 5.0, J_{gem} 11.7), 3.77 (2H, s, C<u>H</u>$_2$Ph), 3.89 (1H, t, H3, $J_{3,2}$ = $J_{3,4}$ 6.9), 7.28 - 7.37 (5H, m, Ar); δ_C (CD_3OD,100 MHz): 21.0 (NHCO<u>C</u>H$_3$), 46.0 (C3), 61.3 (CH_2Ph), 65.4 (C1, C5), 70.5 (C2, C4), 127.5,

128.4, 129.3, 137.2 (Ar), 169.7 (C=O),; *m/z* (ESI+ve): 265 ([M + H]$^+$, 100%).

3-Acetamido-2,4-imino-2,3,4-trideoxy-*meso*-ribitol 2.106

10% Palladium on charcoal (10% wt., 5 mg) was added to a solution of **2.105** (40 mg, 0.15 mmol) in 1,4-dioxane/water (1:1, 2 mL). The reaction mixture was flushed with argon and hydrogen., stirred for 20 h when mass spectrometry showed the completion of the reaction, filtered and concentrated *in vacuo* to give a residue that was purified with a short column of DOWEX® 50WX8-200 (as illustrated above) to yield diol **2.106** (19 mg, 73%) as a light yellow glass.

HRMS *m/z* (ESI+ve): found 175.1074 [M + H]$^+$; $C_7H_{15}N_2O_3^+$ requires 175.1075; ν_{max} (thin film): 3283 (br, OH, NH); δ_H (D_2O, 400 MHz): 1.98 (3H, s, NHCOC$\underline{H}_3$), 3.61 (2H, dd, H1 and H5, $J_{1(5),2(4)}$ 6.4, J_{gem} 11.7), 3.65 (2H, dd, H1' and H5', $J_{1'(5'),2(4)}$ 4.9, J_{gem} 11.7), 3.74 (2H, m, H2 and H4), 4.00 (1H, t, H3, $J_{3,2}$ = $J_{3,4}$ 7.3); δ_C (D_2O,100 MHz): 22.3 (NHCO$\underline{C}H_3$), 48.3 (C3), 62.2 (C1, C5), 64.1 (C2, C4), 174.2 (C=O); *m/z* (ESI+ve): 175 ([M + H]$^+$, 100%).

Methyl 3-azido-*N*-benzyl-2,4-imino-2,3,4-trideoxy-L-ribonate 2.107

The bicycle **2.101** (69 mg, 0.27 mmol) was dissolved in 1,4-dioxane/2M HCl (1:5, 5 mL) at 40 °C overnight. TLC analysis (cyclohexane/ethyl acetate, 2:1) indicated the complete conversion of starting material (R_f 0.70) to product (R_f 0.05). The mixture was diluted with ethyl acetate (15 mL) and washed with sodium bicarbonate (sat., aq., 2 × 10 mL). The aqueous layers were combined and extracted with ethyl acetate (2 x 10 mL). The organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude lactol which was dissolved in anhydrous MeOH (5 mL) at 0 °C together with K_2CO_3 (111 mg, 0.81 mmol). A sonicated solution of iodine

(82 mg, 0.324 mmol) in anhydrous methanol (0.5 mL) was then added dropwise to the reaction mixture which was stirred at 0 °C for 1 hour when mass spectrometry showed the formation of desired product ($[M + H]^+$ 277) and TLC (cyclohexane/ethyl acetate, 2:1) showed the formation of one major spot (R_f 0.50). Excess iodine was quenched with sodium sulfite (sat., aq.) until a white precipitate appeared. The reaction mixture was diluted with excess water to dissolve all the precipitate and extracted with diethyl ether (4 × 20 mL). The organics were combined, dried and concentrated *in vacuo* to give a crude product which was purified by flash column chromatography (cyclohexane/ethyl acetate, 3:1 to 1:1) to afford the ester **2.107** as a yellow oil (62 mg, 85%).

HRMS (ESI+ve): found 277.1299 $[M + H]^+$; $C_{13}H_{17}N_4O_3^+$ requires 277.1301; $[\alpha]_D^{20}$ -39.6 (*c*, 0.50 in CH_3Cl); ν_{max} (thin film): 2106 (s, N_3), 1715 (s, C=O); δ_H($CDCl_3$, 400 MHz): 3.14 (1H, dd, H5, $J_{5,4}$ 2.0, J_{gem} 12.1), 3.18-3.21(1H, m, H4), 3.35 (1H, dd, H5', $J_{5',4}$ 2.5, J_{gem} 12.1), 3.64 (1H, d, H2, $J_{2,3}$ 6.3), 3.67 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.4), 3.98 (1H, d, C$\underline{H}_2$Ph, J_{gem}12.6), 4.15 (1H, t, H3, $J_{3,2}$ = $J_{3,4}$ 6.3), 7.27 - 7.34 (5H, m, Ar); δ_C ($CDCl_3$, 100 MHz): 52.3 (CH_3), 54.0 (C3), 60.3 (C5, $\underline{C}H_2$Ph), 67.2 (C2), 69.1 (C4), 171.5 (C=O); *m/z* (ESI+ve): 277 ($[M + H]^+$, 100%);

Methyl 3-azido-*N*-benzyl-2,4-imino-2,3,4-trideoxy-L-ribonamide 2.108

Methylamine (0.45 mL, 3.62 mmol) and calcium chloride (20 mg, 0.18 mmol) were added to a solution of ester **2.107** (50 mg, 0.181 mmol) in methanol (2 mL) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 4 h until mass spectrometry showed the completion of the reaction ($[M + H]^+$ 276). The reaction mixture was filtered and concentrated to dryness *in vacuo* to give the amide **2.108** as a yellow oil (40 mg, 80%).

HRMS (ESI+ve): found 276.1450 [M + H$^+$]; $C_{13}H_{18}N_5O_2^+$ requires 276.1455; $[\alpha]_D^{20}$ -10.5 (c, 0.50 in CH_3Cl); ν_{max} (thin film): 3338 (br, OH), 2105 (s, N_3), 1654 (s, C=O); δ_H($CDCl_3$, 400 MHz): 2.61 - 2.63 (3H, d, NHC<u>H</u>$_3$, J 5.1), 3.22 (1H, dt, H4, $J_{4,3}$ 6.1, $J_{4,5}$ = $J_{4,5'}$ 3.0), 3.39 (1H, dd, H5, $J_{5,4}$ 3.3, J_{gem} 12.4), 3.46 (1H, dd, H5', $J_{5',4}$ 3.3, J_{gem} 12.4), 3.55 (1H, d, H2, $J_{2,3}$ 6.3), 3.71 (1H, d, C<u>H</u>$_2$Ph, J_{gem} 12.4), 3.75 (1H, d, C<u>H</u>$_2$Ph, J_{gem} 12.4), 3.87 (1H, t, H3, $J_{3,2}$ = $J_{3,4}$ 6.6), 6.72 (1H, d, N<u>H</u>CH_3, J 5.0), 7.27 - 7.36 (5H, m, Ar); δ_C ($CDCl_3$, 100 MHz): 25.7 (CH_3), 55.7 (C3), 61.2 (C6), 61.3 (C5), 69.7 (C4), 69.8 (C2), 128.2, 128.8, 129.3, 136.2 (Ar), 170.8 (C=O); *m/z* (ESI+ve): 276 ([M + H]$^+$, 100%).

Methyl 3-acetamido-*N*-benzyl-2,4-imino-2,3,4-trideoxy-L-ribonamide 2.110

The azide **2.108** (20 mg, 0.073 mmol) was suspended in a mixture of THF/AcOH/Ac_2O (3:2:1, 1.2 mL). Zinc powder (95 mg, 1.45 mmol) was added and the mixture was stirred to give a mixture. Then copper sulfate (sat, aq., 0.25 mL) was added dropwise and the reaction mixture was stirred for 1 hour at room temperature until TLC analysis (cyclohexane/ethyl acetate, 2:1) showed the complete conversion of starting material (R_f 0.1) to a single product (R_f 0.6). Reaction mixture was filtered, concentrated to dryness to give acetate **2.109** which was dissolved in methanol (1 mL) and treated with sodium methoxide (2 mg, 0.037 mmol). The reaction mixture was stirred at 40 °C overnight until mass spectrometry showed the formation of desired product ([M + H]$^+$ 292). The mixture was then filtered and concentrated *in vacuo* to give product **2.110** as a yellow gum (18 mg, 85%).

HRMS (ESI+ve): found 276.1458 [M + H]$^+$; $C_{15}H_{22}N_3O_3^+$ requires 292.1661; $[\alpha]_D^{20}$ +56.4 (*c*, 0.90 in CH_3Cl); $[\alpha]_D^{20}$ +9.7 (*c* 0.35, MeOH); ν_{max} 3294 (br, OH), 1650 (s, C=O); δ_H($CDCl_3$, 400 MHz): 2.59 - 2.61 (3H, d, CH_3, J 4.8), 3.14 (1H, dt, H4, $J_{4,5}$ = $J_{4,5'}$ 3.8, $J_{4,3}$ 6.8), 3.39 - 3.44 (2H, m, H5, H5'), 3.52 (1H, d, H2, $J_{2,3}$ 7.6), 3.64 (1H, d, C<u>H</u>$_2$Ph, J_{gem} 12.4), 3.67 (1H, m, H3), 3.74 (1H, d, C<u>H</u>$_2$Ph, J_{gem} 12.1);

δ_C($CDCl_3$, 100 MHz): 25.7 (CH_3), 50.6 (C3), 61.5 (C6), 63.6 (C5), 67.8 (C2), 72.3 (C4), 170.7 (C=O), 172.1 (C=O); *m/z* (ESI+ve): 292 ($[M + H]^+$, 100%).

Methyl 3-acetamido-2,4-imino-2,3,4-trideoxy-L-ribonamide 2.111

10% Palladium on charcoal (10% wt., 5 mg) was added to a solution of azetidine **2.111** (12 mg, 0.04 mmol) in water/1,4-dioxane (2:1, 1.5 mL). The reaction mixture was flushed sequentially with argon and hydrogen. The mixture was stirred for 20 h after which mass spectrometry showed the reaction had gone to completion ($[M + H]^+$ 202). The reaction mixture was filtered and concentrated *in vacuo* to afford the debenzylated amide **2.110** (6 mg, 72%) as colorless gum.

HRMS (ESI+ve): found 202.1186 $[M + H]^+$; $C_8H_{16}N_3O_3^+$ requires 202.1186; $[\alpha]_D^{20}$ +112.5 (*c* 0.30, CH_3Cl); ν_{max} 3303 (br, OH), 1645 (s, C=O); δ_H(MeOD, 400 MHz): 2.78 (3H, d, CH_3, *J* 4.8), 3.53-3.56 (2H, m, H5 and H5'), 3.73 (1H, dt, H4, $J_{4,5}$ = $J_{4,5'}$ 3.6, $J_{4,3}$ 7.6), 4.01 (1H, d, H2, $J_{2,1}$ 6.4), 4.24 (1H, m, H3); δ_C(MeOD, 100 MHz): 24.9 (CH_3), 49.9 (C3), 62.1 (C2), 62.9 (C4), 63.4 (C5), 170.1 (C=O); *m/z* (ESI+ve): 202 ($[M + H]^+$, 100%).

2.5.3 Synthesis of 3-hydroxy azetidine acetamides

Methyl 3-*O*-benzyl-*N*-butyl-2,4-dideoxy-2,4-imino-β-L-ribo-pyranoside 2.113

Triflic anhydride (1.62 mL, 9.60 mmol) was added dropwise to a solution of diol **2.112** (610 mg, 2.40 mmol) and anhydrous pyridine (1.40 mL) in anhydrous dichloromethane (35 mL) at -20 °C. Then the reaction mixture was stirred at -20 °C for 2 h until TLC (cyclohexane/ethyl acetate, 1:1) showed the consumption of starting material (R_f 0.28) and the formation of one major product (R_f 0.78). The reaction mixture was diluted with dichloromethane (30 mL) and washed with

aqueous hydrochloride acid (2 M, 2 x 35 mL). The organic layer was dried (MgSO4) and the solvent was removed to afford the crude triflate as a yellow oil (1.00 g).

Butylamine (1.5 mL, 12.0 mmol) was added to a solution of crude triflate in acetonitrile (6 mL) and the reaction mixture was stirred at 50 °C - 60 °C for 2 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the complete conversion of product (R_f 0.20). The residue obtained after removing solvent *in vacuo* was purified by flash column chromatography (cyclohexane/ethyl acetate, 5:1 to 1:2) to yield title compound **2.113** as a brown oil (98%).

HRMS (ESI+ve): found 292.1908 $[M + H]^+$; $C_{17}H_{26}NO_3^+$ requires 292.1907; $[\alpha]_D^{20}$ -39.3 (*c* 1.03, CH_3CN); δ_H (CD_3CN, 400MHz): 0.90 - 0.95 (3H, m, CH_3), 1.32 - 1.40 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 2.96 (2H, t, $NC\underline{H}_2CH_2CH_2$, *J* 6.8), 3.39 (3H, s, OCH_3), 3.59 (1H, dd, H2, $J_{2,1}$ 1.2 $J_{2,3}$ 4.4), 3.68 (1H, dt, H4, $J_{4,5}$ = $J_{4,5'}$ 4.0, $J_{4,3}$ 4.4), 3.80 (1H, dd, H5', $J_{5',4}$ 4.0, J_{gem} 8.0), 4.01 (1H, H3, t, $J_{3,2} = J_{3,4}$ 4.4), 4.32 (1H, dd, H5, $J_{5.4}$ 4.0, J_{gem} 8.0), 4.61 (2H, s, $C\underline{H}_2Ph$), 4.72 (1H, d, H1, $J_{1,2}$ 1.2), 7.34 - 7.41 (5H, m, Ar); δ_C (CD_3CN, 100MHz): 13.7 (CH_3), 20.6, 29.7, 48.2 ($\underline{C}H_2\underline{C}H_2\underline{C}H_2$), 55.3 ($OCH_3$), 62.6 (C5), 66.3 (C2), 66.8 (C4), 71.4 ($\underline{C}H_2Ph$), 79.8 (C3), 100.6 (C1), 138.5, 128.8, 128.4, 128.2 (Ar); *m/z* (ESI+ve): 292 ($[M + H]^+$, 100%),.

Methyl 3-*O*-benzyl-*N*-butyl-2,4-dideoxy-2,4-imino-L-ribonate 2.122

Ribopyranoside **2.113** (139 mg, 0.48 mmol) was dissolved in 2 M aqueous HCl/1,4-dioxane (5:1, 6 mL). The reaction mixture was stirred at 40 °C for 15 h. Then the reaction mixture was diluted with DCM (30 mL) and washed with sodium bicarbonate (sat, 25 mL). Then aqueous layer was back extracted with DCM (2 x 25 mL). After organic layer was combined and washed with brine (sat, 40 mL), solvent was removed *in vacuo* to afford the crude aldehyde.

A solution of the residue and potassium carbonate (199 mg, 1.44 mmol) in anhydrous methanol (5 mL) was stirred at 0 °C under nitrogen atmosphere. Iodine solution (159 mg, 0.63 mmol), which was pre-dissolved in anhydrous methanol (4 mL) by sonication, was added dropwise into the reaction mixture at 0 °C. Then the mixture was stirred under 5 °C for 2 h until the completion of reaction was confirmed by mass spectrum ($[M + Na]^+$ 330). Sodium sulfite solution (sat., aq., 4 mL) was poured into the reaction mixture and distilled water (36 mL) was added subsequently to dissolve the precipitate. After the aqueous layer was exacted by ethyl acetate (4 x 25 mL), the organic layer was dried ($MgSO_4$), filtrated and concentrated *in vacuo* to afford the title compound **2.122** without further purification as a yellow oil (54%).

HRMS (ESI+ve): found 330.1666 $[M + Na]^+$; $C_{17}H_{25}NNaO_4^+$ requires 330.1676; $[\alpha]_D^{20}$ -27.1 (*c* 1.00, CH_3Cl); ν_{max} (thin film): 3455 (br, OH) 1742 (s, C=O); δ_H ($CDCl_3$, 400MHz) 0.92 (3H, t, CH_3, *J* 8.0), 1.28 - 1.40 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 2.65 (2H, t, $NC\underline{H}_2CH_2$, *J* 4.0), 2.94 (1H, br-d, H5, J_{gem} 8.8), 3.31 (H4, 1H, ddd, $J_{4,5}$ 1.2, $J_{4,3}$ 5.2, $J_{4,5'}$ 8.8), 3.47-3.42 (1H, t, H5', $J_{5',4} = J_{gem}$ 8.8), 3.47 (1H, d, H2, $J_{2,3}$ 5.2), 3.74 (3H, s, OCH_3), 4.19 (1H, t, H3, $J_{3,2} = J_{3,4}$ 5.2), 4.49 (1H, d, $C\underline{H}_2Ph$, J_{gem} 12.0), 4.66 (1H, d, $C\underline{H}_2Ph$, J_{gem} 12.0), 7.27 - 7.37 (5H, m, Ar); δ_C ($CDCl_3$, 100MHz) 13.87 (CH_3), 20.4, 29.7 ($NCH_2\underline{C}H_2\underline{C}H_2$), 52.04 ($OCH_3$), 57.2 ($N\underline{C}H_2CH_2CH_2$), 60.7 (C5), 70.2 (C2), 70.8 (C4), 71.7 ($\underline{C}H_2Ph$), 72.5 (C3), 137.4, 128.5, 128.00, 127.9 (Ar), 172.0 (C1); *m/z* (ESI+ve): 308 ($[M + H]^+$, 50%), 330 ($[M + Na]^+$, 100%),

Methyl 3-*O*-benzyl-*N*-butyl-2,4-dideoxy-2,4-imino-L-ribonamide 2.128

Methylamine (0.6 mL, 6.5 mmol, in absolute ethanol) was added into a sealed flask that had been filled with a solution of methyl ester **2.122** (100 mg, 0.32 mmol) and calcium chloride (36 mg, 0.32 mmol) in anhydrous methanol (10 mL) in nitrogen atmosphere. The reaction mixture was

stirred at 40 °C for 2 h until TLC (cyclohexane/ethyl acetate/triethylamine 1:1:0.01) showed the formation of a major product (R_f 0.15). After the solvent was removed *in vacuo*, the mixture was dissolved into aqueous ammonium chloride (aq, 8 mL) and stirred for 15 min. Then HCl (aq, 2M) was added to adjust pH to 5. The mixture stirred at room temperature for 20 min and washed with ethyl acetate (5X10 mL). The organic layer was dried ($MgSO_4$), filtrated and the solvent was removed *in vacuo* to get the residue that was further purified by flash column chromatography (cyclohexane/ethyl acetate/triethylamine 66:33:1 to 33:66:1) to yield the title compound **2.128** as a yellow oil (49 mg, 51%).

HRMS (ESI+ve): found 329.1827 $[M + Na]^+$; $C_{17}H_{27}N_2NaO_3^+$ requires 329.1836; $[\alpha]_D^{20}$ -57.0 (*c* 1.12, CH_3Cl); ν_{max} (thin film) 3330 (br, OH, NH), 1651 (s, C=O); δ_H ($CDCl_3$, 400MHz): 2.86 (3H, d, CH_3, *J* 5.0), 3.20 (1H, dt, H4, $J_{4,5} = J_{4,5'}$ 3.2, $J_{4,3}$ 4.8), 3.48 (1H, d, H2, $J_{2,3}$ 4.8), 3.53 (1H, dd, H5, $J_{5,4}$ 3.2, J_{gem} 12.0), 3.63 (1H, dd, H5', $J_{5',4}$ 3.2, J_{gem} 12.0), 3.91 (1H, t, H3, $J_{3,2} = J_{3,4}$ 4.8), 4.45 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.0), 4.83 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.0), 7.02 (1H, d, NH, *J* 5.0), 7.37 - 7.26 (5H, m, Ar); δ_C ($CDCl_3$, 100MHz): 13.9 (CH_3), 25.8 ($NHCH_3$), 20.5, 30.0 ($\underline{C}H_2\underline{C}H_2CH_3$), 58.0 ($N\underline{C}H_2$), 61.84 (C5), 71.0 (C2), 71.1 ($\underline{C}H_2$Ph), 72.5 (C4), 73.6 (C3), 127.8, 128.00, 128.4, 137.6 (-Ar), 172.0 (C1); *m/z* (ESI+ve): 307 ($[M+H]^+$, 40%), 329 ($[M+Na]^+$, 60%).

Methyl *N*-butyl-3-hydroxyl-2,4-dideoxy-3-hydroxy-2,4-imino-L-ribonamide 2.129

Palladium on charcoal (10% w.t., 8 mg) was added into a solution of protected riboamide **2.128** (50 mg, 0.16 mmol) in 1,4-dioxane/water (1:2, 6 mL). The reaction was flashed with nitrogen, argon and hydrogen gas sequentially and the reaction mixture was stirred vigorously for 21 h at room temperature until TLC (ethyl acetate) indicated the consumption of starting material and

the formation of one product (R_f 0.35). After filtration, the solvent was removed in vacuo to afford the title compound **2.129** as light yellow oil without further purification (34.3 mg, 100%). HRMS (ESI+ve): found 217.1542 $[M + H]^+$; $C_{10}H_{21}N_2O_3^+$ requires 217.1547; $[\alpha]_D^{20}$ -4.6 (c, 0.79, MeOH); ν_{max} (thin film) 1676 (s, C=O); δ_H ($CDCl_3$, 400MHz): 0.87 (3H, t, CH_3, *J* 6.8), 1.25 - 1.54 (6H, m, $\underline{C}H_2\underline{C}H_2\underline{C}H_2$), 2.74 (3H, s, $NC\underline{H}_3$), 3.77 (1H, dd, H5, dd, $J_{5,4}$ 3.2, J_{gem} 13.2), 3.81 (1H, dd, H5', dd, $J_{5',4}$ 3.2, J_{gem} 13.2), 4.14 (1H, dt, H4, $J_{4,4} = J_{4,5'}$ 3.2, $J_{4,3}$ 6.4), 4.32 (1H, t, H3, $J_{3,2} = J_{3,4}$ 6.4), 4.52 (1H, d, H2, $J_{2,3}$ 6.4); δ_C ($CDCl_3$, 100MHz): 14.3 (CH_3), 21.6 ($NHCH_3$), 27.0, 27.9 ($\underline{C}H_2\underline{C}H_2CH_3$), 57.3 ($N\underline{C}H_2$), 59.0 (C5), 66.8 (C2), 74.2 (C4), 78.3 (C3), 176.2 (C1); *m/z* (ESI+ve): 217 ($[M + H]^+$, 100%).

Methyl 3-*O*-benzyl-*N*-butyl-2,4-dideoxy-2,4-imino-5-*O*-mesyl-L-ribonate 2.123

Mesyl chloride (0.09 mL, 1.16 mmol) was added to a solution of methyl ester **2.122** (238 mg, 0.78 mmol) in pyridine (10 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 hour when TLC (cyclohexane/ethyl acetate, 1:1) showed the formation of one product (R_f 0.56). The mixture was diluted with DCM (10 mL) and washed with brine (2 x 10 mL). The organic phase was dried ($MgSO_4$) and solvent was removed *in vacuo* to yield a residue was purified by flash column chromatography (cyclohexane/ethyl acetate, 7:1 to 2:1) to give the product **2.123** as a clear oil (133 g, 100%).

HRMS (ESI+ve): found 408.1457 $[M + Na]^+$; $C_{18}H_{27}NNaO_6S^+$ requires 408.1451; $[\alpha]_D^{20}$ -25.8 (*c* 1.10, CH_3Cl); ν_{max} (thin film): 1744 (s, C=O); δ_H($CDCl_3$, 400 MHz): 0.88 (3H, dd, $CH_2C\underline{H}_3$, *J* 7.1, *J* 7.3), 1.25 – 1.41 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 2.56 – 2.73 (2H, m, $NC\underline{H}_2CH_2CH_2$), 3.09 (3H, s, SCH_3), 3.20 (1H, dt, H4, $J_{4,5} = J_{4,5'}$ 4.3, $J_{4,3}$ 5.6), 3.51 (1H, d, H2, $J_{2,3}$ 5.6), 3.72 (3H, s, OCH_3), 4.06 (1H, t, H3, $J_{3,2} = J_{3,4}$ 5.6), 4.10 (1H, dd, H5, $J_{5,4}$ 4.3, J_{gem} 11.6), 4.26 (1H, dd, H5', $J_{5',4}$ 4.3, J_{gem} 11.6), 4.51 (1H, d,

C<u>H</u>$_2$Ph, 11.6), 4.63 (1H, d, C<u>H</u>$_2$Ph, 11.6), 7.26 – 7.28 (5H, m, Ar); δ_C($CDCl_3$, 100 MHz): 13.8 (CH_3), 20.3, 29.5 (<u>C</u>H$_2$<u>C</u>H$_2$CH$_3$), 37.9 (SCH_3), 52.1 (OCH_3), 58.1 (N<u>C</u>H$_2$), 68.4 (C4), 69.5 (C5), 70.4 (C2), 71.9 (<u>C</u>H$_2$Ph), 73.1 (C3), 127.9, 128.1, 128.5, 137.1 (Ar), 171.6 (C1); *m/z* (ESI+ve): 386 ($[M + H]^+$, 100%), 408 ($[M + Na]^+$, 13%).

Methyl 5-azido-3-*O*-benzyl-*N*-butyl-2,4-imino-2,4,5-trideoxy-L-ribonate 2.124

Sodium azide (70 mg, 0.84 mmol) was added to a solution of the mesylate **2.123** (160 mg, 0.42 mmol) in DMF (3.00 mL) at room temperature. The reaction mixture was heated to 60 °C and stirred for 18 h. After which TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the complete conversion of the starting material (R_f 0.52) and the formation of a major product (R_f 0.81). The reaction mixture was diluted with ethyl acetate (5 mL) and washed with a brine/water mixture (1:1, 2 × 5 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (cyclohexane/ethyl acetate, 5:1) to afford the azide **2.124** (120 mg, 86%) as a light yellow oil.

HRMS (ESI+ve): found 333.1923 $[M + H]^+$; $C_{17}H_{25}N_4O_3^+$ requires 333.1921; $[\alpha]_D^{20}$ -52.7 (*c* 1.15, CH_3Cl); ν_{max} (thin film): 2099 (s, N_3), 1745 (s, C=O); δ_H($CDCl_3$, 400 MHz): 0.88 (3H, dd, $CH_2C\underline{H}_3$, *J* 7.1, *J* 7.3), 1.26 – 1.41 (4H, m, C<u>H</u>$_2$C<u>H</u>$_2$CH$_3$), 2.57 – 2.72 (2H, m, NC<u>H</u>$_2$CH$_2$CH$_2$), 3.20 (1H, q, H4, $J_{4,5}$ = $J_{4,5'}$ = $J_{4,3}$ 5.1), 3.21 (1H, dd, H5, $J_{5,4}$ 5.1, J_{gem} 12.9), 3.37 (1H, dd, H5', $J_{5',4}$ 5.1, J_{gem} 12.9), 3.48 (1H, d, H2, $J_{2,3}$ 5.3), 3.74 (3H, s, OCH_3), 4.02 (1H, dd, H3, $J_{3,4}$ 5.1, $J_{3,2}$ 5.4), 4.51 (1H, d, C<u>H</u>$_2$Ph, 11.9), 4.64 (1H, d, C<u>H</u>$_2$Ph, 11.9), 7.29 – 7.37 (5H, m, Ar); δ_C($CDCl_3$, 100 MHz): 13.9 (CH_3), 20.3, 29.6 (<u>C</u>H$_2$<u>C</u>H$_2$CH$_3$), 52.1 (OCH_3), 53.0 (C5), 58.2 (N<u>C</u>H$_2$), 69.2 (C4), 70.5 (C2), 71.7 (<u>C</u>H$_2$Ph), 74.1 (C3), 127.9, 128.0, 128.5, 137.3 (Ar), 171.8 (C1); *m/z* (ESI+ve): 333 ($[M + H]^+$, 100%), 355 ($[M + Na]^+$,

11%).

1-*O*-Acetyl-5-acetamido-3-*O*-benzyl-*N*-butyl-2,4-imino-2,4,5-trideoxy-L-ribitol 2.125

Lithium aluminium hydride (1 M in THF, 1.44 mL, 1.44 mmol) was added dropwise to a solution of the azide **2.124** (120 mg, 0.36 mmol) in THF (3 mL) at −78 °C under argon. The reaction mixture was stirred for 1.5 h, after which mass spectrometry indicated the disappearance of a peak corresponding to the starting material ($[M + H]^+$ 333) and the presence of a peak corresponding to the intermediate amine ($[M + Na^+]^+$ 301). The reaction was quenched with saturated aqueous ammonium chloride and the reaction mixture concentrated *in vacuo*. The crude residue was dissolved to a mixture of acetic anhydride/pyridine (1:1, 3 mL). The reaction mixture was stirred at room temperature for 1 h, after which the mixture was concentrated *in vacuo* and the crude residue was purified by flash chromatography (cyclohexane/ethyl acetate/methanol, 1:1:0 to 0:1:0.05) to afford the diacetate **2.125** (120 mg, 92%) as a brown oil.

HRMS (ESI+ve): found 385.2090 $[M + Na]^+$; $C_{20}H_{30}N_2NaO_4^+$ requires 385.2098; $[\alpha]_D^{20}$ +31 (*c* 1.05, $CHCl_3$); ν_{max} 1741 (s, OCO), 1655 (s, NHCO); δ_H(CD_3OD, 400 MHz): 0.93 (3H, m, CH_3), 1.31 - 1.45 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.96 (3H, s, CH_3), 2.05 (3H, s, CH_3), 2.60 (2H, t, $C\underline{H}_2CH_2CH_2$, *J* 8.0), 3.03 - 3.09 (2H, m, H2, H4), 3.23 (1H, dd, H1, $J_{1,2}$ 6.4, J_{gem} 13.9), 3.40 (1H, dd, H1', $J_{1',2}$ 4.3, J_{gem} 13.9), 3.67 (1H, t, H3, $J_{3,2} = J_{3,4}$ 5.4), 3.99 (1H, dd, H5, $J_{5,4}$ 5.3, J_{gem} 11.6), 4.06 (1H, dd, H5', $J_{5',4}$ 5.1, J_{gem} 13.0), 4.47 (1H, d, $C\underline{H}_2Ph$, J_{gem} 11.6), 4.51 (1H, d, $C\underline{H}_2Ph$, J_{gem} 11.6), 7.27 - 7.36 (5H, m, Ar); δ_C(CD_3OD, 100 MHz): 13.3 (CH_3), 19.8 ($\underline{C}H_2CH_2$), 20.6 (CH_3), 21.6 (CH_3), 30.4 ($CH_2\underline{C}H_2$), 42.1 (C1), 58.7 ($N\underline{C}H_2$), 65.3 (C5), 69.4, 70.1 (C2, C4), 71.8 ($\underline{C}H_2Ph$), 74.9 (C3), 127.9, 128.5, 138.4, 147.1 (Ar), 171.5 (C=O), 172.4 (C=O); *m/z* (ESI+ve): 385 ($[M + Na]^+$, 100%).

5-Acetamido-3-*O*-benzyl-*N*-butyl-2,4-imino-2,4,5-trideoxy-L-ribitol 2.126

Sodium methoxide (2.2 mg, 0.04 mmol) was added to a solution of the diacetate **2.125** (140 mg, 0.39 mmol) in methanol (3 mL) at room temperature under argon. The reaction mixture was heated to 60 °C for 18 h, after which mass spectrometry indicated the consumption of a peak corresponding to the starting material ($[M + H]^+$ 363) and the presence of a peak corresponding to the monoacetylated product ($[M + H]^+$ 321). The reaction mixture was concentrated *in vacuo* to yield a residue that was dissolved in ethyl acetate (5 mL) and washed with water (2 x 5 mL). The organic fraction was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to yield the acetamido azetidine **2.126** (53 mg, 42%) as a yellow oil.

HRMS (ESI+ve): found 321.2168 $[M + H]^+$; $C_{18}H_{29}N_2O_3^+$ requires 321.2173; $[\alpha]_D^{20}$ +54 (*c* 0.26 in $CHCl_3$); ν_{max} (thin film): 1655 (s, CONH); δ_H($CDCl_3$, 400 MHz): 0.88 (3H, dd, CH_3, *J* 7.1, *J* 7.3), 1.25 - 1.39 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.96 (3H, s, CH_3), 2.56 (2H, dd, $C\underline{H}_2CH_2CH_2$, *J* 7.3, *J* 7.8), 3.02 (1H, ddd, H4, $J_{4,5}$ 2.2, $J_{4,5'}$ 3.0, $J_{4,3}$ 5.3), 3.09 – 3.51 (2H, m, H1, H2), 3.43 (1H, dd, H5, $J_{5,4}$ 2.2, J_{gem} 11.9), 3.54 – 3.60 (2H, m, H1', H5'), 3.84 (1H, dd, H3, $J_{3,2}$ 5.1, $J_{3,4}$ 5.3), 4.44 (1H, d, $C\underline{H}_2Ph$, J_{gem} 11.6), 4.51 (1H, d, $C\underline{H}_2Ph$, J_{gem} 11.6), 6.36 (1H, br-d, NH, *J* 4.0), 7.26 - 7.35 (5H, m, Ar); δ_C($CDCl_3$, 100 MHz): 13.9 (CH_3), 20.5, 30.4 ($\underline{C}H_2\underline{C}H_2$), 23.0 ($CH_3$), 41.3 (C1), 57.6 ($N\underline{C}H_2$), 61.6 (C5), 68.7 (C4), 71.3 (C3), 71.6 ($\underline{C}H_2Ph$), 71.8 (C2), 127.7, 127.8, 128.4, 137.7 (Ar), 170.4 (C=O); *m/z* (ESI+ve): 321 ($[M + H]^+$, 100%), 343 ($[M + Na]^+$, 10%).

5-Acetamido-*N*-butyl-2,4-imino-2,4,5-trideoxy-L-ribitol 2.127

Palladium (10% on carbon, 3 mg) was added to a solution of **2.126**(27 mg, 0.08 mmol) in

1,4-dioxane/water (1:1, 2 mL). The reaction vessel was evacuated and flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 18 h at room temperature until mass spectrometry indicated the absence of the starting material ([M + H]$^+$ 321). The reaction mixture was filtered and concentrated *in vacuo* to afford the acetamido azetidine **2.127** (25 mg, 98%) as a yellow oil.

HRMS (ESI+ve): found 253.1533 [M + Na]$^+$; $C_{11}H_{22}N_2NaO_3^+$ requires 253.1523; $[\alpha]_D^{20}$ +23 (*c* 0.5 in MeOH); ν_{max} (thin film): 1650 (s, CONH); δ_H(CD_3OD, 400 MHz): 0.96 (3H, t, CH_3, *J* 7.3), 1.34 - 1.52 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.99 (3H, s, CH_3), 2.66 (2H, ddd, $C\underline{H}_2CH_2CH_2$, *J* 2.9, *J* 7.3, J_{gem} 11.7), 2.92 (1H, br-ddd, H4, $J_{4,5'}$ 3.9, $J_{4,5}$ 4.4, $J_{4,3}$ 5.5), 2.97 (1H, br-dt, H2, $J_{2,1'}$ 4.8, $J_{2,1}$ = $J_{2,3}$ 5.5), 3.21 (1H, dd, H1, $J_{1,2}$ 5.8, J_{gem} 14.1), 3.51 (1H, dd, H1', $J_{1',2}$ 4.4, J_{gem} 14.1), 3.62 (1H, dd, H5, $J_{5,4}$ 4.4, J_{gem} 12.0), 3.66 (1H, dd, H5', $J_{5',4}$ 3.9, J_{gem} 12.0), 3.81 (1H, dt, H3, $J_{3,2}$ = $J_{3,4}$ 5.5); δ_C (CD_3OD, 100 MHz): 12.9 (CH_3), 20.3, 30.0 ($\underline{C}H_2\underline{C}H_2$), 21.2 ($CH_3$), 41.4 (C1), 58.4 ($N\underline{C}H_2$), 62.0 (C5), 66.3 (C3), 71.1 (C2), 73.8 (C4), 172.2 (C=O); *m/z* (ESI+ve): 231 ([M + H]$^+$, 100%), 253 ([M + Na]$^+$, 10%).

Methyl *N*,3-*O*-dibenzyl-2,4-dideoxy-2,4-imino-5-*O*-mesyl-D-ribonate 2.131

Mesyl chloride (0.08 mL, 2.45 mmol) was added to a solution of the methyl ester **2.130**[7] (520 mg, 1.52 mmol) in pyridine (5.0 mL) at room temperature under argon. The reaction mixture was stirred for 1.5 h, after which TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the complete consumption of the starting material (R_f 0.46) and the formation of a major product (R_f 0.64). The reaction mixture was diluted with ethyl acetate (10 mL), and the mixture was washed with HCl (2M, aq., 2 × 10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo to afford the mesylate **2.131** (562 mg, 88%) as a yellow oil, which was

used without further purification.

HRMS *m/z* (ESI +ve): found 442.1299 $[M + Na]^+$; $C_{21}H_{25}NNaO_6S^+$ requires 442.1295; $[\alpha]_D^{20}$ +39.0 (*c* 0.41, $CHCl_3$); ν_{max} (thin film): 1740 (s, C=O); δ_H (400 MHz, $CDCl_3$): 2.93 (3H, s, $SO_2C\underline{H}_3$), 3.32 (1H, a-q, H4, $J_{4,3} = J_{4,5} = J_{4,5'}$ 5.0), 3.62 (1H, d, H2, $J_{2,3}$ 4.0), 3.62 (3H, s, CH_3), 3.77 (1 H, d, $NC\underline{H}_2Ph$, J_{gem} 12.8), 3.84 (1 H, dd, H5, $J_{5,4}$ 4.4, J_{gem} 11.2), 3.88 (1 H, d, $NC\underline{H}_2Ph$, J_{gem} 12.8), 4.00 (1 H, dd, H5', $J_{5',4}$ 4.4, J_{gem} 11.2), 4.10 (1H, dd, H3, $J_{3,2}$ 4.0, $J_{3,4}$ 5.2), 4.49 (1H, d, $OC\underline{H}_2Ph$, J_{gem} 11.6), 4.60 (1H, d, $OC\underline{H}_2Ph$, J_{gem} 11.6), 7.27-7.37 (10H, m, Ar); δ_C (100 MHz, $CDCl_3$): 37.6 ($SO_2\underline{C}H_3$), 52.5 (CH_3), 60.0 ($N\underline{C}H_2Ph$), 67.5 (C2), 68.3 (C5), 68.7 (C4), 72.4 ($O\underline{C}H_2Ph$), 72.9 (C3), 128.1, 128.4, 128.7, 128.7, 128.9, 130.1 ($Ar\underline{C}H$), 136,9 ($Ar\underline{C}C$), 169.7 (C1); *m/z* (ESI +ve): 420 ($[M+H]^+$, 100%).

Methyl 5-azido-*N*,3-*O*-dibenzyl-2,4-imino-2,4,5-trideoxy-D-ribonate 2.132

Sodium azide (110 mg, 1.69 mmol) was added to a solution of the mesylate **2.131** (500 mg, 1.30 mmol) in DMF (5.00 mL) at room temperature. The reaction mixture was heated to 60 °C and stirred for 24 h. After which TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the complete consumption of the starting material (R_f 0.64) and the formation of a major product (R_f 0.78). The reaction mixture was diluted with ethyl acetate (10 mL) and washed with a 1:1 brine/water mixture (2 × 10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash chromatography (cyclohexane/ethyl acetate, 7:1 to 5:1) to afford the azide **2.132** (372 mg, 78%) as a yellow oil.

HRMS *m/z* (ESI +ve): found 389.1590 $[M + Na]^+$; $C_{20}H_{22}N_4NaO_3^+$ requires 389.1584; $[\alpha]_D^{20}$ +70.3 (*c* 0.92, $CHCl_3$); ν_{max} 2099 (s, N_3), 1741 (s, C=O); δ_H (400 MHz, $CDCl_3$): 2.89 (1H, dd, H5, $J_{5,4}$ 4.4, J_{gem} 12.8), 3.02 (1H, d, H5', $J_{5'4}$ 4.8, J_{gem} 12.8), 3.22 (1 H, a-q, H4, $J_{4,3} = J_{4,5} = J_{4,5'}$ 4.8), 3.58 (1H, d, H2, $J_{2,3}$

5.2), 3.66 (3H, s, CH_3), 3.74 (1H, d, NC$\underline{H}_2$Ph, J_{gem} 12.6), 3.93 (1H, d, NC$\underline{H}_2$Ph H6', J_{gem} 12.6), 4.10 (1H, a-t, H3, $J_{3,2} = J_{3,4}$ 5.2), 4.47 (1H, d, OC$\underline{H}_2$Ph, J_{gem} 11.6), 4.61 (1H, d, OC$\underline{H}_2$Ph, J_{gem} 11.6), 7.26-7.37 (10H, m, Ar); δ_C (100 MHz, $CDCl_3$): 52.1 (CH_3), 52.4 (C5), 61.3 (N$\underline{C}H_2$Ph), 68.5 (C4), 69.7 (C2), 71.9 (O$\underline{C}H_2$Ph), 74.3 (C3), 127.8, 128.0, 128.1, 128.5, 128.6, 129.8 (Ar$\underline{C}$H), 137.4 (Ar$\underline{C}$C), 171.5 (C1); *m/z* (ESI +ve): 367 ($[M + H]^+$, 100%).

1-*O*-Acetyl-5-acetamido-*N*,3-*O*-dibenzyl-2,4-imino-2,4,5-trideoxy-D-ribitol 2.133

Lithium aluminium hydride (1 M in THF, 2.84 mL, 2.84 mmol) was added dropwise to a solution of the azide **2.132** (237 mg, 0.71 mmol) in THF (5 mL) at –78 °C under argon. The reaction mixture was stirred for 3.5 h, after which mass spectrometry indicated the absence of a peak corresponding to the starting material ($[M + Na]^+$ 389) and the presence of a peak corresponding to the intermediate amine ($[M + Na^+]^+$ 335). The reaction was quenched with ammonium chloride (sat., aq.) and the reaction mixture concentrated *in vacuo*. The crude residue was dissolved to a mixture of acetic anhydride/pyridine (1:1, 6 mL). The reaction mixture was stirred at room temperature for 15 h, after which the mixture was concentrated *in vacuo* and the crude residue was purified by flash chromatography (cyclohexane/ethyl acetate/methanol, 1:1:0 to 0:1:0.01) to afford the diacetate **2.133** (207 mg, 79%) as a yellow oil. HRMS *m/z* (ESI +ve): found 397.2125 $[M + H]^+$; $C_{23}H_{29}N_2O_4^+$ requires 397.2122; $[\alpha]_D^{20}$ –20.5 (*c* 0.39, MeOH); ν_{max} (thin film): 1737 (s, C=O), 1651 (s, C=O); δ_H (400 MHz, $CDCl_3$): 2.04 (6 H, a-s, NHCOC$\underline{H}_3$, OCOC$\underline{H}_3$), 2.94 (1H, m, H5), 3.09 - 3.33 (3H, m, H2, H4, H5'), 3.70 (2H, m, H3, NC$\underline{H}_2$Ph), 3.86 - 3.91 (1H, m, NC$\underline{H}_2$Ph), 3.93 (1H, dd, H1, $J_{1,2}$ 5.6, J_{gem} 10.0), 3.98 (1H, dd, H1', $J_{1',2}$ 5.6, J_{gem} 10.0), 4.44 (1H, d, OC$\underline{H}_2$Ph, J_{gem} 12.0), 4.49 (1H, d, OC$\underline{H}_2$Ph, J_{gem} 12.0), 7.28-7.36 (10H, m, Ar); δ_C

(100 MHz, $CDCl_3$): 21.1 (NHCO<u>C</u>H_3, OCO<u>C</u>H_3), 40.7 (C5), 61.4 (N<u>C</u>H_2Ph), 64.2 (C1), 68.5, 69.5 (C2, C4), 72.0 (O<u>C</u>H_2Ph), 73.0 (C3), 128.0, 128.2, 128.2, 128.6, 128.8, 129.6 (Ar<u>C</u>H), 137.4 (Ar<u>C</u>C), 170.9, 171.0 (O<u>C</u>OCH_3, NH<u>C</u>OCH_3); *m/z* (ESI +ve): 397 ($[M + H]^+$, 100%);

5-Acetamido-*N*,3-*O*-dibenzyl -2,4-imino-2,4,5-trideoxy-D-ribitol 2.134

Sodium methoxide (2.7 mg, 0.05 mmol) was added to a solution of the diacetate **2.133** (191 mg, 0.48 mmol) in methanol (5 mL) at room temperature under argon. The reaction mixture was heated to 60 °C for 25 h, after which mass spectrometry indicated the consumption of a peak corresponding to the starting material ($[M + H]^+$ 397) and the presence of a peak corresponding to the monoacetylated product ($[M + H]^+$ 355). The reaction mixture was concentrated in vacuo, and the residue redissolved in a 1:2 1,4-dioxane/water mixture (5 mL) and loaded onto a short column of DOWEX® 50WX8-200. The column was washed with water, and the product liberated with ammonia (2 M, aq.). The ammoniacal fractions were combined and concentrated *in vacuo* to afford the acetamido azetidine **2.134** (62 mg, 38%) as a light yellow oil.

HRMS *m/z* (ESI +ve): found 355.2017 $[M + H]^+$; $C_{21}H_{27}N_2O_3^+$ requires 355.2016; $[\alpha]_D^{20}$ –12.1 (*c* 0.77 in MeOH); ν_{max} 3301 (br, OH, NH), 1650 (s, CO); δ_H (400 MHz, CD_3OD): 1.90 (3H, s, NHCOC<u>H</u>$_3$), 2.95 (1H, dd, H5, $J_{5,4}$ 5.2, J_{gem} 13.8), 3.06 (1H, dt, H2, $J_{2,1'} = J_{2,3}$ 4.3, $J_{2,1}$ 5.4), 3.14 (1H, dd, H1, $J_{1,2}$ 5.4, J_{gem} 9.6), 3.21 (1H, dd, H5', $J_{5',4}$ 4.3, J_{gem} 13.8), 3.26 (1H, dd, H1', $J_{1',2}$ 4.5, J_{gem} 9.6), 3.28 (1 H, dd, H3, $J_{3,4}$ 2.8, $J_{3,2}$ 4.2), 3.71 - 3.77 (3H, m, H-4, NC<u>H</u>$_2$Ph), 4.48 (2H, a-s, NC<u>H</u>$_2$Ph), 7.24 - 7.37 (10H, m, Ar); δ_C (100 MHz, CD_3OD): 22.6 (NHCO<u>C</u>H_3), 42.4 (C5), 61.3 (C1), 62.7 (N<u>C</u>H_2Ph), 68.3 (O<u>C</u>H_2Ph), 72.5 (C2), 72.8 (C4), 75.4 (C3), 128.5, 128.8, 128.9, 129.4, 130.6 (Ar<u>C</u>H), 139.2, 139.5 (Ar<u>C</u>C), 173.3 (NH<u>C</u>OCH_3); *m/z* (ESI +ve): 355 ($[M + H]^+$, 100%).

5-Acetamido-2,4-imino-2,4,5-trideoxy-D-ribitol hydrochloride 2.52L

Palladium (10% on carbon, 5 mg) and 1 M aqueous HCl (0.05 mL) were added to a solution of **2.134** (62.0 mg, 0.18 mmol) in 1,4-dioxane/water (1:1, 2 mL). The reaction vessel was evacuated and flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 5 days at room temperature until mass spectrometry indicated the absence of the starting material ($[M+H]^+$ 355). The reaction mixture was filtered and concentrated *in vacuo* to afford the acetamide **2.52L** (40.3 mg, 100%) as a yellow gum.

HRMS *m/z* (ESI +ve): found 175.1075 $[M + H]^+$; $C_7H_{15}N_2O_3^+$ requires 175.1077; $[\alpha]_D^{20}$ +11.3 (*c* 0.42, MeOH); ν_{max} 3336 (br, OH, NH), 1634 (s, CO); δ_H (400 MHz, D_2O): 1.91 (3 H, s, $NHCOC\underline{H}_3$), 3.49 (1 H, dd, H5, $J_{5,4}$ 6.4, J_{gem} 15.0), 3.56 (1 H, dd, H5', $J_{5',4}$ 4.4, J_{gem} 15.0), 3.74 (1 H, dd, H1, $J_{1,2}$ 3.2, J_{gem} 13.2), 3.77 (1 H, dd, H1', $J_{1',2}$ 3.2, J_{gem} 13.2), 4.17–4.23 (2 H, m, H2, H4), 4.40 (1 H, a-t, H3, $J_{3,2} = J_{3,4}$ 6.8); δ_C (100 MHz, D_2O): 22.1 ($NHCO\underline{C}H_3$), 38.9 (C5), 57.9 (C1), 65.2, 65.4 (C2, C4), 67.1 (C3), 176.1 ($NH\underline{C}OCH_3$); *m/z* (ESI+ve): 175 ($[M + H]^+$, 100%);

2.5.4 Synthesis of peptides

Methyl *N*-benzyl-5-*O*-(*tert*-butyldimethylsilyl)-3-fluoro-2,4-imino-2,3,4-trideoxy-L-ribonate 2.135

tert-Butyldimethylsilyl chloride (111 mg, 0.74 mmol) was added to a solution of methyl ester **2.63L** (157 mg, 0.62 mmol) in DMF (10 mL) and the reaction mixture was stirred at rt for 2 h. After this time TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the disappearance of starting material (R_f 0.50) and the formation of a major product (R_f 0.87). The reaction mixture

was diluted with ethyl acetate (20 mL) and washed with water/brine (sat., aq., 1:1) (2 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to yield the title compound **2.135** as a brown oil (218 mg, 95%).

HRMS *m/z* (ESI+ve): found 390.1878 $[M + Na]^+$; $C_{19}H_{30}FNNaO_3Si^+$ requires 390.1871; $[\alpha]_D^{20}$ -16.4 (*c* 0.90, $CHCl_3$); ν_{max}(thin film): 1745 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 0.00, 0.01 (2 x 3H, 2 x s, CH_3), 0.87 (9H, s, $C(CH_3)_3$), 3.27 (1H, dq, H4, $J_{4,3} = J_{4,5} = J_{4,5'}$ 5.4, $J_{4,F}$ 21.5), 3.47 (1H, ddd, H5, *J* 1.0, $J_{5,4}$ 5.4, J_{gem} 10.8), 3.51 (1H, dd, H5', $J_{5',4}$ 5.7, J_{gem} 10.8), 3.64 (1H, dd, H2, $J_{2,3}$ 5.1, $J_{2,F}$ 21.5), 3.65 (3H, s, OCH_3), 3.82 (1H, d, $C\underline{H}_2Ph$, J_{gem} 12.9), 3.91 (1H, d, $C\underline{H}_2Ph$, J_{gem} 12.9), 4.85 (1H, dt, H3, $J_{3,2} = J_{3,4}$ 5.1, $J_{3,F}$ 56.2), 7.25 - 7.35 (5H, m, Ar); δ_C ($CDCl_3$, 100 MHz): -5.5, -5.4 (2 x $C\underline{H}_3Si$), 18.2 ($\underline{C}(CH_3)_2$), 25.8 ($C(\underline{C}H_3)_2$), 51.9 ($O\underline{C}H_3$), 60.9 ($\underline{C}H_2Ph$), 63.7 (d, C5, $J_{5,F}$ 4.0), 67.8 (d, C2, $J_{2,F}$ 21.5), 69.3 (d, C4, $J_{4,F}$ 19.1), 86.2 (d, C3, $J_{3,F}$ 214.6), 127.6, 128.3, 129.7, 136.0 (Ar), 170.6 (d, C1, $J_{1,F}$ 4.8); δ_F ($CDCl_3$, 376 MHz): -179.0 (dt, $J_{F,2} = J_{F,4}$ 21.8, $J_{F,3}$ 56.1); *m/z* (ESI+ve): 368 ($[M + H]^+$, 100%), 390 ($[M + Na]^+$, 30%).

Methyl *N*-benzyl-5-*O*-(*tert*-butyldimethylsilyl)-3-fluoro-2,4-imino-2,3,4-trideoxy-L-ribonamide 2.137

Methylamine (0.30 mL, 2.6 mmol, in absolute ethanol) was added to a solution of methyl ester **2.135** (42 mg, 0.11 mmol) and calcium chloride (12.0 mg, 0.11 mmol) in anhydrous methanol (1.0 mL). The reaction mixture was stirred at 45 °C for 2 h when the completion of reaction was confirmed by mass spectrometry ($[M+H]^+$ 367). The pH of the reaction mixture was adjusted to pH 5 using NH_4Cl (sat. aq.)/water (1:3, 2 mL) and the mixture was extracted with ethyl acetate (3 x 15 mL). The organic layer was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to

yield the amide **2.137** as a yellow oil (41 mg, 100%).

HRMS *m/z* (ESI+ve): found 389.2037 $[M + Na]^+$; $C_{19}H_{31}FN_2NaO_2Si^+$ requires 389.2031; $[\alpha]_D^{20}$ -4.4 (*c* 0.82, $CHCl_3$); ν_{max} (thin film): 1681 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 0.01 (6H, s, CH_3), 0.88 (9H, s, $C(CH_3)_3$), 2.62 (3H, d, NCH_3, $J_{CH3,NH}$ 4.9), 3.32-3.42 (1H, m, H4), 3.38 (1H, dd, H5, *J* 3.3, J_{gem} 11.1), 3.53 (1H, dd, H5', $J_{5',4}$ 3.5, J_{gem} 10.9), 3.68 (1H, dd, H2, $J_{2,3}$ 4.5, $J_{2,F}$ 24.1), 3.73 (1H, d, $C\underline{H}_2Ph$, J_{gem} 12.5), 3.80 (1H, d, $C\underline{H}_2Ph$, J_{gem} 12.2), 4.73 (1H, dt, H3, $J_{3,2} = J_{3,4}$ 4.4, $J_{3,F}$ 56.2), 6.88 (1H, q, NH, $J_{NH,CH3}$ 5.0), 7.25 - 7.35 (5H, m, Ar); δ_C ($CDCl_3$, 100 MHz): -5.6 (CH_3), -5.5 (CH_3), 18.2 ($\underline{C}(CH_3)_2$), 25.4 (NCH_3), 25.7 ($C(\underline{C}H_3)_2$), 61.7 ($\underline{C}H_2Ph$), 62.2 (d, C5, $J_{5,F}$ 4.8), 70.4 (d, C4, $J_{4,F}$ 19.9), 70.5 (d, C2, $J_{2,F}$ 20.7), 86.2 (d, C3, $J_{3,F}$ 216.2), 127.9, 128.6, 129.2 (ArCH), 136.6 (ArC), 170.4 (d, C1, $J_{1,F}$ 5.6); δ_F ($CDCl_3$, 376 MHz): -176.3 (dt, $J_{F,2} = J_{F,4}$ 24.3, $J_{F,3}$ 56.4); *m/z* (ESI+ve): 367 ($[M + H]^+$, 100%), 389 ($[M + Na]^+$, 30%).

Methyl *N*-(*N*-benzyl-8-fluoro-7,9-imino-8,7,9-trideoxy-10-*O*-(*tert*-butyldimethyl silyl)-L-ribonamido)-3-fluoro-2,4-imino-2,3,4-trideoxy-5-*O*-(*tert*-butyldimethyl silyl)-L-ribonamide 2.139

Potassium carbonate (19 mg, 0.14 mmol) was added to a solution of methyl ester **2.135** (40 mg, 0.11 mmol) in 1,4-dioxane/water (2 mL, 1:1). The reaction mixture was stirred at 40 °C for 26 h until mass spectrometry indicated completion of the hydrolysis (*m/z* (ESI-ve): 352 $[M-H]^-$) and the solvent was removed *in vacuo* to give a crude residue of acid **2.136**.

10% Palladium on charcoal (10% wt., 5 mg) was added to a solution of **2.137** (40 mg, 0.11 mmol) in 1,4-dioxane/water (3 mL, 1:2). The reaction was flushed with argon and hydrogen gas sequentially and then stirred vigorously for 5 h at room temperature under hydrogen until mass

spectrometry showed the completion of reaction ($[M+H]^+$ 277). After filtration, the solvent was removed *in vacuo* to afford a residue of **2.138** that was used without further purification.

N,*N*,*N*',*N*'-Tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 50 mg, 0.13 mmol) was added to a solution of the crude acid **2.136** (57 mg) and amine **2.138** (30 mg) in anhydrous DMF (1.5 mL). After stirring for 20 min, triethylamine (0.02 mL) was added to the reaction mixture which was stirred rt for a further 20 h until TLC analysis (cyclohexane/ethyl acetate, 1:1) showed the consumption of the starting materials and formation of one major product (R_f 0.75). The reaction mixture was diluted with ethyl acetate (20 mL) and washed with half saturated brine (20 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate, 1:1) to give the pure peptide **2.139** as a yellow oil (30 mg, 45%).

HRMS *m/z* (ESI+ve): found 634.3288 $[M + Na]^+$; $C_{30}H_{51}F_2N_3NaO_4Si_2^+$ requires 634.3278; $[\alpha]_D^{20}$ -44.0 (*c* 0.93, $CHCl_3$); ν_{max} (thin film): 1682 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 0.01, 0.03, 0.06, 0.07 (12H, 4 x s, CH_3), 0.85 (9H, s, $C(CH_3)_3$), 0.88 (9H, s, $C(CH_3)_3$), 2.74 (3H, d, NCH_3, $J_{CH3,NH}$ 4.9), 3.36 (1H, dq, H9, $J_{9,8} = J_{9,10} = J_{9,10'}$ 5.0, $J_{9,F}$ 21.9), 3.56 (1H, dd, H7, $J_{7,8}$ 4.9, $J_{7,F}$ 20.3), 3.50 - 3.64 (1H, m, H4), 3.57 - 3.63 (1H, m, H5), 3.70 (2H, br-s, $C\underline{H}_2Ph$), 3.74 (2H, br-s, H10), 4.19 (1H, d, H5', J_{gem} 12.2), 4.31 (1H, br-d, H2, $J_{2,F}$ 23.7), 4.79 (1H, dt, H8, $J_{8,7} = J_{8,9}$ 4.8, $J_{8,F}$ 56.1), 5.08 (1H, br-d, H3, $J_{3,F}$ 55.3), 7.26 - 7.35 (5H, m, Ar), 7.43 (1H, br-s, NH); δ_C ($CDCl_3$, 100 MHz): -5.7, -5.4 (2 x $\underline{C}H_3$), 18.2 ($\underline{C}(CH_3)_2$), 25.7 ($NHCH_3$), 25.8 ($C(\underline{C}H_3)_3$), 62.5 (C5, $\underline{C}H_2Ph$), 64.1 (C10), 66.0 (C7), 67.0 (C2), 68.6 (d, C4, $J_{4,F}$ 25.4), 70.9 (d, C9, $J_{9,F}$ 18.3), 85.5 (d, C3, $J_{3,F}$ 194.7), 86.1 (d, C8, $J_{8,F}$ 218.6), 128.3, 128.6, 128.9, 136.2 (Ar), 167.7, 167.8 (C1, C6); δ_F ($CDCl_3$, 376 MHz): -177.7 (dt, $J_{F,7} = J_{F,9}$ 21.2, $J_{F,8}$ 56.1), -184.4 (dt, $J_{F,2} = J_{F,4}$ 22.9, $J_{F,3}$ 55.3); *m/z* (ESI+ve): 612 ($[M + H]^+$, 100%), 634 ($[M + Na]^+$, 30%).

[Numbering as shown in Scheme 2.18]

Methyl 3-azido-*N*-benzyl-5-*O*-(*tert*-butyldimethylsilyl)- 2,4-imino-2,3,4-trideoxy-L-ribonate 2.140

tert-Butyldimethylsilyl chloride (90 mg, 0.60 mmol) and imidazole (56 mg, 0.72 mmol) was added to a solution of methyl ester **2.107** (138 mg, 0.50 mmol) in DMF (3 mL) and the reaction mixture was stirred at room temperature for 2 h when TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the disappearance of starting material (R_f 0.50) and the formation of a major product (R_f 0.80). The reaction mixture was diluted with ethyl acetate (10 mL) and washed with 1:1 water/brine (sat., aq., 2 x 10 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to yield the silyl ether **2.140** as a brown oil (188 mg, 96%).

HRMS (ESI+ve): found 413.1978 [M + Na^+]; $C_{19}H_{30}N_4NaO_3Si^+$ requires 413.1979; $[\alpha]_D^{20}$ +55 (*c* 0.36, CH_3Cl); ν_{max} 2105 (s, N_3), 1747 (s, C=O); δ_H($CDCl_3$, 400 MHz): 0.00 (6H, s, 2 x CH_3), 0.86 (9H, s, C($C\underline{H}_3$)$_3$), 3.13 (2H, ddd, H4, $J_{4,5}$ 5.1, $J_{4,3}$ 6.6, $J_{4,5'}$ 6.9), 3.36 (1H, dd, H5, $J_{5,4}$ 5.1, J_{gem} 10.5), 3.41 (1H, dd, H5', $J_{5',4}$ 6.9, J_{gem} 10.5), 3.47 (1H, d, H2, $J_{2,3}$ 6.6), 3.7 (3H, s, OCH_3), 3.71 (1H, d, $C\underline{H}_2$Ph, J_{gem} 12.5), 3.90 (1H, t, H3, $J_{3,2}$ = $J_{3,4}$ 6.6), 3.92 (1H, d, $C\underline{H}_2$Ph, J_{gem} 12.5); δ_C($CDCl_3$, 100 MHz): -5.46 (2 x $\underline{C}H_3$), 18.3 (3 x $\underline{C}H_3$), 25.8 ($O\underline{C}H_3$), 52.1 (C3), 56.9 ($\underline{C}H_2$Ph), 61.0 (C5), 64.5 (C2), 69.0 (C4), 127.7, 128.3, 129.7, 138.1 (Ar), 171.0 (C=O); *m/z* (ESI+ve): 413 ([M + Na^+], 100%).

Methyl 3-(8-azido-*N*-benzyl-10-*O*-(*tert*-butyldimethylsilyl)-7,9-imino-8,7,9-trideoxy-L-ribonamido)-*N*-benzyl-5-*O*-(*tert*-butyldimethylsilyl)-2,4-imino-2,3,4-trideoxy-L-ribonamide 2.143

Potassium carbonate (27.6 mg, 0.20 mmol) was added to a solution of methyl ester **2.140** (60 mg, 0.15 mmol) in 1,4-dioxane/water (3 mL, 1:1). The reaction mixture was stirred at 60 °C for 18 h until mass spectrometry indicated completion of the hydrolysis ([M - H]$^-$ 375) and the solvent was removed *in vacuo* to give the sodium salt **2.142** (80 mg).

10% Palladium on charcoal (10% wt., 5 mg) was added to a solution of **2.140** (60 mg, 0.15 mmol) in 1,4-dioxane/water (3 mL, 1:1). The reaction mixture was flushed with argon and hydrogen gas sequentially and then stirred vigorously for 30 min at room temperature under hydrogen until mass spectrometry showed the completion of the reaction ([M + H]$^+$ 365). After filtration, the solvent was removed *in vacuo* to afford crude amine **2.141** (60 mg) that was used without further purification.

N,*N*,*N*',*N*'-Tetramethyl-*O*-(1H-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU, 68 mg, 0.18 mmol) was added to a solution of the crude acid **2.142** (60 mg) and amine **2.141** (60 mg) in anhydrous DMF (3 mL). After stirring for 20 min, triethylamine (0.03 mL, 0.21 mmol) was added to the reaction mixture that was then stirred at room temperature for a further 20 h until TLC analysis (cyclohexane/ethyl acetate, 1:1) showed the consumption of the starting materials and formation of one major product (R_f 0.78). The reaction mixture was diluted with ethyl acetate (10 mL) and washed with half saturated brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate, 3:1) to give the pure dipeptide **2.143** as a yellow oil (85 mg, 78%).

HRMS (ESI+ve): found 745.3892 [M + Na]$^+$; $C_{37}H_{58}N_6NaO_5Si_2^+$ requires 745.3899; $[\alpha]_D^{20}$ +12.9 (*c* 0.92, CH_3Cl); ν_{max} 2106 (s, N_3), 1744 (s, OCO), 1681 (s, NHCO); δ_H(CD_3CN, 400 MHz): 0.00 (6H, s,

$\underline{C}H_3$), 0.01 (6H, s, CH_3), 0.88 (9H, s, $C(\underline{C}H_3)_3$), 0.93 (9H, s, $C(CH_3)_3$), 2.88 (1H, d, H2, $J_{2,3}$ 6.9), 2.88 (1H, dt, H4, $J_{4,5} = J_{4,5'}$ 5.2, $J_{4,3}$ 7.1), 3.21 (1H, dt, H9, $J_{9,10} = J_{9,10'}$ 2.7, $J_{9,8}$ 6.4), 3.42 (1H, dd, H5, $J_{5,4}$ 5.2, J_{gem} 11.0), 3.43 (1H, d, H7, $J_{7,8}$ 6.4), 3.46 (1H, dd, H5', $J_{5',4}$ 5.2, J_{gem} 11.0), 3.47 (3H, s, OCH_3), 3.57 (1H, d, $C\underline{H}_2Ph$, J_{gem} 12.3), 3.58 (1H, dd, H10, $J_{10,9}$ = 2.7, J_{gem} 9.0), 3.61 (1H, dd, H10', $J_{10',9}$ 2.7, J_{gem} 9.0), 3.67 (1H, t, H8, $J_{8,7} = J_{8,9}$ 6.4), 3.70 (1H, d, $C\underline{H}_2Ph$, J_{gem} 13.1), 3.81 (1H, d, $C\underline{H}_2Ph$, J_{gem} 13.1), 3.87 (1H, d, $C\underline{H}_2Ph$, J_{gem} 13.1), 4.03 (1H, dt, H3, $J_{3,NH} = J_{3,2}$ 6.9, $J_{3,4}$ 7.1), 6.78 (1H, d, NH, $J_{NH,3}$ 6.9), 7.27-7.40 (10H, m, 2 x Ar); δ_C(CD_3CN, 100 MHz): -5.78 (CH_3), -5.8 (CH_3), -5.7 (CH_3), -5.6 (CH_3), 25.7 ($C(\underline{C}H_3)_3$), 25.8 ($C(\underline{C}H_3)_3$), 45.7 (C3), 51.5 ($O\underline{C}H_3$), 57.4 (C8), 61.1 ($\underline{C}H_2Ph$), 61.5 ($\underline{C}H_2Ph$), 63.9 (C10), 65.1 (C5), 68.8 (C2), 69.8, 69.9 (C7, C9), 70.0 (C4), 127.7, 128.3, 128.5, 129.2, 129.8, 130.0, 127.7, 127.8 (2 x Ar), 169.7 (C=O), 171.3 (C=O); *m/z* (ESI+ve): 745 ($[M + Na]^+$, 100%). [Numbering as shown in Scheme 2.19]

Methyl *N*-benzyl-3-(*N*-benzyl-8-(13-azido-*N*-benzyl-16-*O*-(*tert*-butyldimethylsilyl)-12,14-imino-12,13,14-trideoxy-L-ribonamido)-10-*O*-(*tert*-butyldimethylsilyl)-7,9-imino-7,8,9-trideoxy-L-ribonamido)-5-*O*-(*tert*-butyldimethylsilyl)-2,4-imino-2,3,4-trideoxy-L-ribonamide 2.145

Potassium carbonate (27.6 mg, 0.20 mmol) was added to a solution of methyl ester **2.140** (60 mg, 0.15 mmol) in 1,4-dioxane/water (3 mL, 1:1). The reaction mixture was stirred at 60 °C for 18 h until mass spectrometry indicated completion of the hydrolysis ($[M - H]^-$ 375) and the solvent was removed *in vacuo* to give the crude acid **2.142** (90 mg).

10% Palladium on charcoal (10% wt., 5 mg) was added to a solution of dipeptide **2.143** (80 mg, 0.11 mmol) in 1,4-dioxane/water (3 mL, 1:1). The reaction mixture was flushed with argon and

hydrogen gas sequentially and then stirred vigorously for 3 h at room temperature under hydrogen until mass spectrometry showed the completion of reaction ($[M + H]^+$ 697). After filtration, the solvent was removed *in vacuo* to afford crude amine **2.144** (70 mg) that was used without further purification.

N,*N*,*N*',*N*'-Tetramethyl-*O*-(1H-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU, 68 mg, 0.18 mmol) was added to a solution of the crude acid **2.142** (90 mg) and amine **2.144** (60 mg) in anhydrous DMF (3 mL). After stirring for 20 min, triethylamine (0.03 mL, 0.21 mmol) was added to the reaction mixture which was then stirred at room temperature for a further 24 h until TLC analysis (cyclohexane/ethyl acetate, 2:1) showed the formation of one major product (R_f 0.81). The reaction mixture was diluted with ethyl acetate (10 mL) and washed with half saturated brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate, 10:1 to 4:1) to afford the tripeptide **2.145** as a yellow oil (85 mg, 72%).

$[\alpha]_D^{20}$ +16.5 (*c* 1.1, CH_3Cl); ν_{max} 2105 (s, N_3), 1746 (s, OC=O), 1681 (s, NHC=O); δ_H(CD_3CN, 400 MHz): 0.00 (6H, s, CH_3), 0.05 (6H, s, CH_3), 0.06 (6H, s, CH_3), 0.89 (9H, s, $C(CH_3)_3$), 0.90 (9H, s, $C(CH_3)_3$), 0.91 (9H, s, $C(CH_3)_3$), **Ring A**: 2.91 (1H, d, H2, $J_{2,3}$ 6.8), 2.94 (1H, dt, H4, $J_{4,5} = J_{4,5'}$ 5.0, $J_{4,3}$ = 7.1), 3.45 (3H, s, OCH_3), 3.48 (2H, d, H5, $J_{5,4}$ 5.0), 3.96 (1H, ddd, H3, $J_{3,2}$ 6.8, $J_{3,4}$ 7.1, $J_{3,NH}$ 8.1), 7.04 (1H, d, NH, $J_{NH,3}$ 8.1), **Ring B**: 3.03 (1H, d, H7, $J_{7,8}$ 6.9), 3.09 (1H, dt, H9, $J_{9,10} = J_{9,10'}$ 4.7, $J_{9,8}$ 6.9), 3.49-3.51 (2H, m, H10), 3.83 (1H, dt, H8, $J_{8,9} = J_{8,7}$ 6.9, $J_{8,NH}$ 8.5), 7.02 (1H, d, NH, $J_{NH,8}$ 8.5), **Ring C**: 3.22 (1H, dt, H14, $J_{14,15} = J_{14,15'}$ 5.1, $J_{14,13}$ 6.1), 3.43 (1H, d, H12, $J_{12,13}$ 6.4), 3.53 (2H, d, H15, $J_{15,14}$ 5.1), 3.69 (1H, dd, H13, $J_{13,14}$ 6.1, $J_{13,12}$ 6.4), 3.60 (1H, d, CH_2Ph, J_{gem} 12.4), 3.65 (1H, d, CH_2Ph, J_{gem} 13.0), 3.67 (1H, d, CH_2Ph, J_{gem} 12.5), 3.81 (1H, d, CH_2Ph, J_{gem} 12.5), 3.83 (1H, d, CH_2Ph, J_{gem} 13.0),

3.84 (1H, d, CH_2Ph, J_{gem} 12.4), 7.26-7.41 (15H, m, 3 x Ar); δ_C(CD_3CN, 100 MHz): -5.2 - -5.0 (6 x CH_3), 26.27 ($C(\underline{C}H_3)_3$), 26.31 ($C(\underline{C}H_3)_3$), 26.38 ($C(\underline{C}H_3)_3$), **Ring A:** 46.4 (C3), 52.0 (OCH_3), 65.7 (C5), 69.6 (C2), 70.8 (C4), 172.0 (C1), **Ring B**: 47.5 (C8), 65.1 (C10), 70.9 (C9), 71.1 (C7), 171.0 (C6), **Ring C**: 58.3 (C13), 64.6 (C15), 70.3 (C14), 70.4 (C12), 170.8 (C11), 61.8 ($\underline{C}H_2Ph$), 61.9 ($\underline{C}H_2Ph$), 62.3 ($\underline{C}H_2Ph$), 128.2 - 138.8 (3 x Ar); *m/z* (ESI+ve): 528 ($[0.5M + H]^+$, 100%). [Numbering as shown in Scheme 2.19]

2.6 Appendix

1. NMR assigment of **2.145** was shown in below table:

Label	^{1}H			^{13}C		
	d (ppm)	mult	$^{3}J_{HH}$ (Hz)	d (ppm)	mult	$^{1}J_{CH}$ (Hz)
AC1	--	--	--	172.03		
AC2	2.905	d	6.8	69.58		
AC3	3.963	ddd	6.8 / 7.1 / 8.1	46.35		
AC4	2.940	dt	7.1 / 5.0	70.77		
AC5	3.476	d	5.0	65.71		
AN3	7.038	d	8.1	--	--	--
AC6	3.453	s	NA	51.98		
BC1	--	--	--	170.96		
BC2	3.025	d	6.9	71.12		
BC3	3.830	ddd	6.9 / 6.9 / 8.5	47.50		
BC4	3.090	dt	6.9 / 4.7	70.94		
BC5	3.510 3.490	m m	-- --	65.10		
BN3	7.017	d	8.5	--	--	--
CC1	--	--	--	170.80		
CC2	3.425	d	6.4	70.41		
CC3	3.688	dd	6.4 / 6.1	58.27		
CC4	3.218	dt	6.1 / 5.1	70.33		
CC5	3.526	d	5.1	64.59		
CN3	--	--	--	--	--	--
Bnz1	3.839 3.598	d d	12.4 12.4	62.31		
Bnz2	3.836 3.656	d d	13.0 13.0	61.88		
Bnz3	3.812 3.672	d d	12.5 12.5	61.94		
Ar	7.26-7.41	m		128.2-138.8		
HMBC	AC1 → AC2H, AC3H, AC6H AC3 → AN3H BC1 → BC2H, BC3H, AN3H, AC3H BC3 → BN3H CC1 → CC2H, CC3H, BN3H, BC3H					

2.7 References

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Chapter 3 Scalable syntheses of rare monosaccharides and derivatives from novel triacetonides of seven carbon sugars

3.1 Introduction

An overview of rare sugars has been given in Chapter 1. Most rare sugars are still expensive and difficult to access: efficient syntheses on multigram quantities are required for their use both as building blocks for oligosaccharides and for the studies of their chemical and biological properties.[1] This chapter describes the development of novel and scalable approaches to produce a range of rare hexoses, 6-deoxy-hexoses and their derivatives. All syntheses started from cheap commercially available material or readily accessible compounds. The efficient formation of petrol soluble triacetonides of seven carbon sugars derived from glucose allowed the development of procedures with simple purification and total avoidance of chromatography (Figure 3.1).

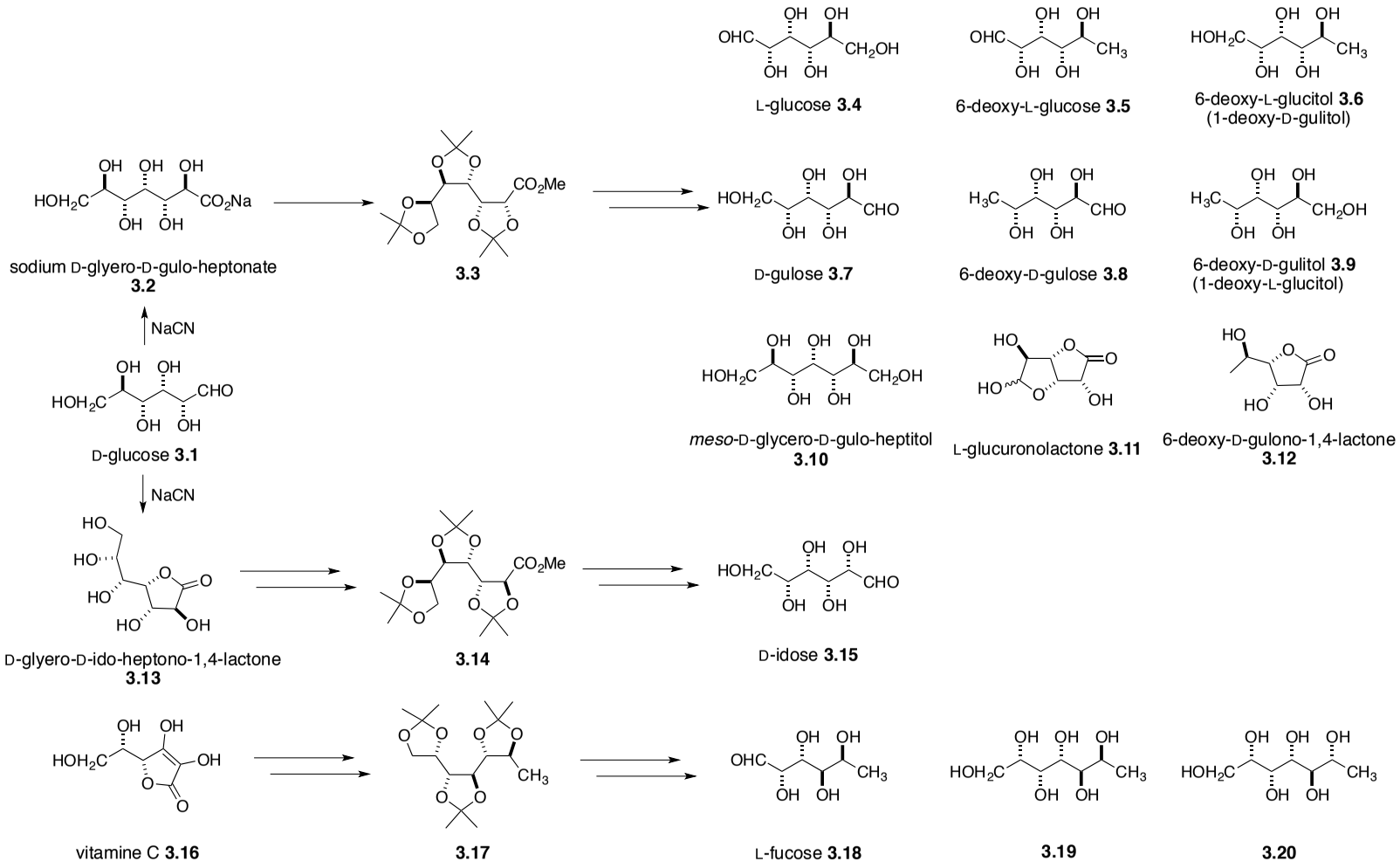


Figure 3.1 Syntheses of various rare monosaccharides from triacetonide intermediates

As shown in Figure 3.1, the *gulo*-heptonate **3.2** is the major product from the reaction of cyanide with glucose **3.1** and is one of the cheapest carbohydrates available – less than $1000 per metric ton on a large scale. Reaction of **3.2** with dimethoxypropane gave the triacetonide **3.3** which was obtained pure by a simple extraction with cyclohexane. The value of **3.3** as a divergent intermediate for access to a number of rare sugars with either an L-*gluco*- or D-*gulo*-configuration is illustrated in this work. These not only include the hexoses themselves, but also the alditols and aldonic acids which also provide starting materials for biotechnological syntheses. The mother liquors from the cyanide reaction provide more cheaply a mixture of **3.2** and the *ido*-epimer **3.13**; the triacetonide of the *ido*-heptonic ester **3.14** was easily transformed by two strategies into D-idose. Glucose has been transformed by biotechnology into vitamin C **3.16** on an industrial scale. Vitamin C **3.16** can be converted to the petrol soluble triacetonide **3.17** and thus provides an approach to a short chemical synthesis of L-fucose. The formation of fully protected seven carbon intermediates was the key step and the petrol/cyclohexane solubility of those novel triacetonides significantly simplified the procedures.

In the following introduction section, the synthesis, biological implications and potent applications of 6 monosaccharides targets in this project are highlighted.

3.1.1 L-Glucose 3.4 and 6-deoxy-L-glucose 3.5

L-Glucose **3.4** (Figure 3.2) has an identical taste compared to its enantiomer D-glucose **3.1** but it is not metabolized in the human body. Its potential application as a zero-calorie sweetener was recognized a long time ago but its high price and osmotic diarrhea side effect prevent its further

development as a safe food. On the other hand, its potential medical usage as a laxative were previously discussed.[2] Additionally, L-glucose **3.4** was found to significantly decrease glomerular filtration rate and affected renal function in rats.[3] Both anomers of L-glucose pentaacetate with a bitter taste were found to introduce insulinotropic effects *in vivo*, suggesting their value in the treatment of diabetes mellitus.[4] An *E. coli.* galactokinase mutant was reported to show a good kinase activity towards L-glucose **3.4**, which will be useful for the construction of complex glycoconjugates containing L-glucose **3.4**.[5]

The classic synthesis of L-glucose **3.4** is a Kiliani-Fischer synthesis from L-arabinose **3.21** (Figure 3.2): complicated separation of C-2 epimers was required and reagents were hazardous to human and environment.[6] In 1969, Sowa recognized the value of D-gulonoheptonic acid for accessing L-glucose **3.4** but the protection strategy was not suitable for a scalable synthesis.[7] A similar route from the benzylidene protected D-glucoheptonolactone also required significant purification procedures.[8] A Lewis acid catalyzed asymmetric cycloaddition was developed to form L-glucose on a milligram scale in 1986.[9] Later, Liptak *et al*. reported the synthesis of L-glucose **3.4** from D-gulono-1,4-lactone **3.23** by swapping its 'head' and 'tail' on a multi-gram scale.[10] The biotechnology of Izumoring is lengthy and not suitable for the mass production of L-glucose.[11]

The reports on 6-deoxy-L-glucose (L-quinovose) **3.5** are limited. This rare 6-deoxy-hexose was found in *O*-polysaccharides of bacteria *Providencia stuartii*[12] and the phenazine L-quinovose ester was identified in a marine actinomycete[13]. It was also synthesized by Kiliani approach from

L-arabinose **3.21**. To date, the main chemical synthesis of 6-deoxy-L-glucose **3.5** is epimerization mediated by acetolysis of its 2-epimer L-rhamnose (6-deoxy-L-mannose) **3.22**.[14] An enzymatic approach of accessing 6-deoxy-L-glucose **3.5** from L-rhamnose **3.22** was recently reported but complex procedures were required (Figure 3.2).[15]

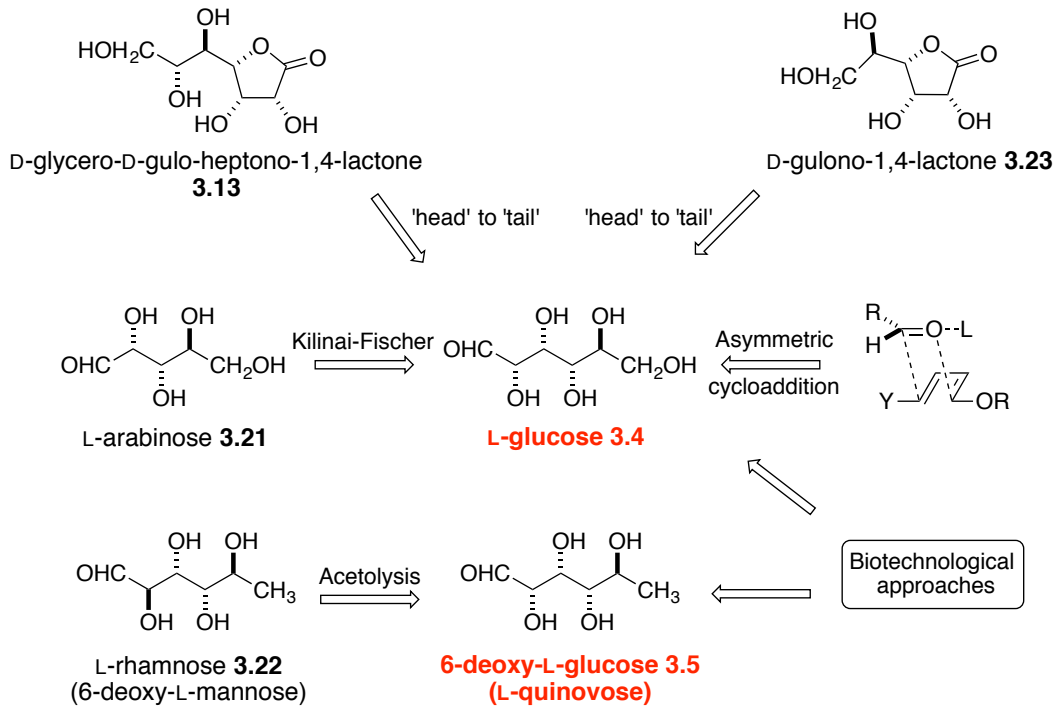


Figure 3.2 The production of L-glucose **3.4** and 6-deoxy-L-glucose **3.5**

3.1.2 D-Gulose 3.7 and 6-deoxy-D-gulose 3.8

D-Gulose **3.7** exists in small quantities in different archaea, bacteria and eukaryotes but its role has not yet been well studied.[16] Although James *et al.* suggested D-gulose **3.7** could be used as a drug formulation or food additive in 1993, research on its application is rare due to its scarcity and high price.[17] 6-Deoxy-D-gulose **3.8** is also rare in nature. The lipopolysaccharide *O*-antigen in *Yersinia enterocolitica* serotype O:8 was found to contain 6-deoxy-D-gulose **3.8** and its biosynthesis was studied.[18] Meier *et al.* reported the synthesis of one biologically important nucleoside diphosphate sugar containing 6-deoxy-D-gulose **3.8**.[19]

There are few reports on chemical synthesis of D-gulose **3.7**. In 1925, Talen reported D-sorbitol **3.26** was oxidized to a mixture of D-gulose and D-sorbose in bromine water.[20] 1,2:5,6-Di-*O*-isopropylidene-α-D-ribo-3-hexulofuranose **3.24**, which was made from D-glucose, was used to access D-gulose **3.7** in 1967.[21] It was also synthesized from D-gulono-1,4-lactone **3.23**, the major product from Kiliani ascension of D-xylose[22], by the reduction by sodium amalgam[23] or organoboron reagents[24]. A biotechnological approach to D-gulose **3.7** from more expensive D-sorbose by L-rhamnose isomerase was reported in 1999 (Figure 3.3).[25]

The chemical synthesis of 6-deoxy-D-gulose **3.8** was first achieved from 5-deoxy-D-xylose **3.27** by a Kiliani synthesis (Figure 3.3).[26] In a later report, D-gulono-1,4-lactone **3.23** was used to produce 6-deoxy-D-gulose **3.8** by the reductive deoxygenation of its 6-hydroxyl group.[27] Recently, Izumori *et al*. developed the deoxyketohexose isomerase for the enzymatic production of several 6-deoxy rare sugars including 6-deoxy-D-gulose **3.8** from L-rhamnose **3.22**.[28]

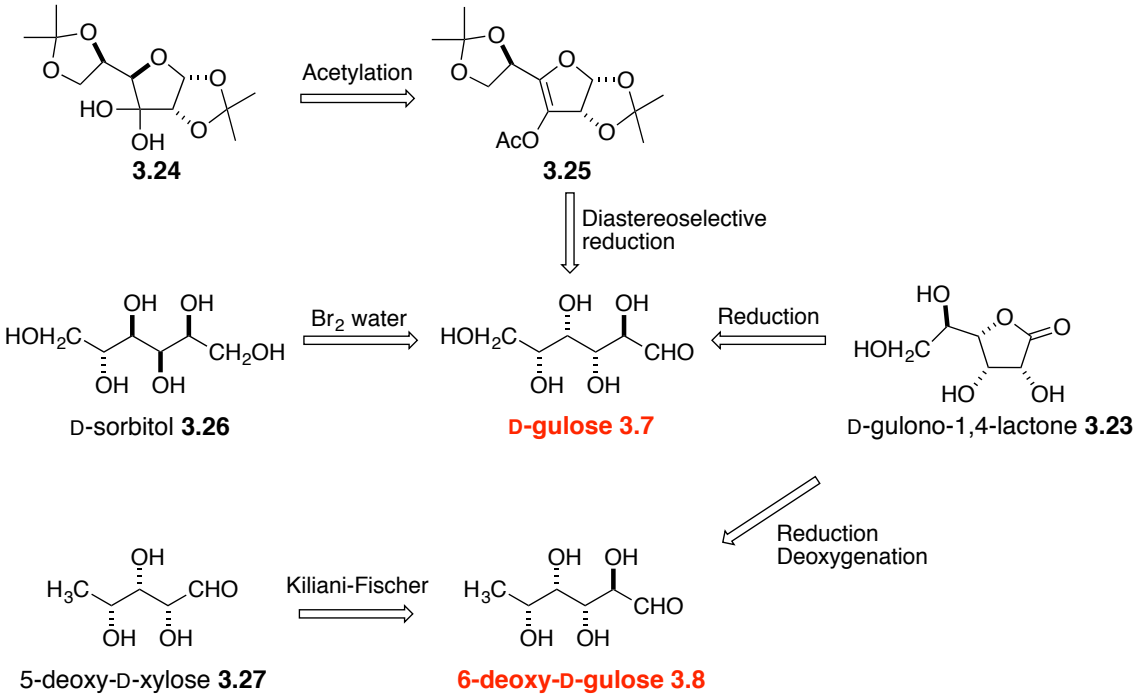


Figure 3.3 The production of D-gulose **3.7** and 6-deoxy-D-gulose **3.8**

3.1.3 D-Idose 3.15

6-Deoxy-idose is unstable and are sensitive to pH.[29] Although no biological activities have been highlighted, D-idose **3.15** has been studied in the absorption of sugars in rats[30], the heat protection effect by sugars in rats[31] and galactose-recognition receptor in rat peritoneal macrophages.[32] L-Idose **3.15** is easily obtained by inversion of C5 in D-glucose **3.1** while D-idose **3.15** has been rarely synthesized. Wiggins *et al.* reported the synthesis of D-idose **3.15** from methyl 2-*O*-p-tolylsulfonyl-β-D-galactopyranose in 1962. However, no details were given.[33] The Henry reaction of nitromethane with D-xylose **3.32** gave the epimeric mixture **3.33** of 1-deoxy-1-nitro-D-iditol and 6-deoxy-6-nitro-L-glucitol (Figure 3.4). The former was isolated by recrystallization and subjected to the Nef reaction to form D-idose **3.15**.[34] More recently, Smith *et al*. developed an approach to several rare hexoses including D-idose by asymmetric glycolate aldol reactions but no details were given for isolation of scalable amounts of D-idose **3.15**.

2-Aminopyridine (2-AP) was used as base catalyst to promote the isomerization of ketohexoses to corresponding aldohexoses including D-idose but complicated purification was required (Chapter 1). One strategy involving the biocatalytic aldol reactions between formaldehyde and glycoaldehyde (**3.34**, **3.35** and **3.36**) to aldohexoses including D-idose was developed in 2015.[35]

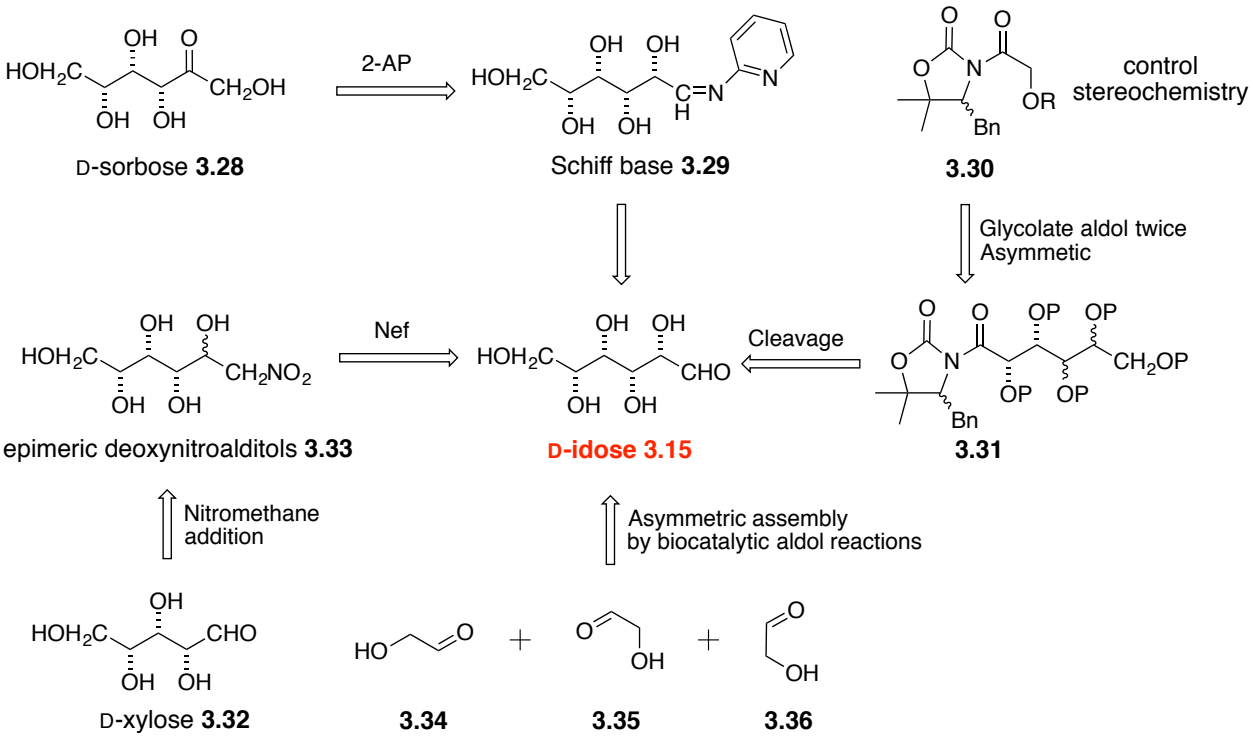


Figure 3.4 The production of D-idose

3.1.4 L-Fucose 3.18

Unlike the rare sugars mentioned above, L-fucose (6-deoxy-L-galactose) **3.18** (Figure 3.5) is a common monosaccharide widely occurring in glycolipids, *N*- and *O*- glycans in mammals.[36] Fucoidan is a sulfated polysaccharide containing fucose, found in various species of brown algae and brown seaweed, and is used as a dietary supplement.[37] Fucosylation plays important roles in various activities including cell signaling and immune response.[38] Abnormal fucosylations are linked with the progression of some diseases such as cancer[39] and atherosclerosis[40]. Fucosylated

human milk oligosaccharides (HMOs) are beneficial to the development of infant's immune system and protect them from diseases.[41] Recent *in vitro* and *in vivo* tests with rats showed L-fucose **3.18**, as a food additive, was not toxic to both mothers and offspring.[42] Accordingly, L-fucose **3.18** and its derivatives are attracting increasing attention in not only academic area but also in food and pharmaceutical industry. The potential market of L-fucose requires its effective synthesis.

L-Fucose **3.18** was isolated from seaweed and identified as early as 1920s. Since then, various chemical and enzymatic approaches have been developed to manufacture L-fucose.[43] Methods include the selective manipulation of the terminal carbons of L-galactose **3.37**, D-galactose **3.38** and D-galacturonic acid **3.39** have been reported (Figure 3.5).[44] Gyula *et al*. developed an approach to access 6-deoxy-L-talose **3.42** from protected D-glucofuranose **3.40** whose C-3 and C-5 were inverted and C-6 was deoxygenated. The following 2-epimerization of free 6-deoxy-L-talose **3.42** in acid afforded L-fucose **3.18**.[45] Currently L-fucose **3.18** is mainly produced by the fermentation of fucosylated polysaccharides such as fucoidans with consequent hazards to the environment.[46]

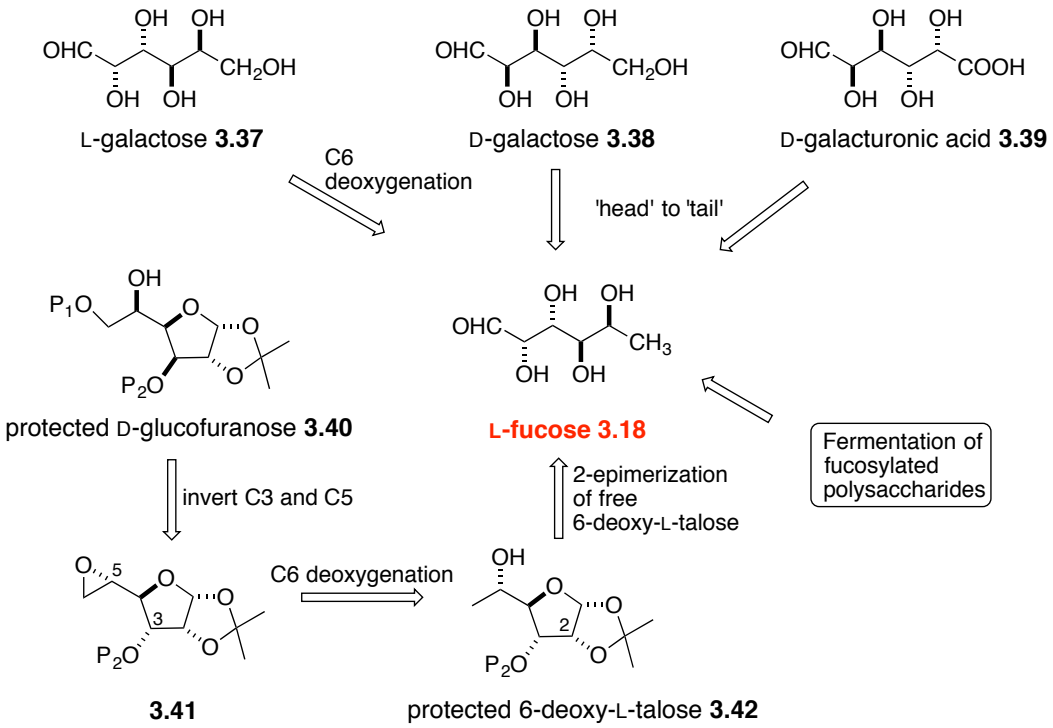


Figure 3.5 The production of L-fucose **3.18**

3.2 Aim

Chemistry and biotechnology provide a synergy for the preparation of rare sugars. It is likely that biotechnology will eventually provide the most efficient route to large scale industrial production but there is an opportunity for chemical syntheses to provide multi gram amounts of monosaccharides as building blocks for novel oligosaccharides and material to allow the initial investigation of novel biological properties.

This work shows the value of readily available and cheap seven carbon sugars for the preparation of rare hexoses and their analogues. The selective manipulation of the 'head' and 'tail' of carbohydrate derivatives has been utilized to access rare sugars. For example, as mentioned above, D-gulono-1,4-lactone **3.23** was used to synthesize L-glucose, D-gulose and

6-deoxy-D-gulose. In light of this, the *meso*-D-glycero-D-gulo-heptitol **3.43** can be considered a bridge connecting cheap D-glucose **3.1** to L-glucose **3.4** and D-gulose **3.7** (Figure 3.6). A route of accessing a range of rare sugars from cheap starting materials can be developed with the 'head' and 'tail' strategy. The main challenges are: i) how to selectively protect the other alcohols in a cheap seven carbon sugar starting material; ii) the procedures should be as simple as possible for the scalable production in order to be comparable or even superior to the established methods; iii) high purities of synthesized sugars with minimum purification are required.

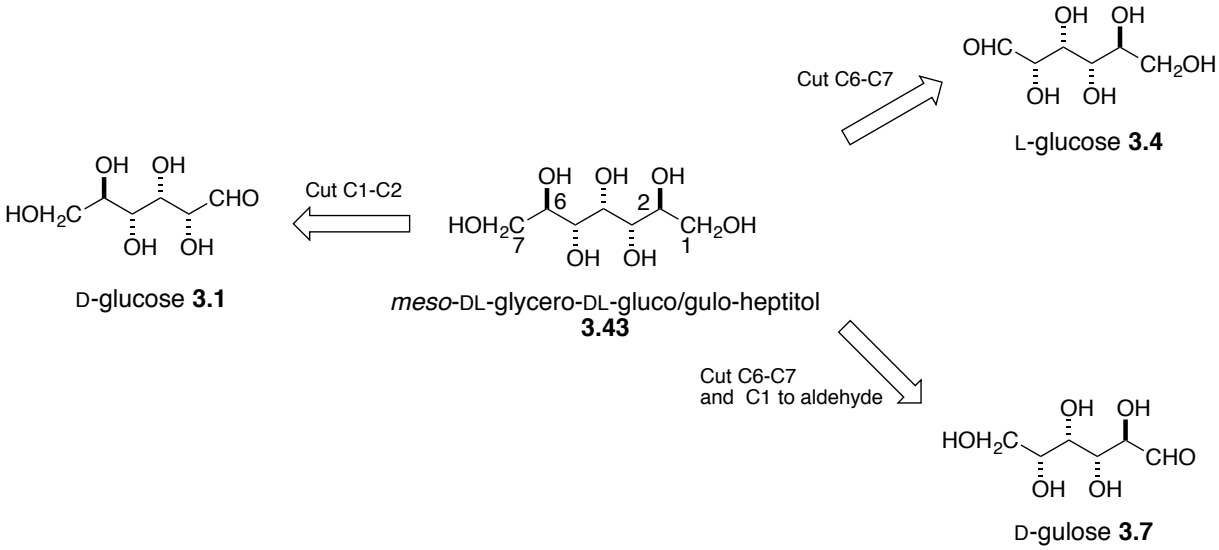


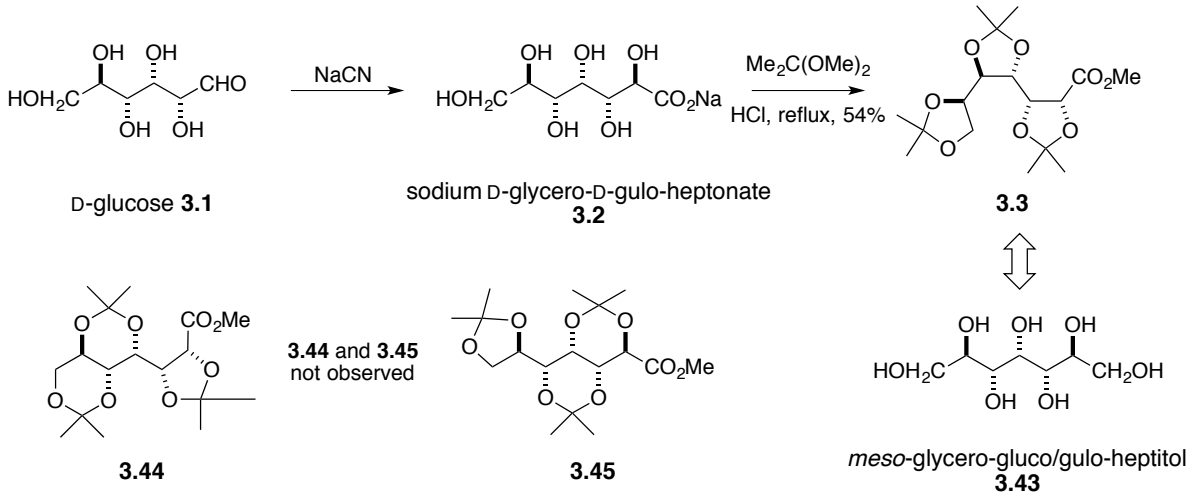
Figure 3.6 A strategy to L-glucose **3.4** and D-gulose **3.7** from D-glucose **3.1**

3.3 Results and discussion

3.3.1 From the Kiliani products derived from D-glucose

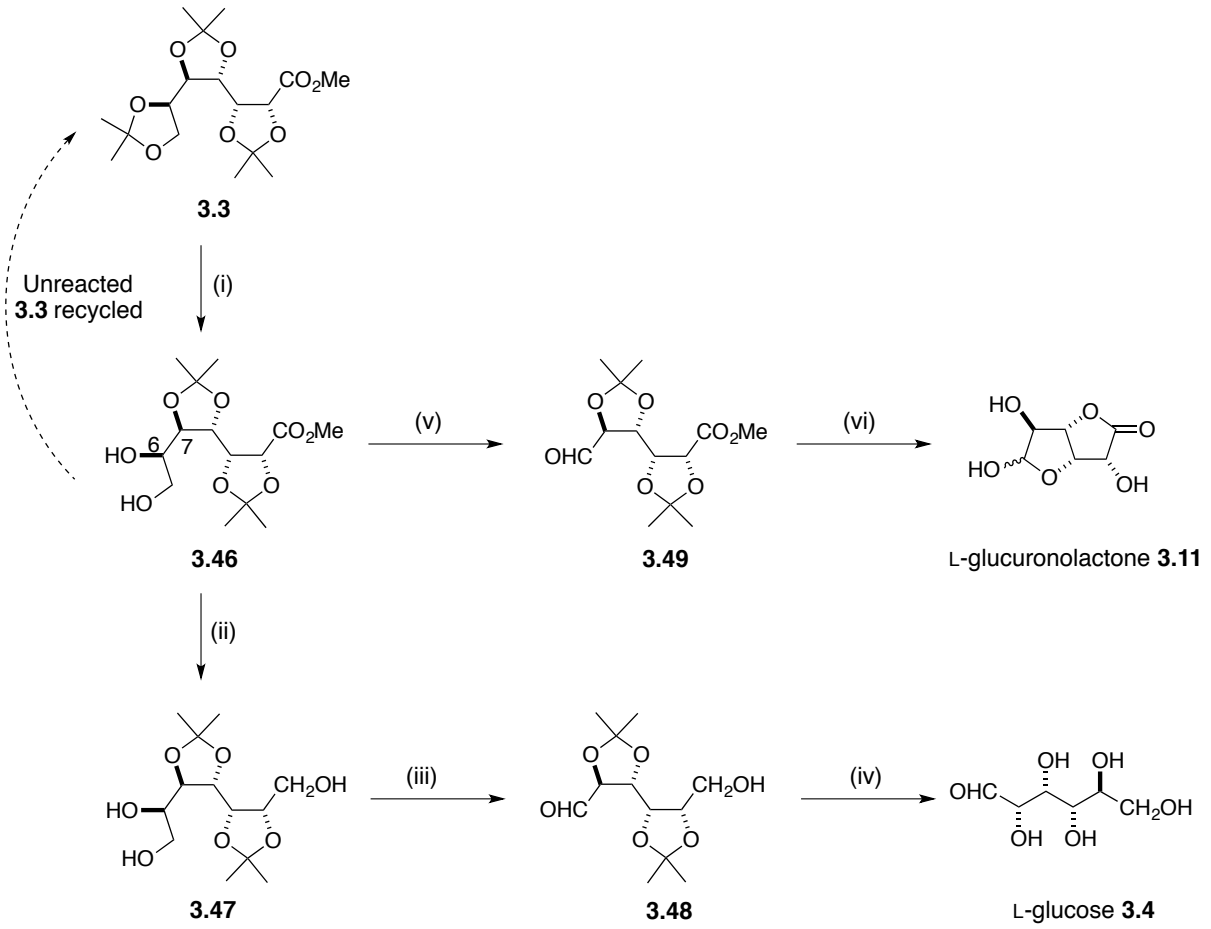
D-Glucose **3.1** with cyanide gives a Felkin-Anh controlled Kiliani ascension with sodium cyanide resulting in a pure crystalline cheap sodium salt of D-glycero-D-gulo-heptonic acid **3.2** ($500 - $1000 per metric ton).[47] First, **3.2** (Scheme 3.2) was treated with 2,2-dimethoxypropane (DMP) in the presence of methanolic HCl at reflux for 1 hour to give a novel triacetonide **3.3** (54%) and

a mixture of partially protected products. Longer heating did not significantly improve the yield. The purification was surprisingly simple: the removal of methanol *in vacuo* gave an aqueous mixture that was extracted by cyclohexane to afford pure triacetonide **3.3** (54%). The ^{1}H NMR spectrum of **3.3** from the extraction was identical with that purified by flash column chromatography. Additionally, according to ^{13}C NMR spectrum, the quaternary carbon signals of **3.3** are at δ = 109.7 – 112.1 ppm. Other possible triacetonides **3.44** and **3.45** (Scheme 3.2) with six-membered rings (quaternary carbon δ = 90 – 100 ppm) were not observed in the entire synthesis. The fully protected **3.3** is the protected chiral equivalent of the *meso*-heptitol **3.43** and allows the selective manipulation of its 'head' and 'tail' to access a series of rare sugars and novel sugar building blocks.



Scheme 3.2 Accessing triacetonide **3.3** from D-glucose **3.1**

3.3.1.1 Synthesis of L-glucose 3.4 and 6-deoxy-L-glucose (L-quinovose) 3.5



(i) 1% H_2SO_4 (aq.), MeOH, 86%; (ii) $LiAlH_4$, THF, 93%; (iii) $NaIO_4$ on silica gel, DCM; (iv) DOWEX® 50WX8-200 resin, water, 100% (2 steps); (v) $NaIO_4$ on silica gel, DCM, 100%; (vi) TFA/water, 100%

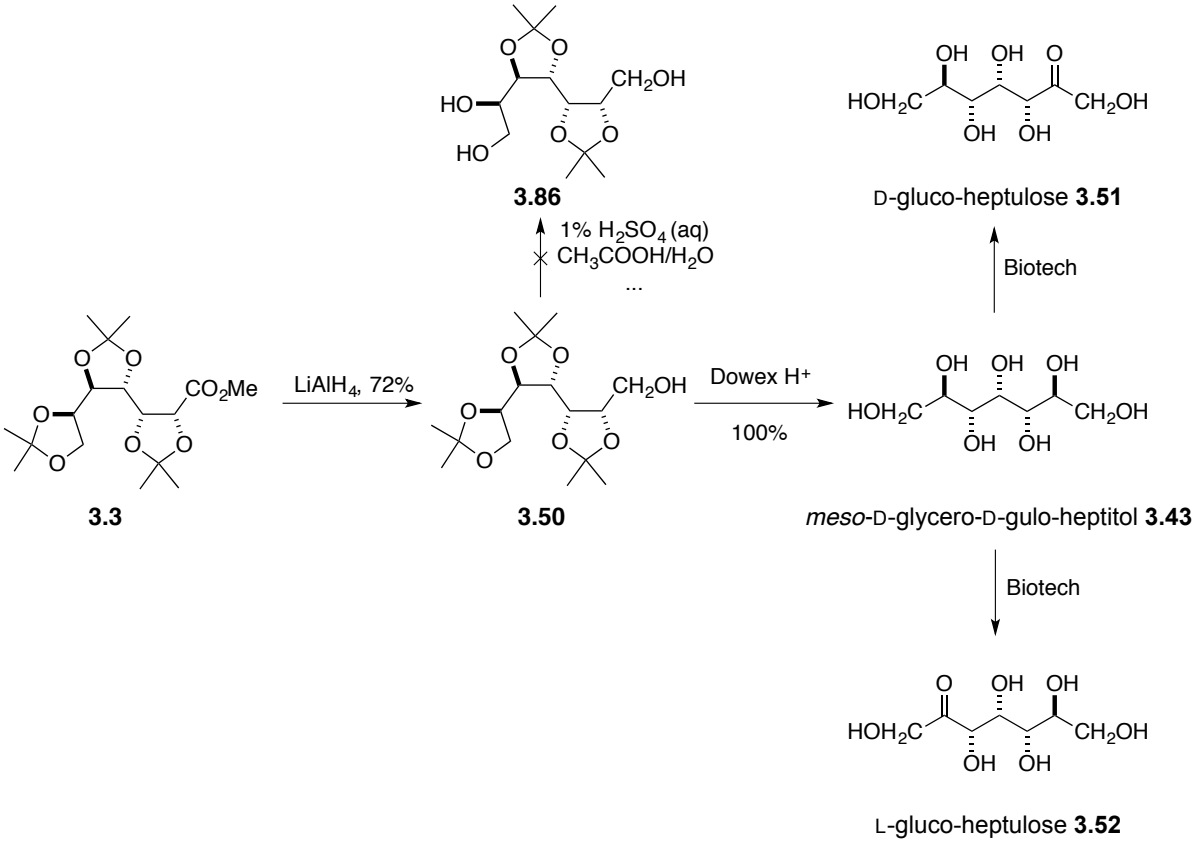
Scheme 3.3 Synthesis of L-glucose **3.3** and L-glucuronolactone **3.21**

Deprotection of terminal acetonide of **3.3** was achieved by stirring in dilute aqueous H_2SO_4 in methanol (Scheme 3.3).[48] The hydrolysis was not complete after 4 hours but other hydrolysis products had started to appear. The reaction mixture was then neutralized with triethylamine. A simple separation of **3.46** and the remaining **3.3** was performed: cyclohexane was used to recover the remains of **3.3** (48%) from the aqueous residue while sequential extraction with ethyl acetate gave clean diol **3.46** (45%) without further purification. The recovered **3.3** was subjected to hydrolysis condition to yield more **3.46** (86% overall yield based on recovered **3.3**).

Reduction of **3.46** with lithium aluminum hydride in THF gave triol **3.47** in high yield (93%). Subsequent cleavage of C-6 and C-7 by Shing's protocol[49] (silica gel-supported periodate in DCM) afforded the diacetonide of L-glucose **3.48** in 1 hour (100%). The hydrolysis of **3.48** by DOWEX® 50WX8-200 resin in water yielded free L-glucose **3.4** quantitatively (43% from salt **3.2**; 80% from triacetonide **3.3**). A 100 g scale of synthesis has been successfully carried out in laboratory conditions. No column chromatography was involved in this approach. NMR spectra of L-glucose **3.4** were identical with the corresponding commercial samples (Appendix 1) and high performance liquid chromatography (HPLC) analysis showed its purity was 99.4% compared with that of commercial sample (99.8%). Treatment of diol **3.46** with silica gel-supported periodate in DCM yielded a stable aldehyde **3.49** (100%) which was subjected to aqueous hydrolysis to give pure L-glucuronolactone **3.11** in an overall yield of 42% (78% from triacetonide **3.3**) (Scheme 3.3). No column was necessary – or gave any improvement in purity – in any of these procedures.

An alternative approach to L-glucose **3.4** was reduction of the triacetonide **3.3** prior hydrolysis of the terminal acetonide (Scheme 3.4). Triacetonide **3.3** was reduced with lithium aluminum hydride to the primary alcohol **3.50** (72%). L-Glucose **3.4** could be formed by the hydrolysis of **3.50** to diol **3.86** and subsequent periodate cleavage. However, the selective deprotection of **3.50** in various acidic conditions failed. Full deprotection of **3.50** in aqueous acid formed *meso*-D-glycero-D-gulo-heptitol **3.43**[50] (68% from **3.3**). Alditols are key intermediates in the biotechnology of Izumoring and **3.43** provides access to many heptoses. Oxidation of the *(R,R)* motif in **3.43** by *Gluconobacter thailandicus* gave D-gluco-heptulose **3.51** and oxidation of the

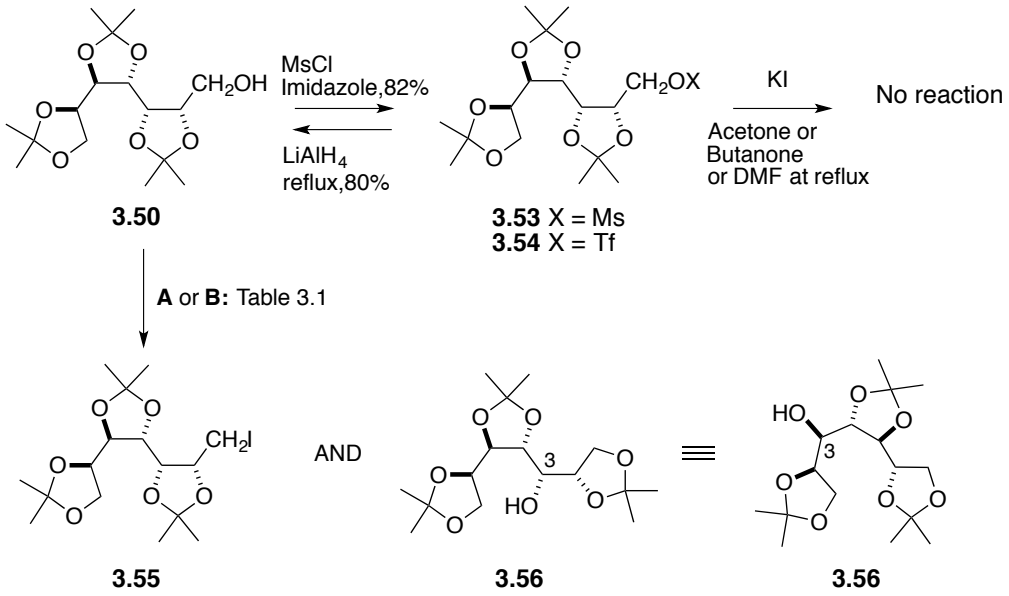
(S,S) motif in **3.43** by *Enterbacter aerogenes* IK7 formed the enantiomer L-gluco-heptulose **3.52**.[51]



Scheme 3.4 Synthesis of *meso*-D-glycero-D-gulo-heptitol **3.23**

To access 6-deoxy-L-glucose (L-quinovose) **3.5**, the deoxygenation of the primary alcohol in **3.50** *via* initial conversion to the iodide **3.55** was required (Scheme 3.5). Initially, reaction of **3.50** with mesyl chloride gave mesylate **3.53** in a yield of 82%. ^{1}H-^{1}H Total Correlated Spectroscopy (TOCSY) confirmed that **3.53** was a primary mesylate (Appendix 2). The Finkelstein reaction is a classic method to synthesize an alkyl iodide from mesylate/triflate in acetone *via* a S_N2 mechanism. However, it was found to be problematic in this case: mesylate **3.53** did not react with potassium iodide in different solvents (acetone, butanone and DMF) at high temperature. Direct reaction of mesylate **3.53** with lithium aluminum hydride at reflux in anhydrous THF gave only primary alcohol **3.50** (80%) as the sole product. Triflation of **3.50** gave a complex

mixture due to the instability of corresponding triflate **3.54**. The Appel reaction gives direct conversion of a primary alcohol to an iodide. As shown in Scheme 3.5 and Table 3.1, the treatment of **3.50** with triphenylphosphine (1.5 eq), iodide (1.5 eq) and imidazole (1.5 eq) with heating generated the desired iodide **3.55** (48%) and a secondary alcohol **3.56** (37%) (Scheme 3.5). The structure of **3.56** was confirmed by NMR spectra with $CDCl_3$ as solvent: i) three quaternary carbon signals (δ = 109.3 ppm, 109.7 ppm, 109.8 ppm) indicated that three five-membered rings remained; ii) the peak of free hydroxyl group (δ = 2.17 ppm) of **3.56** was a doublet in ^1H NMR spectrum; iii) ^1H-^1H Correlation Spectroscopy (COSY) indicated the hydroxyl group is correlated with H-3. This evidence proved the structure of **3.56**. Interestingly, the formation of **3.56** was also observed by leaving **3.50** in $CDCl_3$ for 3 weeks at room temperature. Under the reaction conditions, even though an excess of imidazole (1.5 eq) was added, the reaction mixture was found to be acidic (pH 4 - 5). The isomerization of **3.50** to **3.56** also explained the difficulty of the selective deprotection of **3.50** to diol **3.86** in aqueous acid. To avoid the formation of **3.56**, the Appel reaction was repeated in the presence of more imidazole (5.3 eq) and pH was kept basic during the entire reaction. Iodide **3.55** (69% - 75%) was formed with no formation of secondary alcohol **3.56** (Scheme 3.5). Cyclohexane extraction of the reaction crude gave pure **3.55** with large amount of triphenylphosphine oxide (TPPO). Alternately, quenching and washing the crude residue with hexane removed most of TPPO to allow a pure sample of **3.55** for full characterization.



Scheme 3.5 Attempted deoxyiodination of **3.22**

Table 3.1 Conditions of Appel reaction of **3.50**

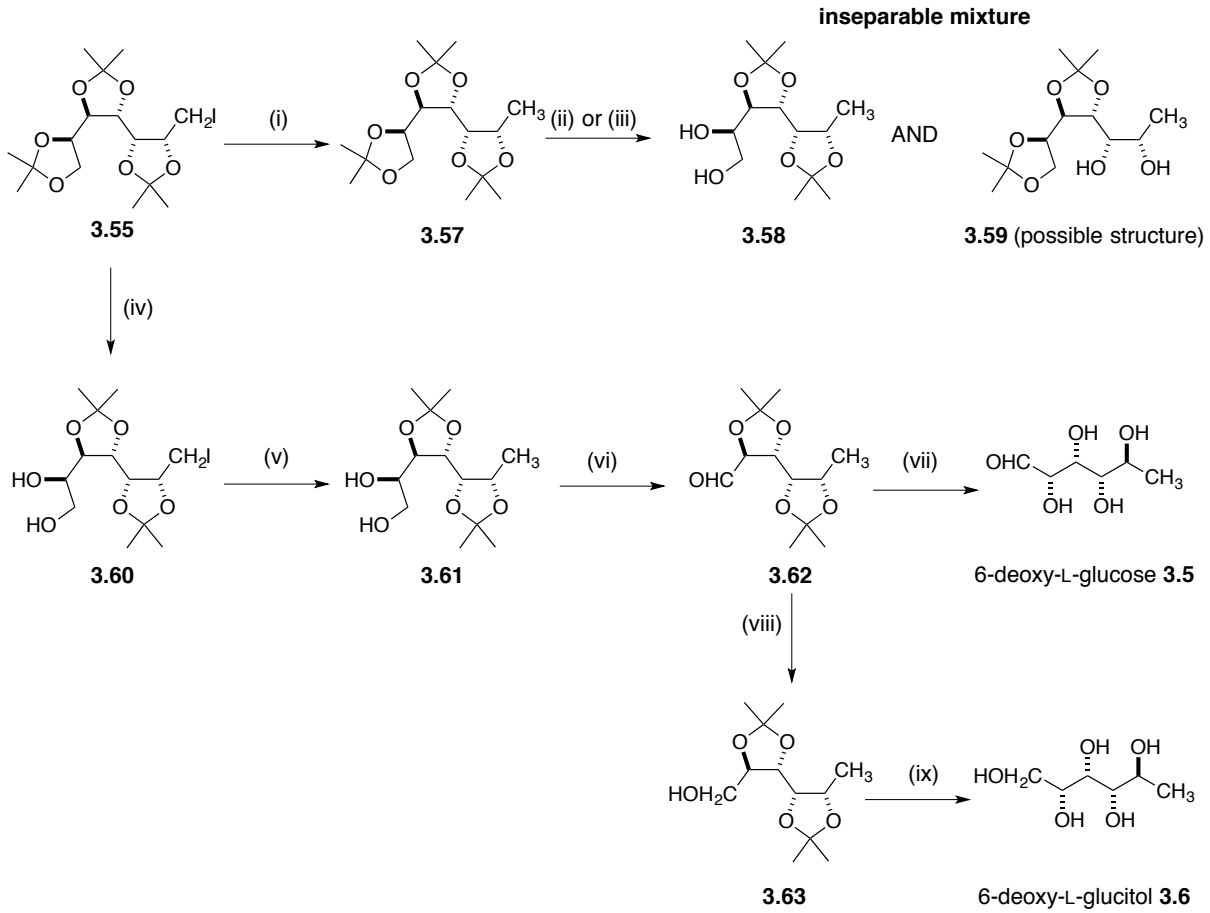
	$P(Ph)_3$	I_2	Imidazole	T (°C)	Time	3.55 (%)	3.56 (%)
A	1.5 eq	1.5 eq	1.5 eq	80	4 h	48[a]	37
B	1.5 eq	1.5 eq	5.3 eq	80	2 h	69 - 75[b]	0

[a]: this condition was repeated twice;

[b]: this condition was repeated 4 times in multigram scales;

The hydrogenation of **3.55** catalyzed by palladium black in presence of triethylamine afforded a fully protected 1-deoxy heptitol **3.57** (80%) (Scheme 3.6). Attempts to selectively remove its terminal acetonide by dilute aqueous H_2SO_4 gave an inseparable mixture of **3.58** and another diacetonide **3.59** (Scheme 3.6), which might be caused by the similarity of the 'head' and 'tail' of **3.57**. The goal of efficient hydrolysis of the terminal acetonide was achieved by altering the order of hydrogenation and hydrolysis: iodide **3.55** was stirred under acetic acid/water (7:3) at room temperature to form the only diol **3.60** (Scheme 3.6). After sequential extractions with cyclohexane and ethyl acetate, the pure diol **3.60** was isolated in a yield of 74% (based on recovered starting material **3.55**). Hydrogenation of **3.60** catalyzed by palladium on charcoal in

presence of triethylamine formed pure **3.61** (83%). After the periodate cleavage of C-6 and C-7 of **3.61** and hydrolysis, 6-deoxy-L-glucose **3.5** was synthesized on a multi-gram scale effectively (15% from salt **3.2**, 28% from triacetonide **3.3**). It is worth noting that the removal of TPPO, as an inert impurity in the whole process, was not necessary. At the end of this synthesis, TPPO could be easily removed by a simple extraction of 6-deoxy-L-glucose **3.33** solution with ethyl acetate. The NMR spectra of **3.5** are identical with that of commercially available 6-deoxy-D-glucose (Appendix 3) and the optical rotation values were of equal magnitude and opposite sign. In addition, the reduction of aldehyde **3.62** with sodium borohydride (Scheme 3.6) to give **3.63** followed by hydrolysis to give 6-deoxy-L-glucitol **3.6** (13% from salt **3.2;** 24% from triacetonide **3.3**).



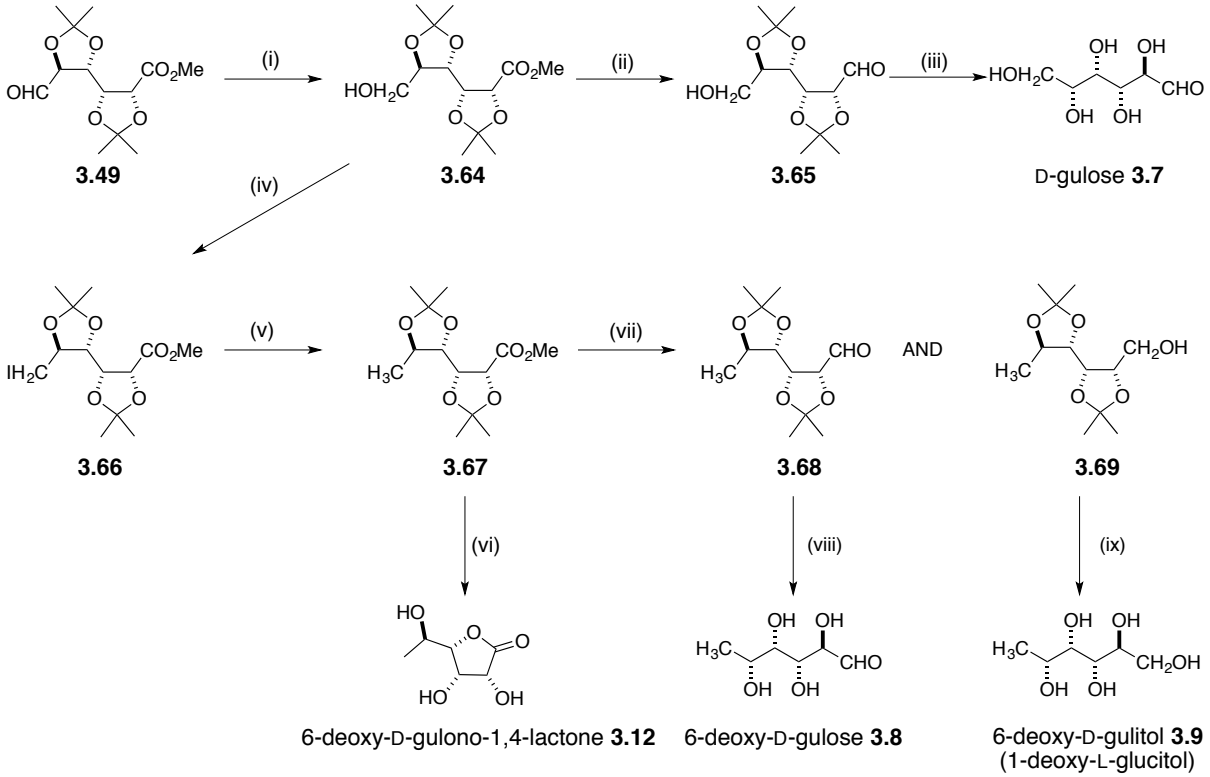
(i) Pd black, H_2, TEA, MeOH, 80%; (ii) 1% H_2SO_4 (aq.), MeOH; (iii) acetic acid/water 7:3; (iv) acetic acid/water 7:3, 74%; (v) Pd/C, H_2, TEA, MeOH, 83%; (vi) $NaIO_4$ on silica gel, 100%; (vii) DOWEX® 50WX8-200 resin, water, 100%; (viii) $NaBH_4$, MeOH, 85%; (ix) DOWEX® 50WX8-200 resin, water, 100%;

Scheme 3.6 Synthesis of 6-deoxy-L-glucose **3.5** and 6-deoxy-L-glucitol **3.6**

3.3.1.2 Synthesis of D-gulose 3.7 and 6-deoxy-D-gulose 3.8

The head to tail strategy was used for the efficient synthesis of D-gulose **3.7** (Scheme 3.7), 6-deoxy-D-gulose **3.8** and their derivatives at different oxidation levels. Reduction of aldehyde **3.49** by sodium borohydride afforded **3.64** in a yield of 96%. The subsequent reduction by DIBALH at -78 °C in THF gave diacetonide of D-gulose **3.65** (86%). This reduction was performed on a 13 g scale without complicate purification procedures. Subsequent acidic hydrolysis of **3.65** gave free D-gulose **3.7** in quantitative yield (38% from salt **3.2;** 71% from triacetonide **3.3**). HPLC analysis showed its purity reached 97.4% compared with that of

corresponding commercial sample (99.8%).



(i) $NaBH_4$, MeOH, 96%; (ii) DIBALH, DCM, -78 °C, 86%; (iii) DOWEX® 50WX8-200 resin, 100%; (iv) $(CF_3SO_2)_2O$, pyridine, -20 °C, then NaI, Butanone, 40 °C, 89% (2 steps); (v) Pd/C, H_2, TEA, MeOH, 91%; (vi) TFA/water, 100%; (vii) DIBALH, DCM, -78 °C, 84% **3.68**, 14% **3.69**; (viii) DOWEX® 50WX8-200 resin, water, 99%; (ix) DOWEX® 50WX8-200 resin, 100%;

Scheme 3.7 Synthesis of D-gulose **3.7**, 6-deoxy-D-gulose **3.8** and their derivatives

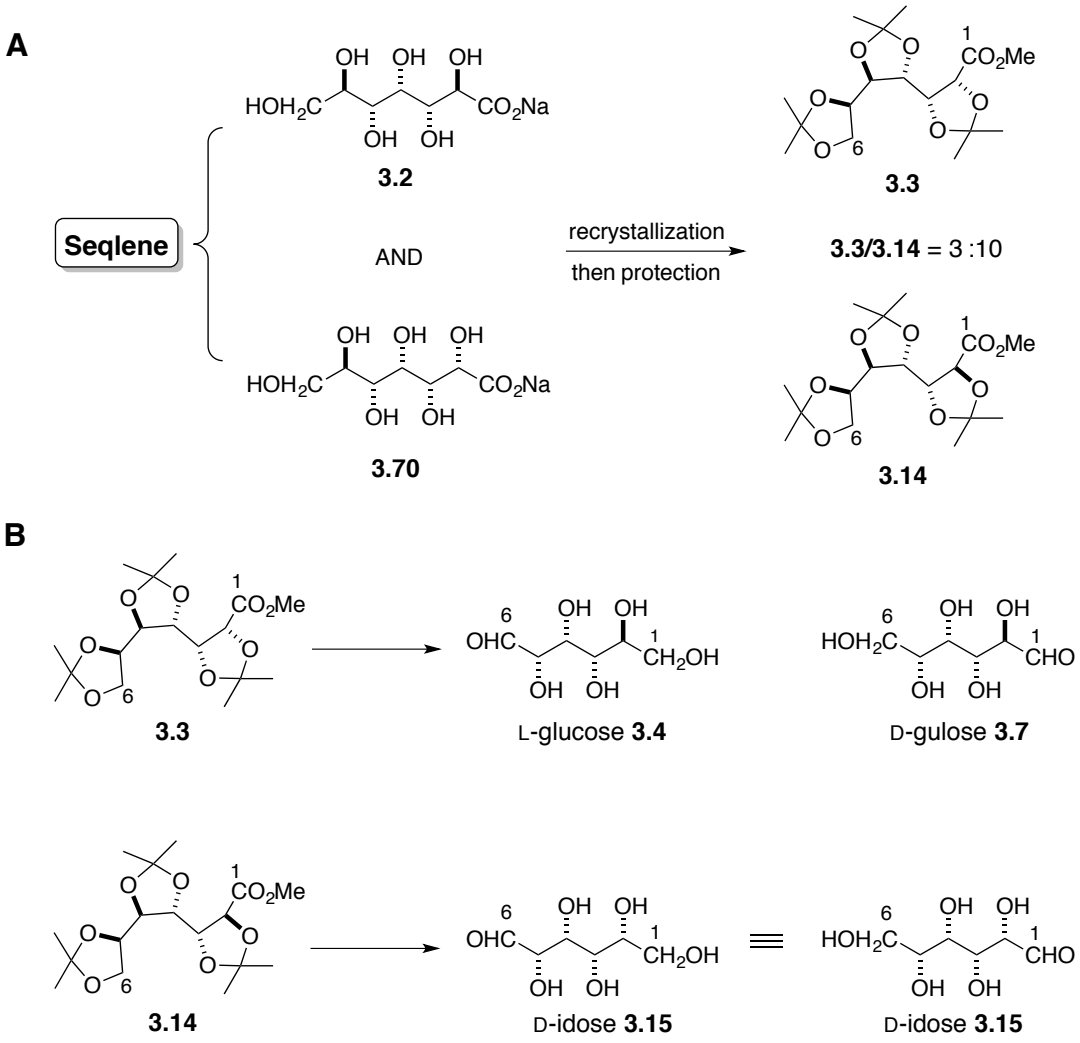
For the preparation of 6-deoxy-D-gulose **3.8**, the primary alcohol **3.64** was efficiently esterified by triflic anhydride in the presence of pyridine to form the corresponding triflate which was treated with potassium iodide in butanone with heating to afford iodide **3.66** (81%, 2 steps). This approach has been performed several times on a multigram scale and is preferable to an Appel reaction due to the ease of purification of the intermediates. Hydrogenation of iodide **3.66** in the presence of palladium on charcoal and triethylamine formed crystalline **3.67** in a yield of 91%. Only extraction with cyclohexane or ethyl acetate was necessary to purify **3.66** and **3.67**. Crystalline 6-deoxy-D-gulono-1,4-lactone **3.12**[52] was formed from the acidic hydrolysis of **3.67**. The reduction of ester **3.67** by DIBALH gave an easily separable mixture of diacetonide

6-deoxy-D-gulose **3.68** (84%) and diacetonide 6-deoxy-D-gulitol **3.69** (14%). After the deprotection by DOWEX® 50WX8-200 resin in water, 6-deoxy-D-gulose **3.8** (27% from salt **3.2**; 50% from triacetonide **3.3**) and 6-deoxy-D-gulitol **3.9** (10% from **3.49**) were produced.

3.3.1.3 Synthesis of D-idose 3.15

The mother liquors from the Kiliani reaction of glucose with cyanide after sodium glucoheptonate has been crystallized provide a commercially available chelating agent called Seqlene ES-50 which contains a 1:1 mixture of **3.2** and **3.70** (Scheme 3.8A). Seqlene finds utility in three major markets: concrete admixture, industrial cleaners and agricultural chelators.[53] As discussed, the *gluco*-heptonate triacetonide **3.3** provides access to L-glucose **3.4** or D-gulose **3.7** depending on whether the resulting aldehyde is derived from C6 or C1 of the acetonide; similar manipulation of the *ido*-heptonate triacetonide gives only D-idose **3.15** regardless of whether the aldehyde comes from C6 or C1 (Scheme 3.8B).

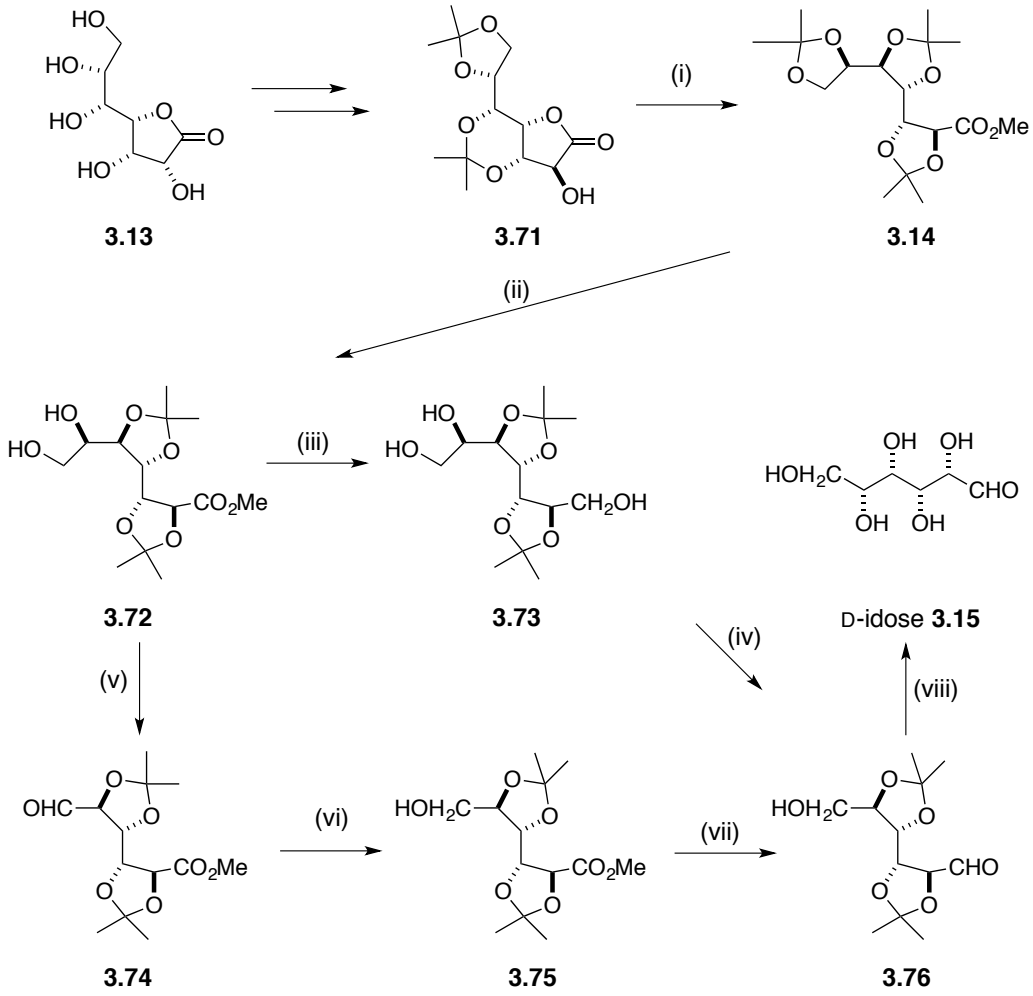
Isbell *et al.* reported the isolation of large quantities (> 100 g) of sodium D-glycero-D-ido-heptonate **3.70** from Seqlene by the recrystallization of a double salt: cadmium D-glycero-D-ido-heptonate • cadmium chloride monohydrate.[54] The double salts obtained from the recrystallization in this reported procedure were directly subjected to the established acetonide protection by DMP in methanolic HCl. A mixture containing **3.3** and **3.14** with a NMR ratio of 10:3 was obtained (Scheme 3.8A). In contrast, direct protection of Seqlene salts gave **3.3** and **3.14** with a ratio of 1:1. Although optimization was required, this approach presented an easily accessible starting material for the scalable production of D-idose **3.15**.



Scheme 3.8 Attempted synthesis of **3.47** from Seqlene: **A** production of triacetonide mixture of **3.3** and **3.14** from Seqlene; **B** production of L-glucose **3.4**, D-gulose **3.7** and D-idose **3.15** from triacetonides **3.3** and **3.14**

In order to test this strategy for the synthesis of D-idose **3.15**, pure *ido*-triacetonide **3.14** was prepared from 3,5:6,7-di-*O*-diisopropylidene-D-*glycero*-D-*ido*-heptonic-γ-lactone **3.71** (Scheme 3.9) which was synthesized from the corresponding D-*glycero*-D-*gulo*-heptonic-γ-lactone **3.13** in large quantities.[55] The treatment of **3.71** with DMP in methanolic HCl at reflux for 2 hours afforded cyclohexane soluble *ido*-triacetonide **3.14** (71%) with other partially protected products. Extraction of the crude residue from the reaction with cyclohexane gave pure **3.14**. The other products were recovered by the subsequent extraction with ethyl acetate and could

be recycled to give an overall yield of 95%. In the next step, over-deprotected products were observed in the hydrolysis of **3.14** with 1% aqueous H_2SO_4 in methanol. However, a milder acidic condition (acetic acid/water/methanol 2:1:3) was employed to give diol **3.72** (61% based on recovered **3.14**) which was purified by the sequential extractions with cyclohexane and ethyl acetate.



(i) $Me_2C(OMe)_2$, (AcCl + dry MeOH), reflux, 95%; (ii) acetic acid/water/methanol 2:1:3, 61%; (iii) $NaBH_4$, MeOH, 95%; (iv) $NaIO_4$ on silica gel, DCM; (v) $NaIO_4$ on silica gel, DCM; (vi) $NaBH_4$, MeOH, 57% (2 steps); (vii) DIBALH, DCM, -78 °C; (viii) DOWEX® 50WX8-200 resin, water, 97% (2 steps from **3.73**), 76% (2 steps from **3.75**);

Scheme 3.9 Synthesis of D-idose **3.15**

From the *ido*-diacetonide **3.72**, two routes were developed to access D-idose **3.15** analogous to those for the preparation of L-glucose and D-gulose from the *gluco*-acetonide **3.3** (Scheme 3.9). i) The reduction of ester **3.72** by an excess amount of sodium borohydride gave triol **3.73** (95%).

Subsequent periodate cleavage of **3.73** gave aldehyde **3.76** which was deprotected by DOWEX® 50WX8-200 resin in water to D-idose **3.15** (91% from diol **3.72**; 53% from lactone **3.71**). ii) The cleavage of C-6 and C-7 bond of diol **3.72** by silica gel-supported periodate gave aldehyde **3.74** which underwent the reduction by sodium borohydride to afford alcohol **3.75** (57%, 2 steps, column chromatography involved). Then DIBALH reduction of ester **3.75** gave corresponding aldehyde **3.76** that was subsequently hydrolyzed by DOWEX® 50WX8-200 resin to D-idose **3.15** with a lower yield (43% from diol **3.72**; 25% from lactone **3.71**). NMR spectra of synthesized D-idose from both of those routes were identical with those of a commercial D-idose sample (Appendix 6).

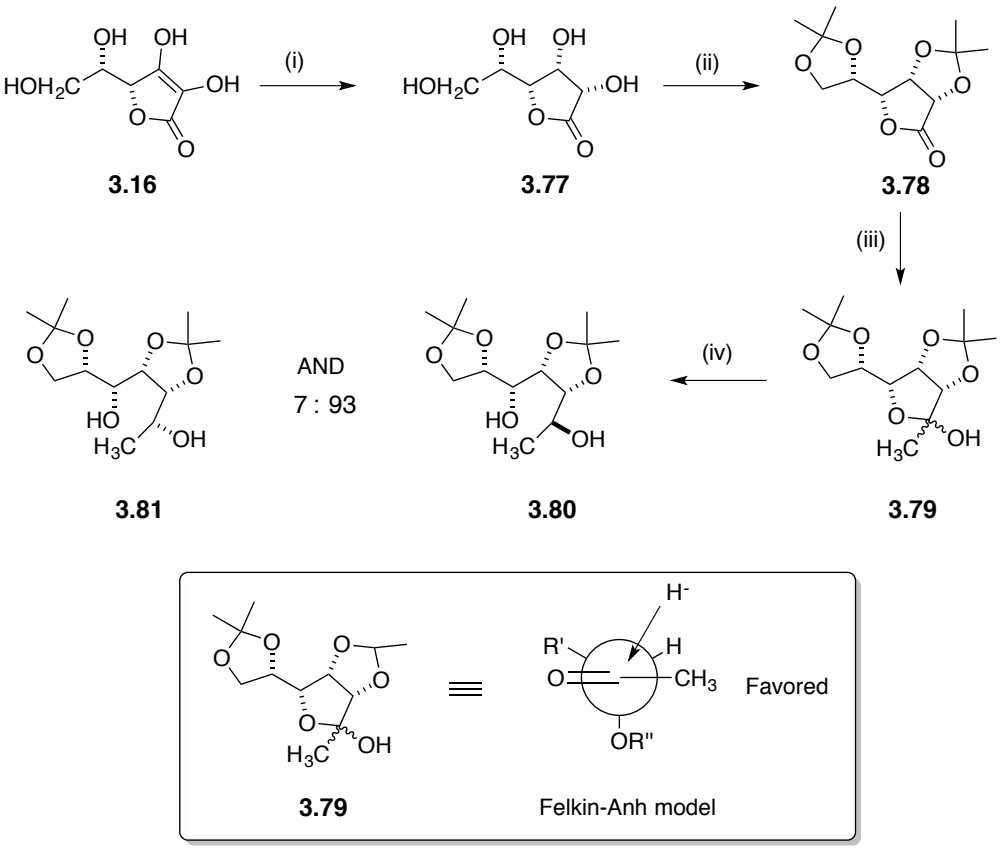
3.3.2 Deoxyheptitols derived from vitamin C

3.3.2.1 Synthesis of L-Fucose 3.18

The scalable synthesis of L-fucose **3.18** was achieved by a similar head and tail strategy. The petrol/cyclohexane solubility of the fully protected seven carbons triacetonide is crucial for avoiding complicated purification procedures in the entire synthesis. This approach was successfully applied in the scalable production of L-fucose from a cheap chemical, vitamin C **3.16** ($ 3 – 4 per kg). (Scheme 3.10)

A well-established industrial procedure allows the transformation of vitamin C **3.16** to L-gulonolactone **3.77** (99%) by palladium catalyzed hydrogenation (Scheme 3.10).[56] The efficient protection of **3.77** gave diacetonide lactone **3.78** (93%) by *p*-toluenesulfonic acid (*p*TSA) in acetone was also previously reported.[57] Addition of methyl lithium to lactone **3.78** afforded

lactol **3.79** as a solid. Although **3.79** could be easily crystallized as α-anomer,[58] crude **3.79** was directly subjected to the reduction by sodium borohydride in methanol to give a mixture of diacetonide **3.80** and its 2-epimer **3.81** in a ratio of 93:7 according to ^{1}H NMR spectrum. No further purification beyond solvent extraction was required. The diastereoselectivity can be rationalized by the Felkin-Ahn controlled addition of hydride.[59] This process has been performed in a 30 g scale in laboratory conditions and is currently being developed on a multi-kilogram scale.

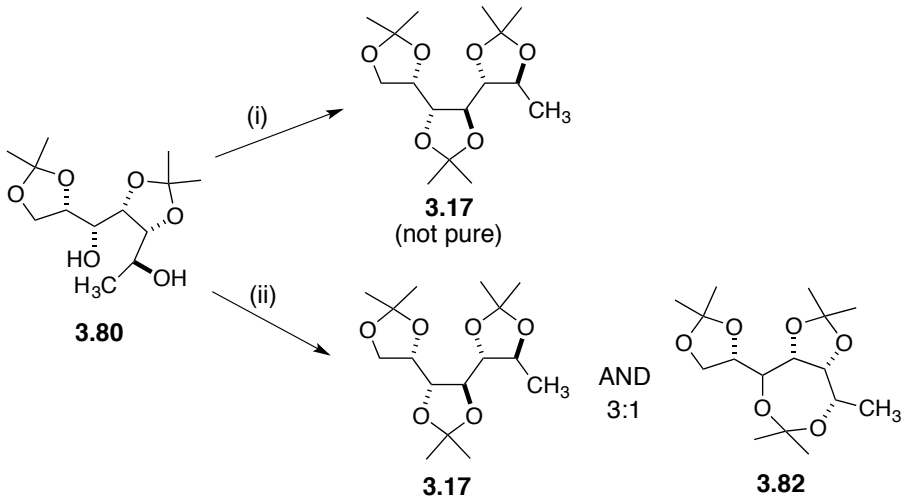


(i) H_2, Pd, 99%[56]; (ii) Acetone, H^+, 93%[57]; (iii) MeLi, THF, 96%; (iv) $NaBH_4$, MeOH, 96%;

Scheme 3.10 Synthesis of L-fucose **3.18** (1)

The epimeric mixture of **3.80** and **3.81** was treated with methanolic HCl in DMP at reflux for 1 hour. After removal of solvent, extraction of the crude product with cyclohexane gave a complex mixture while ^{13}C NMR spectrum of the crude residue indicated the formation of some of the

triacetonide **3.17** with three five-membered acetonide rings. Alternatively, the treatment of the epimeric mixture **3.80** and **3.81** with a catalytic amount of *p*TSA (0.1 eq) in acetone with refluxing overnight gave two cyclohexane soluble products in a ratio of 3:1 (Scheme 3.11). According to the shifts of their quaternary carbons in ^{13}C NMR spectra, the major product was the triacetonide **3.17** (δ = 108.9 ppm, 109.5 ppm and 110.2 ppm) whilst the minor product was a triacetonide with two five-membered rings (δ = 109.6 ppm and 109.0 ppm) and one larger ring (δ = 101.7 ppm). **3.82** showed one possible structure of the minor product. Due to the similar polarities of **3.17** and **3.82**, an inefficient flash column chromatography was required to separate the two products. Therefore, this procedure was not suitable for the development of further synthesis.



3.80

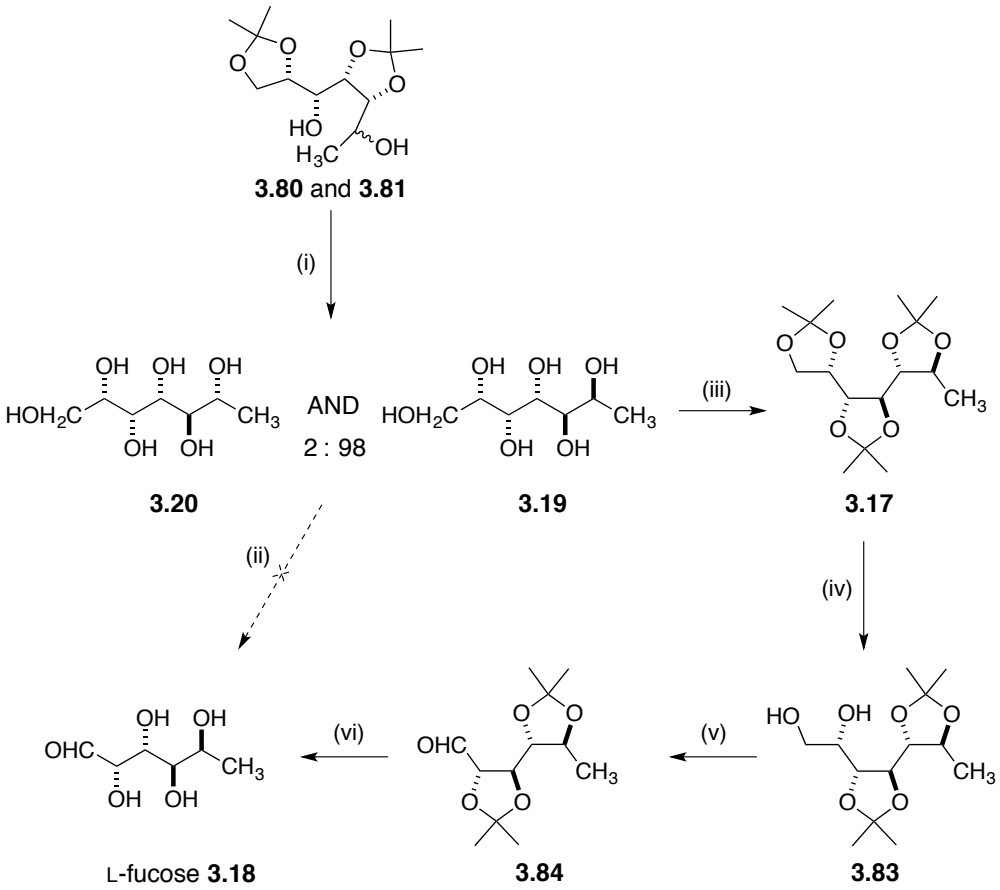
3.17 (not pure)

3.17

3.82

(i) $Me_2C(OMe)_2$, (AcCl + dry MeOH), reflux, 1h; (ii) Acetone, pTSA, overnight

Scheme 3.11 Attempts of full protection of **3.80** (only major epimer is shown)



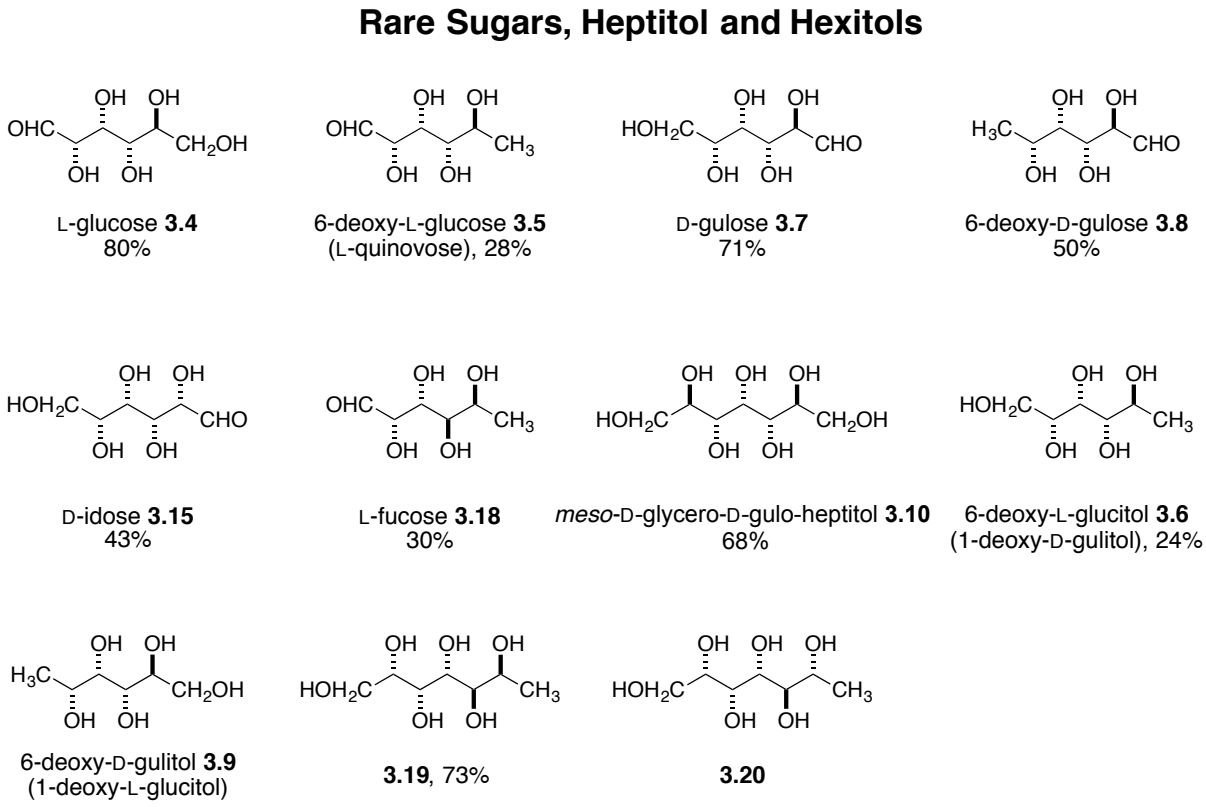
(i) DOWEX® 50WX8-200 resin, water, 81% (crystallization); (ii) $NaIO_4$, water, 2 days; (iii) acetone, *p*TSA, 83%; (iv) acetic acid/water/methanol (2:1:3), 50 °C, 81%; (v) $NaIO_4$ on silica gel, DCM, 80%; (vi) DOWEX® 50WX8-200 resin, water, 100%, recrystallization gave 74%

Scheme 3.12 Synthesis of L-fucose **3.18** (2)

To overcome this problem, the epimeric mixture of **3.80** and **3.81** was completely deprotected to give the 1-deoxy heptitols **3.20** and **3.19** (ratio 7:93). (Scheme 3.12) A simple crystallization of the mixture in acetonitrile/water improved the ratio of **3.20** and **3.19** to 2:98. Previous work suggested that the cleavage of terminal diols in free deoxyheptitols by sodium periodate in water might give the corresponding L-fucose **3.18** and 6-deoxy-D-altrose since the cleavage of the terminal diol was more rapid than other diols.[60] However ^{1}H NMR study of the product from direct periodate oxidation of the free deoxyheptitols in water gave no sign of the formation of L-fucose during the reaction. The protection of 6-deoxy-heptitol **3.19** was achieved by treating the mixture with *p*TSA (0.1 eq) in acetone at reflux for 2 hours. The extraction of the resulting

mixture with cyclohexane gave clean triacetonide **3.17**. The rest of the partially protected residue was recycled by the extraction with ethyl acetate and subjected to the same reaction conditions to afford more **3.17** (83% based on recycled residue). No other triacetonide were detected during this process. Subsequent hydrolysis in acetic acid/water/methanol (2:1:3) afforded diol **3.83** (81% based on recycled starting material) which was purified by sequential extractions with cyclohexane and ethyl acetate. After cleavage of the terminal diol by silica gel-supported periodate and DOWEX® 50WX8-200 resin hydrolysis of resulting aldehyde **3.84**, L-fucose **3.18** (Scheme 3.12) was formed with a purity of 95.9% (HPLC). A simple recrystallization from ethanol improved its purity to 99.3% (HPLC) compared with that of commercial L-fucose sample (99.8%). The overall yield of L-fucose was 30% from **3.78** and 27% from vitamin C **3.16**. A comparison of their NMR spectra showed the synthesized L-fucose had identical spectra to those of a commercial L-fucose sample (Appendix 7).

3.4 Conclusions



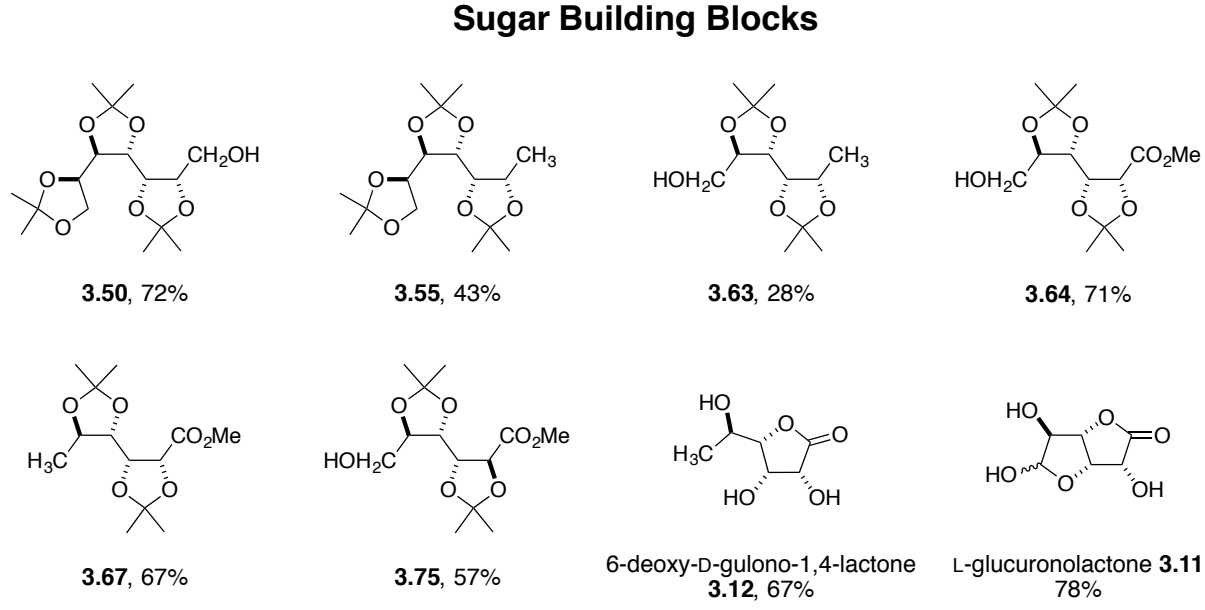


Figure 3.7 Examples of rare sugars and sugar building blocks synthesized in this project

i) A simple and scalable strategy was developed for the synthesis of L-glucose **3.4**, 6-deoxy-L-glucose **3.5**, D-gulose **3.7**, 6-deoxy-D-gulose **3.8**, D-idose **3.15** and L-fucose **3.18** from

readily available cheap starting materials. High yields and high purities were achieved. Crystallization and extraction were the major purification methods used. The solubility of fully protected intermediates in cyclohexane was crucial for the simple purification procedures. Providing multigram amounts of rare sugars will facilitate chemical and biological studies of their properties.

ii) The production of other unnatural sugars maybe achieved by a similar strategy. For example, like in the synthesis of L-fucose **3.15**, methyl lithium can be added to other easily accessible 6-deoxyhexonolactones (*glucono*-, *gulono*- and *galactono*- lactones). A subsequent distereoselective reduction may give 1,2-*syn* diols and 1,2-*anti* diols (Felkin-Ahn controlled and chelation controlled) and a following 'head' and 'tail' cut down strategy may lead to other 6-deoxy-hexoses.

iii) Most of the intermediates obtained in this project are novel and will be valuable building blocks in carbohydrate synthesis. For instance, the terminal free hydroxyl groups in **3.50**, **3.63**, **3.64** and **3.75** can be used to access various 1- or 6-functionalized carbohydrate derivatives such as corresponding halogenated sugars. Also, a simple route to L-glucuronolactone **3.11**, which has been used to produce the enantiomers of DNJNAc and DGJNAc, two sub-micromolar iminosugar inhibitors of hexosaminidases,[61] is readily available.

iv) The scalable synthesis of rare sugars and derivatives provided cheap starting materials for the biotechnological production of other rare sugars. For example, Izumori's group has

successfully transformed the D-gulose synthesized in this project to more expensive rare sugar D-sorbose on a multigram scale by aldose isomerase in water.[51] The hexitols, 6-deoxy-hexitols, heptitols and 6-deoxy-heptitols obtained in this project (**3.10**, **3.6**, **3.9**, **3.19**, **3.20**) would be useful intermediates in the biotechnology of Izumoring and the further development of 6-deoxy-Izumoring – accessing many heptoses and all the 6-deoxy-hexoses.

3.5 Experimental

General Experimental

Izumori's group analyzed the purity of sample by high-performance liquid chromatography (Hitachi GL-611 column, Tokyo, Japan and Shimadzu RID-6A refractive index detector, Kyoto, Japan) at 60 °C, eluted with 10^{-4} M NaOH at a flow rate of 1.0 mL/min. All commercial rare sugar samples were kindly supplied by Carbosynth Limited. Seqlene-ES was provided as a gift by Halstar.

3.5.1 Synthesis of L-glucose 3.4 and 6-deoxy-L-glucose 3.5

Methyl 2,3:4,5:6,7-tri-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptonate 3.3

A methanolic solution of hydrogen chloride [prepared by dropwise addition of acetyl chloride (30.0 mL, 430.1 mmol) to methanol (200 mL) under argon at 0 °C] was added to a suspension of sodium α-D-glucoheptonate hydrate **3.2** (H_2O content 1.5 mol/mol) (100.0 g, 363.6 mmol) in 2,2-dimethoxypropane (500 mL). The reaction mixture was refluxed for 1 hour after which TLC (cyclohexane/ethyl acetate, 1:1) showed the formation of a major product (R_f 0.66). Sodium carbonate (160 g) was added to neutralize (the color of reaction mixture turned from brown to

light yellow), the solids were removed by filtration and the solvent was removed *in vacuo* to give a residue which was extracted with cyclohexane (500 mL). The cyclohexane solution was washed with distilled water (3 x 500 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the pure triacetonide **3.3** (71.0 g, 54%).

HRMS (ESI+ve): found 383.1675 $[M + Na]^+$; $C_{17}H_{28}NaO_8$ requires 383.1676; $[\alpha]_D^{25}$ +13.3 (*c* 2.78, $CHCl_3$); ν_{max} (thin film): 1770 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.29 (3H, s, CH_3) , 1.31 (3H, s, CH_3), 1.32 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.58 (3H, s, CH_3), 3.68 (3H, s, OCH_3), 3.94 – 3.99 (1H, m, H7'), 3.99 – 4.06 (2H, m, H5, H6), 4.12 – 4.07 (1H, m, H7), 4.14 (1H, dd, H4, $J_{4,3}$ 1.3, $J_{4,5}$ 7.3), 4.52 (1H, dd, H3, $J_{3,4}$ 1.3, $J_{3,2}$ 7.8), 4.63 (1H, d, H2, d, $J_{2,3}$ 7.8); δ_C ($CDCl_3$, 100 MHz): 25.4 (CH_3), 25.6 (CH_3), 26.2 (CH_3), 26.5 (CH_3), 26.9 (CH_3), 26.9 (CH_3), 27.3 (CH_3), 52.1 (OCH_3), 67.9 (C7), 75.3 (C2), 76.8, 77.2, 77.4, 77.6 (C3, C4, C5, C6), 109.8 ($\underline{C}(CH_3)_2$), 109.8 ($\underline{C}(CH_3)_2$), 110.7 ($\underline{C}(CH_3)_2$), 170.8 (C1); *m/z* (ESI+ve): 383 ($[M + Na]^+$, 78%), 743 ($[2M + Na]^+$, 100%).

Methyl 2,3:4,5-di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptonate 3.46

Aqueous sulfuric acid (1%, 300 mL) was added dropwise to a stirred solution of **3.3** (71.0 g, 197.0 mmol) in methanol (700 mL) over a period of 15 min. The reaction mixture was stirred at room temperature for 4 hours after which it was neutralized with triethylamine. Methanol was removed *in vacuo* and the residue was extracted with cyclohexane (3 x 150 mL). The combined organics were dried ($MgSO_4$) and concentrated *in vacuo* to recover the starting material **3.3** (34.3 g, 95.2 mmol 48%). The aqueous layer was subsequently extracted with ethyl acetate (6 x 150 mL). The combined organics were dried ($MgSO_4$) and concentrated *in vacuo* to give **3.46** as a white solid (28.1 g, 87.7 mmol 45%, 86% based on recovered starting material).

HRMS (ESI+ve): found 343.1364 $[M + Na]^+$; $C_{14}H_{24}NaO_8$ requires 343.1363; m.p. 87 - 89 °C; $[\alpha]_D^{20}$ +13.0 (*c* 0.72, MeOH); ν_{max} (thin film): 3450 (br, OH), 1763 (s, C=O); δ_H (CD_3CN, 400 MHz): 1.28 (3H, s, CH_3), 1.30 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.52 (3H, s, CH_3), 2.87 (1H, t, OH7, *J* 5.2), 3.26 (1H, d, OH6, *J* 5.2), 3.55–3.64 (2H, m, H6, H7'), 3.65 (3H, s, OCH_3), 3.92 (1H, t, H5, $J_{5,4} = J_{5,6}$ 7.6), 4.19 (1H, dd, H4, $J_{4,5}$ 7.6, $J_{4,3}$ 1.2), 4.54 (1H, dd, H3, $J_{3,4}$ 1.2, $J_{3,2}$ 8.0), 4.68 (1H, d, H2, $J_{2,3}$ 8.0); δ_C (CD_3CN, 100 MHz): 25.8 (CH_3), 26.8 (CH_3), 26.9 (CH_3), 27.5 (CH_3), 52.4 (OCH_3), 64.5 (C7), 74.4 (C6), 76.0 (C2), 77.6 (C5), 77.9 (C3 and C4), 110.2 ($\underline{C}(CH_3)_2$), 111.0 ($\underline{C}(CH_3)_2$), 171.4 (C1); *m/z* (ESI+ve): 343 ($[M + Na]^+$, 100%).

2,3:4,5-Di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptitol 3.47

Lithium aluminum hydride solution (1 M in THF, 123 mL, 122.8 mmol) was added dropwise to a stirred solution of **3.46** (28.1 g, 87.8 mmol) in THF (130 mL) at -40 °C. The reaction mixture was refluxed for 30 min after which TLC analysis (ethyl acetate) showed no remaining starting material (R_f 0.58) and formation of a single product (R_f 0.17). The excess hydride was quenched by dropwise addition of NH_4Cl (35 mL, sat. aq.) at 0 °C and the resulting mixture was dried ($MgSO_4$), filtered (eluting with ethyl acetate) and concentrated *in vacuo* to give the triol **3.47** (23.9 g, 93%) as a colorless oil.

HRMS (ESI+ve): Found 315.1405 $[M + Na]^+$; $C_{13}H_{24}NaO_7$ requires 315.1414; $[\alpha]_D^{20}$ +44.1 (*c* 0.51, MeOH); ν_{max} (thin film): 3392 (br, OH); δ_H (CD_3OD, 500 MHz): 1.35 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.48 (3H, s, CH_3), 3.55 (1H, dd, H7, $J_{7,6}$ 6.0 , J_{gem} 11.0), 3.61 (1H, ddd, H6, $J_{6,7'}$ 3.0, $J_{6,7}$ 6.0, $J_{6,5}$ 7.5), 3.73 (1H, dd, H7', $J_{7',6}$ 3.0, J_{gem} 11.0), 3.81 (1H, dd, H1, $J_{1,2}$ 5.0, J_{gem} 11.5), 3.83 (1H, dd, H1', $J_{1',2}$ 6.5, J_{gem} 11.5), 3.96 (1H, t, H5, $J_{5,4} = J_{5,6}$ 7.5), 4.05 (1H, dd, H4, $J_{4,3}$ 1.0, $J_{4,5}$ 7.5), 4.31 -

4.34 (1H, m, H2), 4.38 (1H, dd, H3, $J_{3,4}$ 1.0, $J_{3,2}$ 7.5); δ_C (CD_3OD, 125 MHz): 25.5 (CH_3), 27.1 (CH_3), 27.3 (CH_3), 27.7 (CH_3), 62.6 (C1), 65.1 (C7), 75.2 (C6), 76.8 (C3), 78.2 (C5), 79.3 (C2), 79.4 (C4), 109.7 ($\underline{C}(CH_3)_2$), 110.8 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 315 ([M + Na]$^+$, 100%).

L-Glucose 3.4

Silica gel-supported $NaIO_4$ (164 g) was added portionwise to a vigorously stirred solution of **3.47** (23.9 g, 81.8 mmol) in DCM (400 mL). After 30 min, TLC analysis (ethyl acetate) showed no remaining starting material (R_f 0.17) and formation of a single product (R_f 0.74). The mixture was dried ($MgSO_4$), filtered and the silica gel was thoroughly washed with CH_2Cl_2 (4 x 200 mL). The solvents were removed *in vacuo* to afford the aldehyde **3.48** (21.4 g, 100%). The crude aldehyde was dissolved in water (180 mL) and DOWEX® 50WX8-200 resin (~12 g, pre-washed with water) was added. After 24 h, TLC analysis (ethyl acetate) showed no remaining starting material and formation of a single product (baseline). The resin was filtered and washed with water. Removal of water *in vacuo* afforded pure L-glucose **3.4** (14.8 g, 100% from **3.48**, 43% from **3.3**) as a syrup which slowly formed a white solid, $[\alpha]_D^{20}$ -43.6 (*c* 1.8, water, eq) compared to commercial sample $[\alpha]_D^{20}$ -47.1 (*c* 2.1, water) [lit.[8] $[\alpha]_D^{23}$ -52.0 (*c* 0.80, water)]. The ^{1}H NMR spectrum was identical to that of a commercial sample (Appendix 1).

Methyl 2,3:4,5-di-*O*-isopropylidene-L-glucuronate 3.49

Silica gel-supported $NaIO_4$ (55.0 g) was added to a vigorously stirred solution of **3.46** (8.0 g, 24.9 mmol) in CH_2Cl_2 (320 mL). The reaction mixture was stirred at room temperature for 30 min until TLC analysis (cyclohexane/ethyl acetate, 1:1) showed the disappearance of starting

material (R_f 0.14) and the formation of a single product (R_f 0.28). The solids were removed by filtration and the solvent evaporated to yield the aldehyde **3.49** (7.2 g, 100%).

HRMS (ESI+ve): Found 343.1365 $[M + MeOH + Na]^+$; $C_{14}H_{24}NaO_8^+$ requires 343.1363; $[\alpha]_D^{20}$ -5.3 (*c* 0.62, DCM); ν_{max} (thin film): 1765 (s, CHO), 1733 (s, COO); ^{1}H NMR ($CDCl_3$, 400 MHz): 9.79 (1H, d, H6, $J_{6,5}$ 1.6), 4.69 (1H, d, H2, $J_{2,3}$ 7.6), 4.46 - 4.43 (2H, m, H5, H3), 4.31 (1H, dd, H4, $J_{4,3}$ 7.2, $J_{4,5}$ 2.4), 3.73 (3H, s, OCH_3), 1.61 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.37 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 201.1 (C6), 170.2 (C1), 112.1 ($\underline{C}(CH_3)_2$), 111.4 ($\underline{C}(CH_3)_2$), 81.5 (C5), 76.6 (C3), 75.2 (C2), 74.7 (C4), 52.4 (OCH_3), 26.8 ($\underline{C}H_3$), 26.6 ($\underline{C}H_3$), 26.5 ($\underline{C}H_3$), 25.6 ($\underline{C}H_3$); *m/z* (ESI+ve): 343 ($[M + MeOH + Na]^+$, 100%).

L-Glucurono-3,6-lactone 3.11

A solution of aldehyde **3.49** (10.1 g, 35.1 mmol) in TFA/water (9:1, 100 mL) was stirred at room temperature for 1 hour. Then the reaction mixture was heated to 50 °C with continued stirring for 2 hours until TLC analysis (ethyl acetate/ethanol/water, 45:5:1) showed the formation of two major spots (R_f 0.39, 0.58). The solvent was removed *in vacuo* to afford a residue, which was dissolved in water (100 mL). After extraction with ethyl acetate (3 x 100 mL), aqueous layer was concentrated *in vacuo* to yield L-glucuronolactone **3.11** as a white solid (5.6 g, 91% from **3.3**, 42% from **3.2**).

m.p. 158 - 162 °C; $[\alpha]_D^{20}$ -18.1 (*c* 1.0, water) [lit.[8] m.p. 165 - 167 °C; $[\alpha]_D^{22}$ -18.0 (*c* 2.0, water)]; The ^{1}H NMR spectrum was identical to that of an authentic sample of **3.11**.

2,3:4,5:6,7-Tri-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptitol 3.50

Lithium aluminum hydride solution (1 M in THF, 19.4 mL, 19.4 mmol) was added dropwise to a stirred solution of **3.3** (7.0 g, 19.4 mmol) in THF (40 mL) at 0 °C. The reaction mixture was refluxed for 1 hour after which TLC analysis (cyclohexane/ethyl acetate, 2:1) showed formation of a single product (R_f 0.33). The excess hydride was quenched by dropwise addition of NH_4Cl (~5 mL, sat. aq.) at 0 °C and the resulting mixture was dried ($MgSO_4$), filtered (eluting with ethyl acetate) and concentrated *in vacuo* to give a residue that was dissolved in ethyl acetate (50 mL) and washed with water (2 x 50 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to give pure product **3.50** (4.6 g, 72%) as a clear oil.

HRMS (ESI+ve): Found 355.1726 ($[M + Na]^+$); $C_{16}H_{28}NaO_7^+$ requires 355.1727; $[\alpha]_D^{20}$ +33 (*c* 0.83, MeOH); ν_{max} (thin film): 3300 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.34 (3H, s, CH_3), 1.35 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.85 (1H, br, OH), 3.73 (1H, dd, H1, $J_{1,2}$ 5.2, J_{gem} 12.4), 3.78 (1H, dd, H1', $J_{1',2}$ 5.2, J_{gem} 12.4), 3.99 – 4.05 (4H, m, H4, H5, H6, H7), 4.12 – 4.15 (1H, m, H7'), 4.25 (1H, dt, H2, $J_{2,1} = J_{2,1'}$ 5.2, $J_{2,3}$ 6.6), 4.33 (1H, br-d, H3, $J_{3,2}$ 6.6); δ_C ($CDCl_3$, 100 MHz): 25.3 (CH_3), 25.5 (CH_3), 26.4 (CH_3), 26.8 (CH_3), 27.0 (CH_3), 27.4 (CH_3), 61.8 (C1), 67.9 (C7), 75.0 (C3), 77.4 (C2), 77.5, 77.7, 78.0 (C4, C5, C6), 108.4 ($\underline{C}(CH_3)_2$), 109.6 ($\underline{C}(CH_3)_2$), 110.1 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 355 ($[M + Na]^+$, 100%).

meso-D-*glycero*-D-*gulo*-Heptitol 3.43

DOWEX® 50WX8-200 resin (~300 mg, pre-washed with water) was added into a solution of **3.50** (1.3 g, 3.9 mmol) in water/1,4-dioxane (30 mL, 1:1). After stirring at room temperature for 24 h, TLC (cyclohexane/ethyl acetate, 2:1) showed the disappearance of starting material (R_f 0.33). After filtration, solvent was removed *in vacuo* to obtain unprotected heptitol **3.43** (780 mg,

94%) as a white solid. ^{13}C spectrum fitted previous report.[50]

HRMS (ESI+ve): found 235.0789 $[M + Na]^+$; $C_7H_{16}NaO_7^+$ requires 235.0788; m.p.: 120 – 122 °C [lit.[62] m.p.: 134 °C]; $[\alpha]_D^{20}$ 0 (*c* 1.0, water); ν_{max} (thin film): 3370 (br, OH); δ_H (D_2O, 400 MHz): 3.62 – 3.67 (2H, m, H1, H7), 3.74 (2H, dd, H3(H5), *J* 2.9, *J* 7.6), 3.76 – 3.82 (4H, m, H1', H2, H6, H7'), 4.01 (1H, t, H4, $J_{4,3}$ = $J_{4,5}$ 2.9); δ_C ($CDCl_3$, 100 MHz): 61.7 (C1/C7), 67.5 (C4), 70.3 (C2/C6), 72.1 (C3/C5); *m/z* (ESI+ve): 235 ($[M + Na]^+$, 100%).

1-*O*-Mesyl-2,3:4,5:6,7-tri-*O*-isopropylidene-D-glycero-D-gulo-heptitol 3.53

Mesyl chloride (0.014 mL, 0.18 mmol) and triethylamine (0.06 mL, 0.45 mmol) were added to a solution of **3.50** (50 mg, 0.15 mmol) in DCM (2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 12 hours when TLC (cyclohexane/ethyl acetate 2:1) showed the formation of one product (R_f 0.65) and the consumption of starting material (R_f 0.33). The reaction mixture was diluted with DCM (8 mL) and washed with distilled water (10 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to yield a residue that was purified by flash column chromatography (cyclohexane/ethyl acetate 5:1) to yield mesylate **3.53** as a white solid (50 mg, 82%).

HRMS (ESI+ve): found 433.1497 $[M + Na]^+$; $C_{16}H_{28}NaO_7^+$ requires 433.1503; m.p. 76 – 78 °C; $[\alpha]_D^{20}$ = +9.9 (*c* 1.08, CH_3CN); ν_{max} (thin film): fingerprint region only; δ_H (CD_3CN, 500 MHz): 1.31 (3H, s, CH_3), 1.35 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.39 (6H, s, 2 x CH_3), 1.48 (3H, s, CH_3), 3.08 (3H, s, $SO_2C\underline{H}_3$), 3.90 (1H, dd, H7, $J_{7,6}$ 4.1, J_{gem} 11.0), 3.92 - 3.94 (1H, m, H6), 3.97 (1H, dd, H4, $J_{4,3}$ 1.3, $J_{4,5}$ 7.9), 4.07 – 4.09 (1H, m, H5), 4.39 (1H, dd, H1, $J_{1,2}$ 8.2, J_{gem} 10.7), 4.41 (1H, dd, H3, $J_{3,4}$ 1.3, $J_{3,2}$ 7.2), 4.45 (1H, dd, H1', $J_{1',2}$ 3.5, J_{gem} 10.7), 4.53 (1H, H2, ddd, $J_{2,1'}$ 3.5, $J_{2,3}$ 7.2, $J_{2,1}$ 8.2); δ_C (CD_3CN, 100

MHz): 25.5 (CH_3), 25.6 (CH_3), 27.08 (CH_3), 27.11 (CH_3), 27.2 (CH_3), 27.6 (CH_3), 37.7 ($SO_2\underline{C}H_3$), 68.2 (C7), 71.3 (C1), 75.7 (C3), 76.3 (C2), 77.9 (C5), 78.5 (C6), 78.9 (C4), 110.0 ($\underline{C}(CH_3)_2$), 110.5 ($\underline{C}(CH_3)_2$), 110.7 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 433 ([M + Na]$^+$, 100%).

1,2:4,5:6,7-Tri-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptitol 3.56

and

1-Deoxy-1-iodo-2,3:4,5:6,7-tri-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptitol 3.55

Imidazole (16 mg, 0.23 mmol), triphenylphosphine (60 mg, 0.23 mmol) and iodine (58 mg, 0.23 mmol) were added into a solution of **3.50** (50 mg, 0.15 mmol) in toluene (3 mL). After stirring at 80 °C for 2 hours, TLC (cyclohexane/ethyl acetate, 2:1) showed the formation of two products (R_f 0.56, 0.42) and some remaining starting material (R_f 0.33). The reaction mixture was stirred for a further of 2 hours until all starting material disappeared. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (cyclohexane/ethyl acetate, 5:1 to 1:1) to give the iodide **3.55** (32 mg, 48%) and secondary alcohol **3.56** (20 mg, 37%).

Full data for iodide **3.55** is available in the following experimental**.**

3.56: HRMS (ESI+ve): found 355.1731 [M + Na]$^+$; $C_{16}H_{28}NaO_7^+$ requires 355.1727; $[\alpha]_D^{20}$ +3.9 (*c* 1.2, MeOH); ν_{max} (thin film): 3400 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.32 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.43 (3H, s, CH_3), 2.17 (1H, d, OH, *J* 9.2), 3.68 (1H, dt, H3, $J_{3,4}$ 0.9, $J_{3,OH} = J_{3,2}$ 9.2,), 3.93 (1H, dd, H7, $J_{7,6}$ 5.2, J_{gem} 8.4), 3.95 (1H, t, H5, $J_{5,4} = J_{5,6}$ 8.0), 4.03 – 4.09 (4H, m, H2, H6, H1, H1'), 4.15 (1H, dd, H7', $J_{7,6}$ 6.0, J_{gem} 8.4), 4.20 (1H, dd, H4, $J_{4,3}$ 0.9, $J_{4,5}$ 8.0); δ_C ($CDCl_3$, 100 MHz): 25.3 (CH_3), 25.4 (CH_3), 26.7 (CH_3), 26.8 (CH_3), 27.0 (CH_3), 27.2 (CH_3), 66.6 (C7), 67.9 (C1), 70.0 (C3), 76.9 (C2, C5, C6), 79.1 (C4), 109.3 ($\underline{C}(CH_3)_2$), 109.7

($\underline{C}(CH_3)_2$), 109.8 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 355 ([M + Na]$^+$, 100%).

1-Deoxy-1-iodide-2,3:4,5:6,7-tri-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptitol 3.55

Imidazole (13.1 g, 192.6 mmol), triphenylphosphine (12.1 g, 46.1 mmol) and iodine (11.7 g, 46.2 mmol) were added to a solution of **3.50** (12.0 g, 36.1 mmol) in toluene (250 mL). After stirring at 80 °C for 2 hours, TLC (cyclohexane/ethyl acetate, 2:1) showed the formation of one major product (R_f 0.78) and the disappearance of starting material (R_f 0.47). The solvent was removed *in vacuo* and the residue was stirred with hexane (100 mL) for 5 min. Silica gel (~12 g) was added and the silica gel was washed with hexane (3 x 100 mL). After removing hexane *in vacuo*, pure iodide **3.55** was obtained as a colorless oil (11.0 g, 69%).

HRMS (ESI+ve): found 465.0745 [M + Na]$^+$; $C_{16}H_{27}NaO_6I^+$ requires 465.0745; $[\alpha]_D^{20}$ +11.1 (*c* 1.24, MeOH); ν_{max} (thin film): fingerprint region only; δ_H ($CDCl_3$, 400 MHz): 1.34 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.37 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.50 (3H, s, CH_3), 3.39 (1H, dd, H1, $J_{1,2}$ 8.2, J_{gem} 10.0), 3.48 (1H, dd, H1', $J_{1',2}$ 5.5, J_{gem} 10.0), 3.92 (1H, a-t, H5, $J_{5,4} = J_{5,6}$ 8.1), 3.98 (1H, dd, H7, $J_{7,6}$ 4.5, J_{gem} 8.5), 4.02 – 4.07 (2H, m, H4, H6), 4.14 (1H, dd, H7', $J_{7',6}$ 6.0, J_{gem} 8.5), 4.31 (1H, dd, H3, $J_{3,4}$ 2.0, $J_{3,2}$ 6.0), 4.51 (1H, ddd, H2, $J_{2,1'}$ 5.5, $J_{2,3}$ 6.0, $J_{2,1}$ 8.2); δ_C ($CDCl_3$, 100 MHz): 4.27 (C1), 25.3 (CH_3), 25.4 (CH_3), 26.7 (CH_3), 26.8 (CH_3), 27.1 (CH_3), 27.3 (CH_3), 67.9 (C7), 76.0 (C3), 77.2 (C6), 77.7 (C5), 78.3 (C2, C4), 108.8 ($\underline{C}(CH_3)_2$), 109.8 ($\underline{C}(CH_3)_2$), 110.0 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 355 ([M + Na]$^+$, 100%).

1-Deoxy-2,3:4,5:6,7-tri-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptitol 3.57

Palladium black (10 % wt., ~30 mg) and triethylamine (0.09 mL, 0.68 mmol) was added to a

solution of **3.55** (300 mg, 0.68 mmol) in methanol (10 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. Then the reaction mixture was stirred vigorously at room temperature under a hydrogen atmosphere for 24 h until TLC (cyclohexane/ethyl acetate 1:1) showed the formation of one product (R_f 0.74) and the disappearance of starting material (R_f 0.78). The reaction mixture was filtered and evaporated to dryness *in vacuo*, the residue was dissolved in cyclohexane (10 mL) and washed with water (aq., sat, 20 mL). Then the aqueous layer was back extracted with cyclohexane (2 x 10 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to yield triacetonide **3.57** (172 mg, 80%) as a light yellow oil.

HRMS (ESI+ve): found 339.1778 $[M + Na]^+$; $C_{16}H_{28}NaO_6^+$ requires 339.1778; $[\alpha]_D^{20}$ +33.3 (*c* 1.0, $CHCl_3$); ν_{max} (thin film): fingerprint region only; δ_H ($CDCl_3$, 400 MHz): 1.34 (3H, s, CH_3), 1.35 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.41 (3H, d, H1, $J_{1,2}$ 6.5), 1.42 (6H, s, 2 x CH_3), 1.51 (3H, s, CH_3), 3.85 (1H, dd, H4, $J_{4,3}$ 1.4, $J_{4,5}$ 7.9), 3.90 (1H, t, H5, $J_{5,6} = J_{5,4}$ 8.0), 3.97 (1H, dd, H7, $J_{7,6}$ 4.9, J_{gem} 8.4), 4.03 (1H, ddd, H6, $J_{6,7}$ 4.9, $J_{6,7'}$ 6.0, $J_{6,5}$ 8.0), 4.13 (1H, dd, H7', $J_{7',6}$ 6.0, J_{gem} 8.4), 4.20 (1H, dd, H3, $J_{3,4}$ 1.4, $J_{3,2}$ 6.6), 4.40 (1H, quint, H2, $J_{2,3} = J_{2,1} = J_{2,1'} = J_{2,1''}$ 6.4); δ_C ($CDCl_3$, 100 MHz): 15.2 (C1), 25.4 (CH_3), 25.5 (CH_3), 26.7 (CH_3), 26.8 (CH_3), 27.0 (CH_3), 27.2 (CH_3), 68.1 (C7), 72.9 (C2), 76.2 (C3), 77.4, 77.5 (C5, C6), 79.1 (C4), 108.0 ($\underline{C}(CH_3)_2$), 109.6 ($\underline{C}(CH_3)_2$), 109.7 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 339 ($[M + Na]^+$, 100%).

1-Deoxy-2,3:4,5-di-*O*-isopropylidene-1-iodo-D-*glycero*-D-*gulo*-heptitol 3.60

A solution of the triacetonide **3.55** (9.0 g, 20.4 mmol) in acetic acid/water (7:3, 50 mL) was stirred at room temperature for 16 hours when TLC (cyclohexane/ethyl acetate, 1:1) showed

the formation of a new spot (R_f 0.34) and some starting material (R_f 0.78). Then solvent was removed *in vacuo* to obtain a residue that was mixed with water (70 mL). Cyclohexane (3 x 70 mL) was used to extract the remaining starting material **3.26** out. Then the aqueous layer was extracted with dichloromethane (3 x 70 mL). After removing dichloromethane *in vacuo*, pure diol **3.60** (4.0 g, 49%) was obtained as a colorless oil. The remaining starting material **3.55** (~3.0 g) from the cyclohexane phase was recycled and used to repeat the procedure above to obtain more diol **3.60** (6.0 g in total, 74%);

HRMS (ESI+ve): found 425.0431 $[M + Na]^+$; $C_{13}H_{23}NaO_6I^+$ requires 425.0432; $[\alpha]_D^{20}$ +21.6 (*c* 1.15, MeOH); ν_{max} (thin film): 3380 (br, OH); δ_H (CD_3CN, 400 MHz): 1.34 (3H, s, CH_3), 1.35 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.46 (3H, s, CH_3), 2.85 (1H, t, OH-7, J 5.6), 3.22 (1H, d, OH-6, J 5.4), 3.42 (1H, dd, H1, $J_{1,2}$ 9.0, J_{gem} 10.3), 3.49 (1H, a-ddd, H7, $J_{7,OH}$ 5.4, $J_{7,6}$ 5.6, J_{gem} 10.8), 3.54 (1H, dd, H1', $J_{1,2}$ 4.9, J_{gem} 10.3), 3.57 – 3.67 (2H, m, H6, H7'), 3.87 (1H, t, H5, $J_{5,4} = J_{5,6}$ 7.8), 4.10 (1H, dd, H4, $J_{4,3}$ 1.5, $J_{4,5}$ 7.8), 4.34 (1H, dd, H3, $J_{3,4}$ 1.5, $J_{3,2}$ 6.9), 4.55 (1H, ddd, H2, $J_{2,1'}$ 4.7, $J_{2,3}$ 6.9, $J_{2,1}$ 9.0); δ_C (CD_3CN, 100 MHz): 4.7 (C1), 25.7 (CH_3), 27.2 (CH_3), 27.4 (CH_3), 27.6 (CH_3), 64.6 (C7), 74.4 (C6), 77.0 (C3), 77.8 (C5), 78.9 (C4) 79.2 (C2), 109.2 ($\underline{C}(CH_3)_2$), 110.3 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 425 ($[M + Na]^+$, 100%).

1-Deoxy-2,3:4,5-di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptitol 3.61

Palladium on charcoal (10 % wt., 500 mg) and triethylamine (2.0 mL, 14.4 mmol) were added to a solution of **3.60** (5.8 g, 14.4 mmol) in methanol (40 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. Then the reaction mixture was stirred vigorously at room temperature under hydrogen atmosphere for 12 h until TLC (cyclohexane/ethyl acetate 1:1) showed the formation of one product (R_f 0.25) and the

disappearance of starting material (R_f 0.36). After being filtered and evaporated to dryness *in vacuo*, the residue was dissolved in ethyl acetate (50 mL) and washed with water (aq., sat, 50 mL). Then the aqueous layer was back extracted with ethyl acetate (2 x 50 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to yield desired product **3.61** (3.2 g, 83%) as a crystalline solid without further purification.

HRMS (ESI+ve): Found 299.1466 $[M + Na]^+$; $C_{13}H_{24}NaO_6^+$ requires 299.1465; m.p. 40 - 42 °C; $[\alpha]_D^{20}$ +59 (*c* 1.0, MeOH); ν_{max} (thin film): 3420 (br, OH); δ_H (CD_3OD, 400 MHz): 1.36 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.41 (3H, d, H1, *J* 6.0), 1.42 (3H, s, CH_3), 1.50 (3H, s, CH_3), 3.58 (1H, dd, H7, $J_{7,6}$ 6.4, J_{gem} 11.0), 3.63 (1H, ddd, H6, $J_{6,7'}$ 2.6, $J_{6,7}$ 6.4, $J_{6,5}$ 7.8), 3.77 (1H, dd, H7', $J_{7,6}$ 2.6, J_{gem} 11.0), 3.92 (1H, t, H5, $J_{5,4} = J_{5,6}$ 7.8), 4.00 (1H, dd, H4, $J_{4,3}$ 0.7, $J_{4,5}$ 7.8), 4.29 (1H, br-d, H3, $J_{3,2}$ 6.6), 4.46 (1H, quint, H2, $J_{2,3} = J_{2,1}$ 6.6); δ_C (CD_3OD, 100 MHz): 15.7 (C1), 25.8 (CH_3), 27.2 (CH_3), 27.6 (CH_3), 27.7 (CH_3), 65.4 (C7), 74.5 (C2), 75.5 (C6), 78.0 (C5), 78.1 (C3) 80.2 (C4), 109.3 ($\underline{C}(CH_3)_2$), 110.7 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 299 ($[M + Na]^+$, 100%).

6-Deoxy-2,3:4,5-di-*O*-isopropylidene-L-glucose 3.62

Silica gel-supported $NaIO_4$ (23.2 g) was added portionwise to a vigorously stirred solution of **3.61** (3.2 g, 11.6 mmol) in CH_2Cl_2 (50 mL). After 2 hours, TLC analysis (ethyl acetate) showed no remaining starting material (R_f 0.53) and formation of a single product (R_f 0.66). The mixture was dried ($MgSO_4$), filtered and the silica gel was thoroughly washed with CH_2Cl_2 (4 x 50 mL). The solvents were removed *in vacuo* to afford the aldehyde **3.62** (2.6 g, 93%) as a light brown foam.

HRMS (ESI+ve): found 267.1209 ($[M + Na]^+$); $C_{12}H_{20}NaO_5^+$ requires 267.1203; $[\alpha]_D^{20}$ +42 (*c*, 1.3

in $CHCl_3$); ν_{max} (thin film): 3423 (br, OH), 1730 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.37 (3H, s, CH_3), 1.38 (3H, d, H6, $J_{6,5}$ 6.6), 1.42 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.52 (3H, s, CH_3), 3.97 (1H, dd, H3, $J_{3,4}$ 2.0, $J_{3,2}$ 7.9), 4.10 (1H, dd, H4, $J_{4,3}$ 2.0, $J_{4,5}$ 6.6), 4.34 (1H, dd, H2, $J_{2,1}$ 1.8, $J_{2,3}$ 7.9), 4.41 (1H, quint, H5, $J_{5,4} = J_{5,6}$ 6.6), 9.82 (1H, d, H1, $J_{1,2}$ 1.8); δ_C ($CDCl_3$, 100 MHz): 15.0 (C6), 25.4 (CH_3), 26.3 (CH_3), 26.7 (CH_3), 26.8 (CH_3), 72.6 (C5), 75.7, 75.8 (C2, C3), 81.3 (C4), 108.5 ($\underline{C}(CH_3)_2$), 111.7 ($\underline{C}(CH_3)_2$), 201.7 (C1); *m/z* (ESI+ve): 299 ([M + MeOH + Na]$^+$, 100%).

6-Deoxy-L-glucose (L-quinovose) 3.5

DOWEX® 50WX8-200 (~300 mg, pre-washed with water) was added into a solution of **3.62** (2.3 g, 9.4 mmol) in water/1,4-dioxane (10 mL, 1:1). After stirring at room temperature for 18 h, TLC showed the disappearance of starting material (Rf 0.66). Then resin was filtered off and the solvent was removed *in vacuo* to obtain deprotected sugar **3.5** (1.5 g, 100%) as a light yellow syrup that was recrystallized from acetonitrile/ethanol (6:1) to obtain **3.5** (1.0 g, 67%) as a crystalline solid (α/β pyranoside ratio 1:2.5).

HRMS (ESI+ve): found 165.0757 [M + H]$^+$; $C_6H_{13}O_5^+$ requires 165.0757; m.p. 126 – 128 °C; $[\alpha]_D^{20}$ -47.7 (*c* 1.12, water); ν_{max} (thin film): 3380 (br, OH); δ_H (D_2O, 400 MHz): 1.19 (3H, d, H6α, $J_{6,5}$ 6.3), 1.21 (3H, d, H6β, $J_{6,5}$ 6.1), 3.07 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.5), 3.08 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 9.3), 3.17 (1H, dd, H2β, $J_{2,1}$ 7.9, $J_{2,3}$ 9.3), 3.36 (1H, t, H3β, $J_{3,2} = J_{3,4}$ 9.3), 3.42 (1H, dq, H5β, $J_{5,6}$ 6.2, $J_{5,4}$ 9.5), 3.47 (1H, dd, H2α, $J_{2,1}$ 3.8, $J_{2,3}$ 9.9), 3.58 (1H, t, H3α, $J_{3,2} = J_{3,4}$ 9.5), 3.85 (1H, dq, H5α, $J_{5,6}$ 6.3, $J_{5,4}$ 9.6), 4.55 (1H, d, H1β, $J_{1,2}$ 7.9), 4.55 (1H, d, H1α, $J_{1,2}$ 3.8); δ_C (D_2O, 100 MHz): 17.9 (C6α, C6β), 68.6 (C5α), 72.9 (C2α), 73.1 (C5β), 73.6 (C3α), 75.6 (C2β), 76.1 (C4β), 76.4 (C4α), 76.7 (C3β), 93.2 (C1α), 96.9 (C1β); *m/z* (ESI+ve): 165 ([M + H]$^+$, 100%). [The ^{1}H NMR spectrum of **3.33** is identical to that of

an authentic sample of the enantiomer D-quinovose – see Appendix 3]

6-Deoxy-2,3:4,5-di-*O*-isopropylidene-L-glucitol 3.63

Sodium borohydride (19 mg, 0.49 mmol) was added into a solution of **3.62** (100 mg, 0.41 mmol) in methanol (4 mL). The reaction mixture was stirred at room temperature for 2 hours until mass spectrometry showed the completion of reaction ($[M+Na]^+$ 279). After neutralizing the mixture with acetic acid (~0.5 mL), the solvent was removed *in vacuo* to yield a residue that was dissolved in ethyl acetate (10 mL). Then the resulting solution was washed with water (3 x 10 mL) and the organic phase was dried ($MgSO_4$), filtered and solvente was removed *in vacuo* to yield protected glucitol **3.63** (86 mg, 85%) as a colourless oil.

HRMS (ESI+ve): found 269.1362 $[M + Na]^+$; $C_{12}H_{20}NaO_5^+$ requires 269.1370; $[\alpha]_D^{20}$ +57 (*c* 1.2, $CHCl_3$); ν_{max} (thin film): 3461 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.36 (3H, s, CH_3), 1.40 (3H, d, H6, $J_{6,5}$ 6.6), 1.41 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.52 (3H, s, CH_3), 3.62 (1H, dd, H1, $J_{1,2}$ 3.4, J_{gem} 12.4), 3.87 (1H, dd, H1', $J_{1',2}$ 3.4, J_{gem} 12.4), 3.88 (1H, dd, H3, $J_{3,4}$ 1.5, $J_{3,2}$ 8.7), 4.00 (1H, dd, H4, $J_{4,3}$ 1.5, $J_{4,5}$ 6.7), 4.08 (1H, dt, H2, $J_{2,1} = J_{2,1'}$ 3.4, $J_{2,3}$ 8.8), 4.42 (1H, quint, H5, $J_{5,4} = J_{5,6}$ 6.6); δ_C ($CDCl_3$, 100 MHz): 15.1 (C6), 25.4 (CH_3), 26.7 (CH_3), 27.1 (CH_3), 27.2 (CH_3), 60.7 (C1), 72.8 (C5), 75.2, 75.3 (C3, C4), 77.5 (C2), 108.4 ($\underline{C}(CH_3)_2$), 109.4 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 269 ($[M + Na]^+$, 100%).

6-Deoxy-L-glucitol [1-deoxy-D-gulitol] 3.6

DOWEX® 50WX8-200 (~50 mg, pre-washed with water) was added into a solution of **3.63** (86 mg, 0.35 mmol) in water/1,4-dioxane (10 mL, 1:1). After stirring at room temperature for 20 h, resin was filtered off and solvent was removed *in vacuo* to obtain product **3.6** (58 mg, 100%) as

a colorless syrup.

HRMS (ESI+ve): found 189.0731 [M + Na]$^+$; $C_6H_{14}NaO_5^+$ requires 189.0733; $[\alpha]_D^{20}$ +7.3 (*c* 0.98, water) [lit.[64] $[\alpha]_D^{27}$ +1.45 (*c* 3.105, ethanol)]; ν_{max} (thin film): 3400 (br, OH); δ_H (D_2O, 400 MHz): 1.36 (3H, s, CH_3), 1.40 (3H, d, H6, *J* 6.6), 3.51 (1H, dd, H4, $J_{4,3}$ 2.9, $J_{4,5}$ 6.9), 3.61 (1H, dd, H1, $J_{1,2}$ 6.4, J_{gem} 11.5), 3.72 (1H, dd, H1', $J_{1',2}$ 3.9, J_{gem} 11.5), 3.77 – 3.81 (2H, m, H2, H3), 3.89 (1H, quint, H5, $J_{5,4}$ = $J_{5,6}$ 6.6); δ_C ($CDCl_3$, 100 MHz): 18.5 (C6), 63.1 (C1), 67.6 (C5), 70.5 (C2), 73.2 (C3), 75.5 (C4); *m/z* (ESI+ve): 189 ([M + Na]$^+$, 100%).

3.5.2 Synthesis of D-gulose 3.7 and 6-deoxy-D-gulose 3.8

Methyl 2,3:4,5-di-*O*-isopropylidene-D-gulonate 3.64

Sodium borohydride (977.0 mg, 25.70 mmol) was added in portion to a solution of aldehyde **3.49** (13.50 g, 46.80 mmol) in methanol (250 mL) at 0 °C. After stirring at 0 °C for 1.5 hours, TLC (cyclohexane/ethyl acetate 1:1) indicated the formation of product (R_f 0.40); glacial acetic acid (~2.5 mL) was added dropwise to adjust pH to neutral. The solvent was removed *in vacuo* to give a residue that was dissolved into water (100 mL). After washing with ethyl acetate (3 x 100 mL), organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain pure product **3.64** as a white solid without further purification (13.04 g, 96%).

HRMS *m/z* (ESI+ve): found 313.1252 [M + Na]$^+$, $C_{13}H_{22}O_7Na^+$ requires 313.1257; m.p. 76°C - 78°C; $[\alpha]_D^{20}$ +8.9 (*c* 1.20, MeOH); ν_{max} (thin film): 3502 (br, OH), 1764 (s, C=O); δ_H ($CDCl_3$, 400MHz) 1.34 (3H, s, CH_3), 1.37 (6H, s, 2 x CH_3), 1.60 (3H, s, CH_3), 2.55 (1H, br-s, OH), 3.66 (1H, dd, H6, $J_{6,5}$ 3.6, J_{gem} 12.0), 3.71 (3H, s, OCH_3), 3.82 (1H, dd, H6', $J_{6',5}$ 3.6, J_{gem} 12.0), 4.12 (1H, dd, H4, $J_{4,3}$ 2.0, $J_{4.5}$

8.0), 4.21 (1H, dt, H5, $J_{5,6}$ = $J_{5,6'}$ 3.6, $J_{5,4}$ 8.0), 4.36 (1H, dd, H3, $J_{3,4}$ 2.0, $J_{3.2}$ 7.6), 4.66 (1H, d, H2, $J_{2,3}$ 7.6), δ_C ($CDCl_3$, 100MHz) 25.9 (CH_3), 26.7 (CH_3), 26.8 (CH_3), 27.6 (CH_3), 52.5 (OCH_3), 61.9 (C6), 74.7 (C4), 75.6 (C5), 76.4 (C2), 77.8 (C3), 110.0 ($\underline{C}(CH_3)_2$), 111.4 ($\underline{C}(CH_3)_2$), 170.8 (C1); *m/z* (ESI+ve): 313 ($[M + Na]^+$, 100%).

2,3:4,5-Di-*O*-isopropylidene-D-gulose 3.65

Diisobutylaluminium hydride (1.5 M in toluene, 75.00 ml, 112.50 mmol) was added dropwise to a solution of the methyl ester **3.64** (13.04 g, 45.00 mmol) in dichloromethane (70 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 1.5 hours until TLC (cyclohexane/ethyl acetate 1:1) indicated the formation of one major product (R_f 0.43). Also mass spectrometry showed the formation of desired product peak ($[M + Na]^+$ 315) and disappearance of starting material peak ($[M + MeOH + Na]^+$ 313). Then the mixture was diluted with ethyl acetate (100 mL) and potassium sodium tartrate (sat, *aq*, 50 mL) was added. After stirring for 8 h, the mixture was diluted with water (100 mL) and extracted with ethyl acetate (3 x 150 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain a crude that was further purified *via* flash chromatography (cyclohexane/ethyl acetate 5:1 to 4:1) to yield the hydrated form of aldehyde **3.65** as a syrup (10.00 g, 86%).

HRMS *m/z* (ESI+ve): found 283.1149 $[M + Na]^+$, $C_{12}H_{20}O_6Na^+$ requires 283.1152; $[\alpha]_D^{20}$ +5.4 (*c*, 0.82 in $CHCl_3$), ν_{max} (thin film, cm^{-1}): 3427 (broad, OH); δ_H (400MHz, $CDCl_3$) 1.38 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.52 (3H, s, CH_3), 3.55 (1H, dd, H6, $J_{6,5}$ 10.3, J_{gem} 11.2), 3.67 (1H, ddd, H5, $J_{5,6'}$ 4.0, $J_{5,4}$ 9.3, $J_{5,6}$ 10.0), 3.88 (1H, t, H4, $J_{4,5}$ = $J_{4,3}$ 9.2), 4.11 (1H, t, H2, $J_{2,1}$ = $J_{2,3}$ 8.0), 4.28 (1H, dd, H6', $J_{6',5}$ 4.0, J_{gem} 11.2), 4.39 (1H, dd, H3, $J_{3,2}$ 8.0, $J_{3.4}$ 8.7), 4.75 (1H, d, H1, $J_{1,2}$ 8.2), δ_C

(100MHz, $CDCl_3$) 24.1 (CH_3), 27.0 (CH_3), 27.1 (CH_3), 27.2 (CH_3), 61.9 (C6), 74.8 (C4), 76.9 (C5), 79.6 (C3), 81.4 (C2), 98.1 (C1), 110.0 ($\underline{C}(CH_3)_2$), 111.1 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 315 ([M + MeOH + Na]$^+$, 100%).

D-Gulose 3.7

DOWEX@ 50WX8-200 (5.00 g) was added into a solution of aldehyde **3.65** (10.0 g, 38.5 mmol) in water (120 mL). After stirring at room temperature for 48 hours, mass spectrum indicated that the reaction was complete and the resin was filtered off and water was removed *in vacuo* to yield pure D-gulose **3.7** as a colorless syrup without further purification (6.90 g, 100%).

HRMS *m/z* (ESI+ve): found 203.0525 [M + Na]$^+$, $C_6H_{12}O_6Na^+$ requires 203.0526; $[\alpha]_D^{20}$ -20.0 (*c* 0.89, water, eq) [Commercial sample: $[\alpha]_D^{20}$ -23.0 (*c* 1.40, water, eq), lit.[17b] $[\alpha]_D^{20}$ -24.1 (*c* and solvent not indicated)], ν_{max} (thin film): 3309 (br, OH); ^1H and ^{13}C NMR are identical with that of commercial D-gulose sample (Appendix 4); *m/z* (ESI+ve): 203 ([M + Na]$^+$, 100%).

Methyl 6-deoxy-6-iodo-2,3:4,5-di-*O*-isopropylidene-D-gulonate 3.66

Triflic anhydride (5.90 mL, 35.0 mmol) was added dropwise to a solution of the mixture of **3.64** (7.8 g, 26.9 mmol) and anhydrous pyridine (3.25 mL, 40.4 mmol) in dichloromethane (50 mL) at -20 °C. After stirring at -20 °C for 3 h, TLC (cyclohexane/ethyl acetate, 2:1) showed the formation of a major product (R_f 0.68) and the disappearance of starting material (R_f 0.28). The reaction mixture was diluted with dichloromethane (10 mL), washed with HCl (2 M, aq. 60 mL) and aqueous $NaHCO_3$ (sat, 60 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed in *vacuo* to give a mixture of the crude triflates as a white solid (10.2 g).

Sodium iodide (8.1 g, 53.8 mmol) was added to a solution of the crude triflate in butanone (50 mL). The mixture was wrapped with foil and stirred at 50 °C for 16 hours until mass spectrometry showed the formation of a product ($[M + H]^+$ 401). After removal of solvent *in vacuo*, the residue was dissolve in cyclohexane (80 mL) and washed with water (80 mL). The aqueous layer was back extracted with cyclohexane (2 x 80 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed to yield iodide **3.66** (8.7 g, 81%) as a light yellow oil.

HRMS (ESI+ve): found 423.0273 $[M + Na]^+$; $C_{13}H_{21}NaO_6I^+$ requires 423.0275; $[\alpha]_D^{20}$ +5.9 (*c* 1.08, $CHCl_3$); ν_{max} (thin film): 1765 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.39 (6H, s, 2 x CH_3), 1.40 (3H, s, CH_3), 1.61 (3H, s, CH_3), 3.30 (2H, d, H6, $J_{6,5}$ 5.5), 3.74 (3H, s, OCH_3), 4.00 (1H, dd, H4, $J_{4,3}$ 1.7, $J_{4,5}$ 7.2), 4.16 (1H, dt, H5, $J_{5,6} = J_{5,6'}$ 5.5, $J_{5,4}$ 7.1), 4.50 (1H, dd, H3, $J_{3,4}$ 1.7, $J_{3,2}$ 7.8), 4.70 (1H, d, H2, $J_{2,3}$ 7.8); δ_C ($CDCl_3$, 100 MHz): 5.7 (C6), 25.5 (CH_3), 26.4 (CH_3), 26.7 (CH_3), 27.6 (CH_3), 52.2 (OCH_3), 75.2 (C2), 76.2 (C5), 76.6 (C3), 78.8 (C4), 110.2 ($\underline{C}(CH_3)_2$), 111.1 ($\underline{C}(CH_3)_2$), 170.3 (C1); *m/z* (ESI+ve): 401 ($[M + H]^+$, 100%), 423 ($[M + Na]^+$, 15%).

Methyl 6-deoxy-2,3:4,5-di-*O*-isopropylidene-D-gulonate 3.67

Palladium on charcoal (10 % wt., 1.2 g) and triethylamine (4.2 mL, 30.0 mmol) were added to a solution of **3.66** (12 g, 30.0 mmol) in methanol (200 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. Then the reaction mixture was stirred vigorously at room temperature under a hydrogen atmosphere for 20 hours until mass spectrometry showed the disappearance of starting material ($[M+H]^+$ 401) and the formation of a product ($[M + Na]^+$ 297). The reaction mixture was filtered and evaporated to dryness *in vacuo*, the residue

was dissolved in ethyl acetate (100 mL) and washed with sodium thiosulphate (aq., sat, 100 mL). Then the aqueous layer was back extracted with ethyl acetate (2 x 100 mL) and the organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to yield the deoxy-gulonate **3.67** (7.5 g, 91%) as a white crystalline solid.

HRMS (ESI+ve): found 297.1309 $[M+Na]^+$; $C_{13}H_{22}NaO_6^+$ requires 297.1309; m.p. 38 - 40 °C; $[\alpha]_D^{20}$ +15.9 (*c* 1.38, $CHCl_3$); ν_{max} (thin film): 1766 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.30 (3H, d, H6, $J_{6,5}$ 6.1), 1.35 (6H, s, 2 x CH_3), 1.39 (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.71 (1H, dd, H4, $J_{4,3}$ 1.8, $J_{4,5}$ 8.2), 3.73 (3H, s, OCH_3), 4.18 (1H, dq, H5, $J_{5,6}$ 6.1, $J_{5,4}$ 8.2), 4.34 (1H, dd, H3, $J_{3,4}$ 1.8, $J_{3,2}$ 7.8), 4.69 (1H, d, H2, $J_{2,3}$ 7.8); δ_C ($CDCl_3$, 100 MHz): 17.4 (C6), 25.6 (CH_3), 26.4 (CH_3), 26.5 (CH_3), 27.5 (CH_3), 52.1 (OCH_3), 73.0 (C5), 75.4 (C2, C3), 79.5 (C4), 108.7 ($\underline{C}(CH_3)_2$), 111.1 ($\underline{C}(CH_3)_2$), 170.4 (C1); *m/z* (ESI+ve): 297 ($[M + Na]^+$, 100%).

6-Deoxy-2,3:4,5-di-*O*-isopropylidene-D-gulose 3.68

and

6-Deoxy-2,3:4,5-di-*O*-isopropylidene-D-gulitol 3.69

Diisobutylaluminium hydride (1.0 M in THF, 10.5 ml, 10.5 mmol) was added dropwise to a solution of **3.67** (1.16 g, 4.2 mmol) in dichloromethane (20 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 2 hours until TLC (cyclohexane/ethyl acetate 2:1) indicated the formation of one major product (R_f 0.45) and one minor product (R_f 0.30). Then the mixture was diluted with ethyl acetate (10 mL) and potassium sodium tartrate (sat, aq, ~3 mL) was added. After stirring for 10 h, the mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed

in vacuo to obtain a crude product that was purified by flash column chromatography to yield the aldehyde **3.68** as the major product (856 mg, 84%) as a colorless syrup and small amount of the alcohol **3.69** (150 mg, 14%) as a colorless syrup.

NMR data of 3.68: δ_H (CD_3CN, 400 MHz): 1.26 (3H, d, H6, $J_{6,5}$ 6.1), 1.31 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.54 (3H, s, CH_3), 3.63 (1H, dd, H4, $J_{4,3}$ 1.0, $J_{4,5}$ 8.4), 4.11 (1H, dq, H5, $J_{5,6}$ = $J_{5,6'}$ = $J_{5,6''}$ 6.1, $J_{5,4}$ 8.4), 4.46 (1H, dd, H3, $J_{3,4}$ 1.0, $J_{3,2}$ 8.4), 4.49 (1H, dd, H2, $J_{2,1}$ 2.0, $J_{2,3}$ 8.4), 9.57 (1H, d, H1, $J_{1,2}$ 2.0); δ_C (CD_3CN, 100 MHz): 17.9 (C6), 25.5 (CH_3), 26.8 (CH_3), 26.9 (CH_3), 27.8 (CH_3), 73.8 (C5), 76.5 (C3), 80.3 (C4), 82.0 (C2), 109.5 ($\underline{C}(CH_3)_2$), 111.6 ($\underline{C}(CH_3)_2$), 201.7 (C1).

3.69: HRMS (ESI+ve): found 296.1359 $[M + Na]^+$; $C_{12}H_{22}NaO_5^+$ requires 296.1359; $[\alpha]_D^{20}$ +20.3 (*c* 0.6, water); ν_{max} (thin film): 3370 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.31 (3H, d, H6, $J_{6,5}$ 6.1), 1.37 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.51 (3H, s, CH_3), 2.51 (1H, br-s, OH), 3.59 (1H, dd, H4, $J_{4,3}$ 1.5, $J_{4,5}$ 8.6), 3.79 (2H, H1, $J_{1,2}$ 5.1), 4.08 (1H, dd, H3, $J_{3,4}$ 1.5, $J_{3,2}$ 6.7), 4.23 (1H, dq, H5, $J_{5,6}$ = $J_{5,6'}$ = $J_{5,6''}$ 6.1, $J_{5,4}$ 8.6), 4.27 (1H, dd, H2, $J_{2,1}$ 5.1, $J_{2,3}$ 6.7), 4.49 (1H, dd, H2, $J_{2,1}$ 2.0, $J_{2,3}$ 8.4), 9.57 (1H, d, H1, $J_{1,2}$ 2.0); δ_C ($CDCl_3$, 100 MHz): 17.2 (C6), 25.5 (CH_3), 26.3 (CH_3), 26.8 (CH_3), 27.5 (CH_3), 61.5 (C1), 73.4 (C5), 73.7 (C3), 77.5 (C2), 80.0 (C4), 108.8 ($\underline{C}(CH_3)_2$), 109.1 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 296 ($[M + Na]^+$, 100%).

6-Deoxy-D-gulose 3.8

DOWEX@ 50WX8-200 (100 mg) was added into a solution of aldehyde **3.68** (856 mg, 3.51 mmol) in water/1,4-dioxane (1:1, 10 mL). The mixture was stirred at room temperature for 24 hours until mass spectrometry showed the formation of the desired product ($[M + Na]^+$ 187). Then resin was filtered off and water was removed *in vacuo* to yield 6-deoxy-D-gulose **3.8** (570 mg,

99%) as a colorless syrup (α/β pyranosides ratio 1:10) (1H spectrum is shown in Appendix 5). HRMS (ESI+ve): found 187.0575 $[M + Na]^+$; $C_6H_{12}NaO_5^+$ requires 187.0577; $[\alpha]_D^{20}$ -20.3 (*c* 0.6, water), [lit.[65] $[\alpha]_D^{20}$ -39.3 (*c* 1.0, water)]; ν_{max} (thin film): 3440 (br, OH); NMR data for major β anomer: δ_H (D_2O, 400 MHz): 1.22 (3H, d, H6, $J_{6,5}$ 6.6), 3.57 (1H, dd, H2, $J_{2,3}$ 3.4, $J_{2,1}$ 8.4), 3.62 (1H, dd, H4, $J_{4,5}$ 0.8, $J_{4,3}$ 3.5), 4.07 (1H, t, H3, $J_{3,4} = J_{3,2}$ 3.5), 4.10 (1H, dq, H5, $J_{5,4}$ 0.8, $J_{5,6}$ 6.6), 4.85 (1H, d, H1, $J_{1,2}$ 8.4); δ_C (D_2O, 100 MHz): 15.3 (C6), 69.1 (C2), 69.5 (C5), 71.4 (C3), 72.1 (C4), 93.8 (C1); *m/z* (ESI+ve): 187 ($[M + Na]^+$, 100%).

6-Deoxy-D-gulitol (1-deoxy-L-glucitol) 3.9

DOWEX@ 50WX8-200 (30 mg) was added into a solution of **3.69** (150 mg, 0.61 mmol) was dissolved into water/1,4-dioxane (1:1, 10 mL). The mixture was stirred at room temperature for 24 h until mass spectrometry showed the formation of desired product ($[M + Na]^+$ 187). Then resin was filtered off and water was removed *in vacuo* to yield 6-deoxy-D-gulitol **3.9** (102 mg, 100%) as a colorless syrup.

HRMS (ESI+ve): found 189.0731 $[M + Na]^+$; $C_6H_{14}NaO_5^+$ requires 189.0733; $[\alpha]_D^{20}$ -2.8 (*c* 1.4, water); δ_H (D_2O, 400 MHz): 1.19 (3H, d, H6, $J_{6,5}$ 6.4), 3.61 (1H, t, H4, $J_{4,3} = J_{4,5}$ 6.4), 3.62 (1H, m, H3), 3.64 (1H, dd, H1, $J_{1,2}$, 6.3, J_{gem} 11.8), 4.76 (1H, ddd, H2, $J_{2,1'}$ 2.9, $J_{2,1}$ 6.3, $J_{2,3}$ 8.7), 4.83 (1H, dd, H1', $J_{1',2}$, 2.9, J_{gem} 11.8), 3.90 (1H, quint, H5, $J_{5,4} = J_{5,6}$ 6.4); δ_C (D_2O, 100 MHz): 18.8 (C6), 63.5 (C1), 69.4 (C5), 71.3 (C3), 71.5 (C2), 74.4 (C4); *m/z* (ESI+ve): 189 ($[M + Na]^+$, 100%).

6-Deoxy-D-gulono-1,4-lactone 3.12

A solution of **3.67** (400 mg, 1.46 mmol) in trifluoroacetic acid/water (10 mL, 1:1) was stirred at

room temperature for 16 hours until mass spectrometry showed the formation of desired lactone ([M + Na]$^+$ 185). The solvent was removed *in vacuo* to yield pure lactone **3.12** (236 mg, 100%) as an off-white solid.

HRMS (ESI+ve): found 185.0420 [M + Na]$^+$; $C_6H_{12}NaO_5^+$ requires 185.0420; m.p. 178 - 180 °C; $[\alpha]_D^{20}$ -57 (*c* 0.89, water) [lit.[52] m.p. 180 - 181 °C; $[\alpha]_D^{24}$ -63.8 (*c* 0.88, water)]; ν_{max} (thin film): 1748 (s, C=O); δ_H (D_2O, 400 MHz): 1.28 (3H, d, H6, $J_{6,5}$ 6.4), 4.12 (1H, dq, H5, $J_{5,6}$ 6.4, $J_{5,4}$ 8.6), 4.31 (1H, dd, H4, $J_{4,3}$ 2.7, $J_{4,5}$ 8.6), 4.54 (1H, dd, H3, $J_{3,4}$ 2.7, $J_{3,2}$ 4.7), 4.74 (1H, d, H2, $J_{2,3}$ 4.7); δ_C (D_2O, 100 MHz): 17.4 (C6), 66.4 (C5), 70.1 (C3), 71.5 (C2), 85.5 (C4), 178.7 (C1); *m/z* (ESI+ve): 185 ([M + Na]$^+$, 100%).

3.5.3 Synthesis of D-idose 3.15

Methyl 2,3:4,5:6,7-tri-*O*-isopropylidene-D-*glycero*-D-*ido*-heptonate 3.14

Method 1:

A methanolic solution of hydrogen chloride [prepared by dropwise addition of acetyl chloride (0.8 mL, 11.2 mmol) to methanol (6.6 mL) under argon at 0 °C] was added to a solution of idonolactone **3.71**[55] (3.45 g, 12.0 mmol) in 2,2-dimethoxypropane (50 mL). The reaction mixture was then refluxed for 2 hours when TLC (cyclohexane/ethyl acetate, 1: 1) showed the formation of a major product (R_f 0.70). Sodium carbonate (5 g) was added into reaction mixture to neutralize the pH to 7 (the color of reaction mixture turned from brown to light yellow). After the solids were removed by filtration, the solvent was removed *in vacuo* to give a residue that was dissolved into cyclohexane (50 mL). The solution was washed with distilled water (3 x 50

mL), dried ($MgSO_4$) and solvent was removed *in vacuo* to yield pure triacetonide **3.14** (3.10 g, 8.6 mmol, yield 71%). Further extraction of the aqueous layer by ethyl acetate (3 x 50 mL) gave partially acetonated products; removal of solvent *in vacuo*, afforded a residue (~2.0 g) which was dissolved into acetone (20 mL) and the procedures above were repeated to obtain more **3.14** (4.10 g in total, 95%) as a syrup, which used in the next step without further purification.

Method 2 (from Seqlene without recrystallization):

A solution of Seqlene (2 mL) was fully dried *in vacuo* to give a dark brown residue (~1.3 g). A methanolic solution of hydrogen chloride [prepared by dropwise addition of acetyl chloride (0.4 mL, 5.6 mmol) to methanol (3.3 mL) under argon at 0 °C] was added to a solution of the residue in 2,2-dimethoxypropane (25 mL). The reaction mixture was then refluxed for 5 hours when TLC (cyclohexane/ethyl acetate, 1: 1) showed the formation of a major product (R_f 0.70). Sodium carbonate (2 g) was added into reaction mixture to neutralize the pH to 7 (the color of reaction mixture turned from brown to light yellow). After the solids were removed by filtration, the solvent was removed *in vacuo* to give a residue that was dissolved into cyclohexane (20 mL). The solution was washed with distilled water (3 x 10 mL), dried ($MgSO_4$) and solvent was removed *in vacuo* to yield a mixture of **3.14** and **3.3** (680 mg, 36%) as a NMR ratio of 1:1 (Some impurities were also detected).

Method 3 (from Seqlene with recrystallization):

A solution of Seqlene (10 mL, ~7.2 g solid) was diluted with water (60 mL) and passed through a column containing Amberlite IR-120 (H^+) cation exchange resin column (~40 mL). The eluent was stirred at 100 °C for 5 hours with cadmium carbonate (2.7 g). Then cadmium chloride (2.9 g) was added. After filtration by active carbon, the solution was concentrated *in vacuo* to half of the

volume. Ethanol (~40 mL) was added until the solution became muddy. The solution was left to recrystallize at room temperature overnight to obtain a cadmium salt (1.5 g) of lactones that was subjected to the protection conditions as shown above to form a mixture of triacetonide **3.14** and **3.3** (2.0 g) as a ratio of 10:3 according to ^{1}H NMR.

HRMS *m/z* (ESI+ve): found 383.1673 $[M + Na]^+$, $C_{17}H_{28}O_8Na^+$ requires 383.1676; $[\alpha]_D^{20}$ +18.9 (*c* 1.25, MeOH); ν_{max} (thin film): 1764 (s, C=O); δ_H (CD_3OD, 400MHz): 1.22 (3H, s, CH_3), 1.28 (3H, s, CH_3), 1.29 (6H, s, 2 x CH_3), 1.35 (3H, s, CH_3), 1.36 (3H, s, CH_3), 3.69 (3H, s, OCH_3), 3.82-3.85 (1H, m, H7), 3.92-3.94 (1H, m, H5), 4.00 (1H, dd, H4, $J_{4,3}$ 2.1, $J_{4,5}$ 7.5), 4.01-4.04 (2H, m, H6, H7'), 4.17 (1H, dd, H3, $J_{3,4}$ = 2.1, $J_{3,2}$ 7.8), 4.53 (1H, d, H2, $J_{2,3}$ 7.8), δ_C (CD_3OD, 100MHz) 24.1 (CH_3), 24.8 (CH_3), 25.5 (CH_3), 25.7 (CH_3), 26.2 (CH_3), 26.6 (CH_3), 51.5 (OCH_3), 67.1 (C7), 75.3 (C2), 77.0 (C5), 77.2 (C6), 77.9 (C4), 78.6 (C3), 109.5 ($\underline{C}(CH_3)_2$), 109.6 ($\underline{C}(CH_3)_2$), 111.5 ($\underline{C}(CH_3)_2$), 171.3 (C1); *m/z* (ESI+ve): 383 ($[M + Na]^+$, 100%).

Methyl 2,3:4,5-di-*O*-isopropylidene-D-*glycero*-D-*ido*-heptonate 3.72

A solution of triacetonide **3.14** (4.10 g, 11.4 mmol) in acetic acid/water/methanol (15 mL, 2:1:3) was stirred at 40 °C for 5.5 h until TLC (ethyl acetate) showed the formation of one major product (R_f 0.60). The reaction mixture was concentrate *in vacuo* to ~2 mL and then stirred with $NaHCO_3$ (sat. aq, 40 mL). Cyclohexane (3 x 40 mL) was used to extract the unreacted starting material **3.14** (~2.2 g) on which the process was repeated. The aqueous layer was then washed with dichloromethane (3 x 40 mL), the combined extracts dried ($MgSO_4$), and the solvent removed *in vacuo* to obtain **3.72** (1.20 g, 32%) as a clear oil. The unreacted **3.14** from cyclohexane was recycled by the hydrolysis protocol to obtain more **3.72** (2.21 g, 61% based on

recovered **3.14**).

HRMS *m/z* (ESI+ve): found 343.1360 $[M + Na]^+$, $C_{14}H_{24}O_8Na^+$ requires 343.1363; $[\alpha]_D^{20}$ +63 (*c* 0.46, MeOH); ν_{max} (thin film): 3469 (br, OH), 1760 (s, C=O); δ_H ($CDCl_3$, 400MHz) 1.44 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.51 (3H, s, CH_3), 2.42 (1H, t, OH7, $J_{OH,H7} = J_{OH,H7'}$ 5.5), 2.98 (1H, d, OH6, $J_{OH,H6}$ 5.3), 3.72 – 3.79 (2H, m, H6, H7), 3.83 (3H, s, OCH_3), 3.84 – 3.87 (1H, m, H7'), 4.12 (1H, t, H5, $J_{5,6} = J_{5,4}$ 7.3), 4.26 (1H, dd, H4, $J_{4,3}$ 3.1, $J_{4,5}$ 7.3), 4.33 (1H, dd, H3, $J_{3,4}$ 3.1, $J_{3,2}$ 7.6), 4.70 (1H, d, H2, $J_{2,3}$ 7.6); δ_C ($CDCl_3$, 100MHz) 26.0 (CH_3), 26.6 (CH_3), 26.7 (CH_3), 27.4 (CH_3), 52.6 (OCH_3), 63.9 (C7), 74.7 (C4), 73.0 (C6), 75.5 (C2), 76.8 (C5), 78.4, 78.6 (C3 and C4), 110.0 ($\underline{C}(CH_3)_2$), 111.6 ($\underline{C}(CH_3)_2$), 171.4 (C1); *m/z* (ESI+ve): 343 ($[M + Na]^+$, 100%).

2,3:4,5-Di-*O*-isopropylidene-D-*glycero*-D-*ido*-heptitol 3.73

Sodium borohydride (334 mg, 8.80 mmol) was added into a solution of **3.72** (1.41 g, 4.40 mmol) in methanol (20 mL) at 0 °C and the solution was stirred for 3 hours at room temperature until TLC (ethyl acetate) showed the consumption of starting material (R_f 0.57) and the formation of a new product (R_f 0.37). Acetic acid (~0.5 mL) was added into the solution to adjust the pH to 7 and the solvent was removed *in vacuo* to obtain a crude product which was further purified by flash column chromatography (ethyl acetate/methanol, 50 : 1) to obtain the triol **3.73** as a white solid (1.20 g, 95%).

HRMS *m/z* (ESI+ve): found 315.1415 $[M + Na]^+$, $C_{13}H_{24}O_7Na^+$ requires 315.1414; m.p. 92 °C – 94 °C; $[\alpha]_D^{20}$ +64 (*c*, 0.68 in MeOH); ν_{max} (thin film): 3389 (broad, OH); δ_H (CD_3OD, 400MHz) 1.38 (3H, s, CH_3), 1.40 (9H, s, 3 x CH_3), 3.55 (1H, dd, H7, $J_{7,6}$ 6.3, J_{gem} 11.0), 3.60 – 3.53 (1H, m, H6), 3.65 (1H, dd, H1, $J_{1,2}$ 5.2, J_{gem} 11.9), 3.71 (1H, dd, H1', $J_{1',2}$ 4.0, J_{gem} 11.9), 3.73 (1H, dd, H7', $J_{7',6}$ 2.9, J_{gem}

11.0), 3.99 (1H, dd, H3, $J_{3,4}$ 1.5, $J_{3,2}$ 8.5), 4.03 – 4.05 (2H, m, H4, H5), 4.18 (1H, ddd, H2, $J_{2,1'}$ 4.1, $J_{2,1}$ 5.2, $J_{2,3}$ 8.5); δ_C (CD_3OD, 100MHz) 25.8 (2 x CH_3), 26.2 (CH_3), 26.3 (CH_3), 61.7 (C1), 63.6 (C7), 73.8 (C6), 76.6, 78.5 (C4, C5), 77.4 (C3), 77.9 (C2), 108.9 ($\underline{C}(CH_3)_3$), 109.2 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 315 ($[M + Na]^+$, 100%).

Methyl 2,3:4,5-di-*O*-isopropylidene-D-*glycero*-D-idonate 3.75

Silica gel-supported $NaIO_4$ (4.50 g) was added portionwise to a vigorously stirred solution of **3.72** (791 mg, 2.47 mmol) in DCM (30 mL). After 1 hour, TLC analysis (cyclohexane/ethyl acetate, 1:1) showed no remaining starting material (R_f 0.19) and formation of a single product (R_f 0.70). The mixture was filtered and the silica gel was thoroughly washed with CH_2Cl_2 (4 x 30 mL). The solvents were removed *in vacuo* to afford the crude aldehyde **3.74** (630 mg, 89%). Then sodium borohydride (20.9 mg, 1.36 mmol) was added to a solution of the crude aldehyde **3.74** in methanol (5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 hour until TLC (cyclohexane/ethyl acetate, 1:1) indicated the formation of one major product (R_f 0.53) and a minor product (0.30). After acetic acid (~0.2 mL) was added into the reaction mixture to adjust pH to 7, ethyl acetate (3 x 10 mL) was used to extract the product and organic layer was dried ($MgSO_4$), filtered and the solvent was removed to obtain a residue which was further purified by flash column chromatography (cyclohexane/ethyl acetate, 1:3) to obtain the major product **3.75** as a clear oil (410 mg, 57% 2 steps).

HRMS *m/z* (ESI+ve): found 343.1362 $[M + Na]^+$, $C_{14}H_{24}O_8Na^+$ requires 343.1363; $[\alpha]_D^{20}$ +33 (*c* 1.02, $CHCl_3$); ν_{max} (thin film): 3495 (br, OH), 1759 (s, C=O); δ_H ($CDCl_3$, 400MHz) 1.46 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.48 (3H, s, CH_3), 1.50 (3H, s, CH_3), 2.11 (1H, br-dd, OH6, $J_{OH,H6'}$ 5.0, $J_{OH,6}$ 7.0),

3.71 (1H, ddd, H6, $J_{6,5}$ 4.0, $J_{6,OH}$ 7.3, J_{gem} 12.0), 3.82 (3H, s, OCH_3), 3.88 (1H, dt, H6', $J_{6',5} = J_{6',OH}$ 4.0, J_{gem} 12.0), 4.15 (1H, dd, H4, $J_{H4,H3}$ 3.2, $J_{H4,H5}$ 8.2), 4.21 (1H, dd, H3, $J_{3,4}$ 3.2, $J_{3,2}$ 7.5), 4.24 (1H, q, H5, $J_{5,6} = J_{5,6'}$ 4.0, $J_{5.4}$ 8.2), 4.62 (1H, d, H2, $J_{2,3}$ 7.5), δ_C ($CDCl_3$, 100MHz) 26.0 (CH_3), 26.5 (CH_3), 26.7 (CH_3), 27.3 (CH_3), 52.6 (OCH_3), 61.7 (C6), 75.5 (C2), 76.2 (C4), 77.3, 77.7 (C3 and C5), 109.8 ($\underline{C}(CH_3)_2$), 111.7 ($\underline{C}(CH_3)_2$), 171.0 (C1); *m/z* (ESI+ve): 343 ($[M + Na]^+$, 100%).

D-Idose 3.15

Method 1 (from **3.73**):

Silica gel-supported $NaIO_4$ (4.50 g) was added portionwise to a vigorously stirred solution of the triol **3.73** (668 mg, 2.29 mmol) in DCM (30 mL). After 2 hours, TLC analysis (ethyl acetate) showed no remaining starting material (R_f 0.31) and formation of one elongated spot (R_f 0.56-0.65). The reaction mixture was filtered and the silica gel was thoroughly washed with DCM (4 x 30 mL). The solvents were removed *in vacuo* to afford the crude aldehyde (600 mg, 100%) which was dissolved in water (20 mL) and treated with DOWEX® 50WX8-200 (~400 mg, pre-washed with water). After stirring at room temperature for 24 hours, TLC analysis (ethyl acetate) showed no remaining starting material and formation of a single product (baseline). The resin was filtered and washed with water. Removal of water *in vacuo* afforded D-idose **3.15** (400 mg, 97% from **3.73**; 53% from **3.14**) as a colorless syrup.

$[\alpha]_D^{20}$ +10.7 (*c* 0.55, water) [commercial sample: $[\alpha]_D^{20}$ +10.0 (*c* 0.80, water), lit.[66] $[\alpha]_D^{17}$ +13.7 (*c* 2.47, water)], ^1H and ^{13}C NMR are identical with those of corresponding commercial sample; HPLC showed purity 85%; After HPLC purification, purity reached to 100%; *m/z* (ESI+ve): 203 ($[M + Na]^+$, 100%) HRMS *m/z* (ESI+ve): found 203.0524 ($[M + Na]^+$), $C_6H_{12}O_6Na^+$ requires

203.0526.

Method 2 (from **3.75**):

Diisobutylaluminium hydride (1.0 M in toluene, 4.05 ml, 4.05 mmol) was added dropwise to a solution of **3.75** (391 mg, 1.35 mmol) in DCM (5 mL) at -78 °C. The reaction mixture was stirred at -78°C for 2 hours until TLC analysis (ethyl acetate) showed no remaining starting material (R_f 0.31) and the formation of one spot (R_f 0.56-0.65). Mass spectrometry also showed the formation of desired product peak ($[M + MeOH + Na]^+$ 315) and disappearance of starting material peak ($[M + Na]^+$ 313). The mixture was diluted with ethyl acetate (10 mL) and potassium sodium tartrate (sat., *aq*., 2 mL) was added. After stirring for 8 h, the reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). Organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain an oil that was further purified by flash chromatography (cyclohexane/ethyl acetate 7:1 to 3:1) to yield crude aldehyde **3.76** as a syrup (267 mg, 76%). The crude aldehyde **3.76** was dissolved in water (20 mL) was treated with DOWEX® 50WX8-200 (~300 mg, pre-washed with water). After 24 hours, TLC analysis (ethyl acetate) showed no remaining starting material and formation of a single product (baseline). The resin was filtered and washed with water. Removal of water *in vacuo* afforded D-idose **3.15** (185 mg, 76% from **3.75**; 21% from **3.14**) as a colorless syrup.

$[\alpha]_D^{20}$ +11.7 (*c* 0.65, water, eq) [commercial sample: $[\alpha]_D^{20}$ +10.0 (*c* 0.80, water), lit.[66] $[\alpha]_D^{17}$ +13.7 (*c* 2.47, water)] ^1H and ^{13}C NMR are identical with those of corresponding commercial sample (Appendix 6); HPLC showed purity 77%; *m/z* (ESI+ve): 203 ($[M+Na]^+$, 100%) HRMS *m/z* (ESI+ve): found 203.0525 ($[M + Na]^+$), $C_6H_{12}O_6Na^+$ requires 203.0526.

3.5.4 Synthesis of L-fucose 3.18

1-Deoxy-3,4:6,7-di-*O*-isopropylidene-L-*gulo*-hept-2-ulose 3.79

Methyl lithium (75.8 mL, 121.3 mmol, 1.6 M in diethyl ether) was added dropwise to a solution of the diacetonide **3.78** (31.3 g, 121.3 mmol) in anhydrous THF (350 mL) at -78 °C. After stirring at -78 °C for 1.5 hours, mass spectrometry (sample quenched with one drop sat. aq. ammonia chloride) showed the disappearance of the lactone **3.78** ($[M+Na]^+$ 281). The reaction mixture was quenched with ammonium chloride (sat., *aq*., ~30 mL) for 10 min at -40 °C. The solvent was removed *in vacuo*, and the residue dissolved in ethyl acetate (200 mL). The solution was washed with water (2 x 100 mL), dried ($MgSO_4$) and filtered. The solvent was then removed *in vacuo* to obtain the lactol **3.79** as a white solid (32.0 g, 96%) which was used in the next step without further purification; a small amount was recrystallized as the α-anomer from ethyl acetate for NMR analysis.

HRMS (ESI+ve): found 297.1306 $[M + Na]^+$; $C_7H_{16}O_6Na$ requires 297.1308; m.p. 138 – 139 °C; $[\alpha]_D^{20}$ +4.4 (*c* 0.38, $CHCl_3$) [lit.[58] m.p.: 139 – 141 °C; $[\alpha]_D^{20}$ +4.3 (*c* 1.00, $CHCl_3$)]; ν_{max} (thin film): 3370 (broad, OH); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.30 (3H, s, CH_3), 1.40 (3H, s CH_3), 1.46 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.58 (3H, s, H1), 2.17 (1H, s, OH), 3.71 (1H, t, H7, J_{gem} = $J_{7,6}$ 8.0), 4.09 (1H, dd, H5, $J_{5,4}$ 4.1, $J_{5,6}$ 8.4), 4.23 (1H, dd, H7', $J_{7',6}$ 6.7, J_{gem} 8.0), 4.35 (1H, dq, H6, $J_{6,7}$ = $J_{6,7'}$ = $J_{6,7''}$ 7.8, $J_{6,5}$ 8.4), 4.47 (1H, d, H3, $J_{3,4}$ = 6.0), 4.73 (1H, dd, H4, $J_{4,5}$ 4.1, $J_{4,3}$ 6.0); ^{13}C NMR ($CDCl_3$, 100 MHz): 22.5 (C1), 24.8 (CH_3), 25.4 (CH_3), 26.1 (CH_3), 26.7 (CH_3), 66.1 (C7), 75.7 (C6), 80.7 (C4), 81.5 (C5), 85.4 (C3), 105.8 ($\underline{C}(CH_3)_2$), 109.8 ($\underline{C}(CH_3)_2$), 113.0 (C2); *m/z* (ESI+ve): 297 ($[M + Na]^+$, 100%).

1-Deoxy-3,4:6,7-di-*O*-isopropylidene L-*glycero*-D-*galacto*-heptitol 3.80

Sodium borohydride (13.3 g, 350.1 mmol) was slowly added to a solution of **3.79** (32.0 g, 116.7 mmol) in methanol (130 mL) at 0 °C. After stirring for 1.5 h at 0 °C, TLC (cyclohexane/ethyl acetate, 1:1) indicated the disappearance of starting material (R_f 0.70) and the formation of one product (R_f 0.33). Acetic acid (~10 mL) was added dropwise to the reaction mixture at 0 °C. The solvent was removed *in vacuo*, the residue was suspended in sodium bicarbonate solution (sat. aq, 100 mL) and the reaction mixture extracted with ethyl acetate (4 x 200 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the desired product as a clear oil (30.6 g, 95%) which was used in the next step without further purification. ^{1}H NMR showed the ratio of **3.80** to the epimer **3.81** was 93:7.

HRMS (ESI+ve): found 299.1465 $[M + Na]^+$; $C_7H_{16}O_6Na$ requires 299.1465; ν_{max} (thin film): 3398 (br, OH); **3.80**: ^{1}H NMR (CD_3OD, 400 MHz): 1.16 (3H, d, H1, $J_{1,2}$ 6.4), 1.24 (3H, s, CH_3), 1.25 (3H, s, CH_3), 1.30 (3H, s, CH_3), 1.42 (3H, s, CH_3), 3.70 (1H, dd, H5, $J_{5,4}$ 1.2, $J_{5,6}$ 6.9), 3.74 (1H, a-t, H7, $J_{7,6}$ = J_{gem} 8.0), 3.89 – 3.95 (3H, m, H2, H3, H7'), 3.98 (1H, dd, H4, $J_{4,5}$ 1.2, $J_{4,3}$ 7.0), 4.15 (1H, a-q, H6, $J_{6,7}$ = $J_{6,7'}$ = $J_{6,5}$ 7.0); **3.80**: ^{13}C NMR (CD_3OD, 100 MHz): 19.3 (C1), 23.9 (CH_3), 24.4 (CH_3), 25.2 (CH_3), 25.6 (CH_3), 64.9 (C2), 65.6 (C7), 69.8 (C5), 76.1 (C4), 77.4 (C6), 80.9 (C3), 108.2 ($\underline{C}(CH_3)_2$), 109.1 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 299 ($[M + Na]^+$, 100%).

1-Deoxy-L-*glycero*-D-*galacto*-heptitol 3.19

and

1-Deoxy-L-*glycero*-D-*talo*-heptitol 3.20

DOWEX® 50WX8-200 (~8 g) was added into a solution of the diacetonide mixture **3.80** and **3.81** (30.6 g, 110.5 mmol) in methanol/water (1:1, 100 mL) and the reaction mixture stirred at 60 °C

for 4 hours until mass spectrometry showed the formation of one product ([M+Na]$^+$ 219) and the TLC (cyclohexane/ethyl acetate, 1:1) showed the disappearance of starting material (R_f 0.33). After filtering off the resin, the solution was concentrated to ~30 mL and a mixture of MeOH:CH_3CN (1:3, ~20 mL) was added. After cooling to 0 °C for 10 min, the crystallized heptitol **3.19** (13.2 g, 61%) was filtered off as a white solid and washed with cold methanol. The mother liquor was used for a second crystallization as above to obtain more heptitol **3.19** (17.6 g in total, 81%). ^{1}H NMR and HPLC analysis showed the ratio of **3.19** to its 2-epimer **3.20** increased from 93:7 in the initial product to 98:2 after recrystallization. The remaining mother liquor contained **3.19**:**3.20** in a ratio of ~4:3. Pure **3.19** (a white solid) and **3.20** (a clear oil) were obtained by HPLC for NMR analysis.

3.19: HRMS (ESI+ve): found 219.0835 [M + Na]$^+$; $C_7H_{16}O_6Na$ requires 219.0839; m.p.: 138 – 140 °C; $[\alpha]_D^{20}$ +0.4 (*c* 0.26, water) [lit.[67] m.p.: 150 °C; $[\alpha]_D$ 0 (water)]; ν_{max} (thin film): 3240 (broad, OH); ^{1}H NMR (D_2O, 400 MHz): 1.24 (3H, d, H1, $J_{1,2}$ 6.6), 3.50 (1H, dd, H3, $J_{3,2}$ 1.8, $J_{3,4}$ 9.2), 3.63 (1H, dd, H7, $J_{7,6}$ 6.1, J_{gem} 11.8), 3.71 (1H, dd, H4, $J_{4,5}$ 1.4, $J_{4,3}$ 9.1), 3.75 (1H, dd, H7', $J_{7',6}$ 3.6, J_{gem} 11.6), 3.85 (1H, dt, H6, $J_{6,7'}$ 3.6, $J_{6,7}$ = $J_{6,5}$ 6.1), 3.88 (1H, br-dd, H5, $J_{5,4}$ 1.5, $J_{5,6}$ 6.1), 4.09 (1H, qd, H2, $J_{2,3}$ 1.8, $J_{2,1}$ 6.5); ^{13}C NMR (D_2O, 100 MHz): 19.3 (C1), 63.0 (C7), 66.4 (C2), 70.4 (C5), 71.0 (C6), 73.5 (C4), 73.8 (C3); *m/z* (ESI+ve): 219 ([M + Na]$^+$, 100).

3.20: HRMS (ESI+ve): Found 219.0832 [M + Na]$^+$; $C_7H_{16}O_6Na$ requires 219.0839; $[\alpha]_D^{20}$ -11.0 (*c* 1.20, water); ν_{max} (thin film): 3300 (broad, OH); ^{1}H NMR (D_2O, 400 MHz): 1.17 (3H, d, H1, $J_{1,2}$ 6.6), 3.56 (1H, dd, H4, $J_{4,5}$ 1.4, $J_{4,3}$ 8.9), 3.60 (1H, dd, H7, $J_{7,6}$ 6.0, J_{gem} 11.6), 3.73 (1H, dd, H7', $J_{7',6}$ 3.2, J_{gem} 11.3), 3.75 (1H, dd, H3, $J_{3,2}$ 3.7, $J_{3,4}$ 8.9), 3.83 (1H, dt, H6, $J_{6,7'}$ 3.5, $J_{6,7}$ = $J_{6,5}$ 6.2), 3.85 (1H, br-dd, H5, $J_{5,4}$ 1.4, $J_{5,6}$ 6.2), 4.06 (1H, qd, H2, $J_{2,3}$ 3.7, $J_{2,1}$ 6.5); ^{13}C NMR (D_2O, 100 MHz): 15.6 (C1), 63.0 (C7),

68.2 (C2), 70.3 (C5), 72.2 (C4), 73.6, 73.7 (C3, C6); *m/z* (ESI+ve): 219 ([M + Na]$^+$, 100%).

1-Deoxy-2,3:4,5:6,7-tri-*O*-isopropylidene L-*glycero*-D-*galacto*-heptitol 3.17

p-Toluenesulfonic acid (1.5 g, 8.72 mmol) was added to a suspension of **3.19** (17.4 g, 88.8 mmol) in acetone (170 mL). The reaction mixture was stirred at 80 °C for 2 hours until it became a clear solution and TLC (cyclohexane/ethyl acetate, 2:1) indicated the formation of one major product (R_f 0.71). Excess sodium carbonate (~5 g) was added to the solution with stirring at room temperature for 10 min. The solid was removed by filtration and the solvent was removed *in vacuo.* The residue was suspended in water (100 mL) and extracted with cyclohexane (3 x 70 mL). After the combined cyclohexane phase had been washed with water (70 mL), the solvent was removed *in vacuo* to yield triacetonide **3.17** (17.1 g, 61%) as a white solid. Further extraction of the aqueous layer with ethyl acetate (3 x 100 mL) gave partially acetonated products; removal of the solvent *in vacuo*, afforded a residue (~7.0 g) which was dissolved into acetone (70 mL) and the procedures above were repeated to obtain more **3.17** (23.4 g in total, 83%), which was used in the next step without further purification.

HRMS (ESI+ve): Found 339.1770 [M + Na]$^+$; $C_{16}H_{26}O_6Na$ requires 339.1778; m.p. 40 – 42 °C; $[\alpha]_D^{20}$ +18.5 (*c* 0.68, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.35 (3H, s, CH_3), 1.37 (3H, d, H1, $J_{1,2}$ 5.9), 1.39 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.45 (3H, s, CH_3), 3.50 (1H, t, H3, $J_{3,4}$ = $J_{3,2}$ 8.2), 3.81 (1H, dd, H4, $J_{4,5}$ 6.9, $J_{4,3}$ 8.2), 3.90 (1H, a-dd, H7, $J_{7,6}$ 7.6, J_{gem} 8.0), 4.00 - 4.08 (3H, m, H2, H5, H7'), 4.19 (1H, a-ddd, H6, *J* 5.5, *J* 6.4, $J_{6,7}$ 7.6); ^{13}C NMR ($CDCl_3$, 100 MHz): 18.6 (C1), 25.7 (CH_3), 26.4 (CH_3), 26.7 (CH_3), 27.0 (CH_3), 27.2 (CH_3), 27.3 (CH_3), 66.1 (C7), 76.6 (C6), 76.6 (C2), 78.9 (C4), 81.2 (C5), 83.1 (C3), 108.9 ($\underline{C}(CH_3)_2$), 109.5 ($\underline{C}(CH_3)_2$), 110.2 ($\underline{C}(CH_3)_2$);

m/z (ESI+ve): 339 ([M + Na]$^+$, 100%).

1-Deoxy-2,3:4,5-di-*O*-isopropylidene-L-*glycero*-D-*galacto*-heptitol 3.84

A solution of triacetonide **3.17** in acetic acid/water/methanol (60 mL, 2:1:3) was stirred at 50 °C for 7 hours until TLC (ethyl acetate) showed the formation of one major product (R_f 0.64). The reaction mixture was concentrate *in vacuo* to ~10 mL and then stirred with $NaHCO_3$ (sat. aq, 80 mL). Cyclohexane (3 x 70 mL) was used to extract the unreacted starting material **3.17** (~10 g) on which the process was repeated. The aqueous methanol layer was washed with dichloromethane (3 x 100 mL), the combined extracts dried ($MgSO_4$), and the solvent was removed *in vacuo* to obtain **3.84** (11.9 g, 58%) as a white solid. The unreacted starting material from the cyclohexane layer was used to repeat the hydrolysis one more time following the procedures shown above to obtain more **3.84** (16.7 g, 81% based on recovered **3.17**).

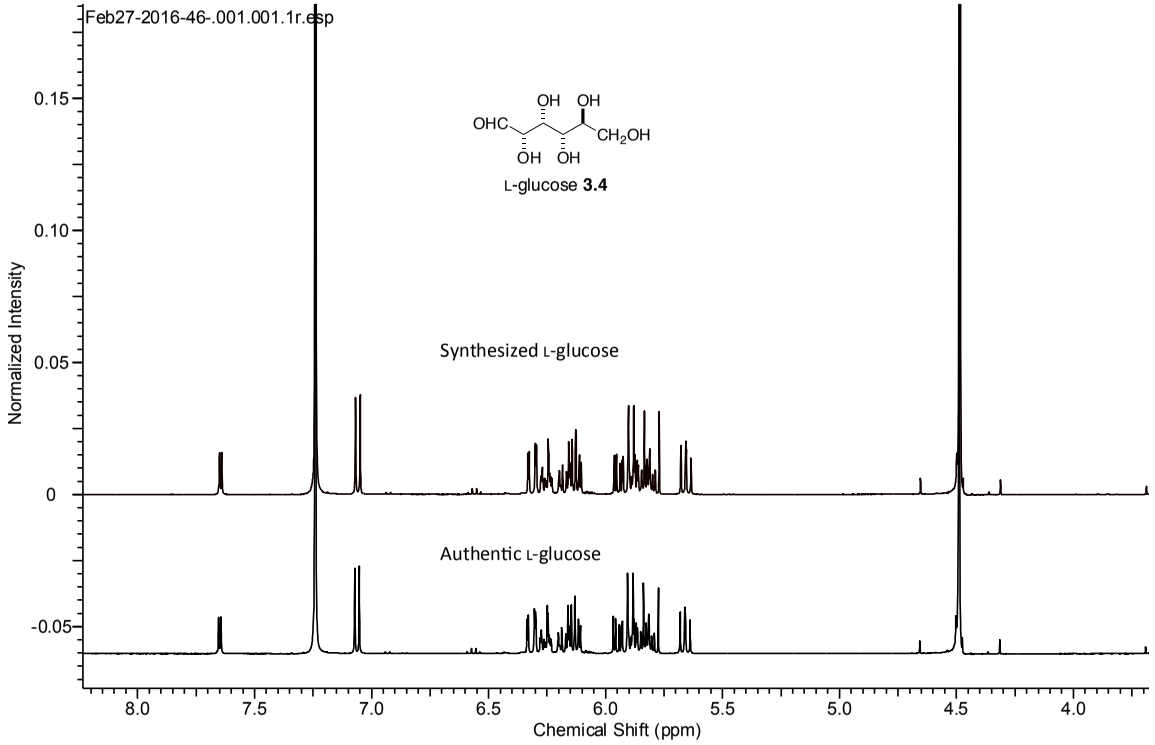
HRMS (ESI+ve): Found 299.1457 [M + Na]$^+$; $C_7H_{16}O_6Na$ requires 299.1465; m.p. 56 – 58 °C; $[\alpha]_D^{20}$ +26.0 (*c*, 0.41 in $CHCl_3$); ν_{max} (thin film): 3355 (broad, OH); ^{1}H NMR (CD_3OD, 400 MHz): 1.36 (3H, d, H1, $J_{1,2}$ 7.0), 1.37 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.43 (3H, s, CH_3), 3.56 (1H, t, H3, $J_{3,2}$ = $J_{3,4}$ = 7.8), 3.63 (1H, dd, H7, $J_{7,6}$ 7.0, J_{gem} 11.0), 3.66 (1H, dd, H7', $J_{7',6}$ 5.8, J_{gem} 11.0), 3.77 (1H, a-ddd, H6, $J_{6,5}$ 2.0, $J_{6,7'}$ 5.8, $J_{6,7}$ 7.1), 4.05 (1H, t, H4, $J_{4,3}$ = $J_{4,5}$ 7.8), 4.08 – 4.11 (2H, m, H2, H5); ^{13}C NMR (CD_3OD, 100 MHz): 17.4 (C1), 25.7 (2 x CH_3), 26.0 (CH_3), 26.2 (CH_3), 63.5 (C7), 70.3 (C6), 77.2, 76.8 (C2, C4), 80.3 (C5), 83.5 (C3), 108.7 ($\underline{C}(CH_3)_2$), 109.5 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 299 ([M + Na]$^+$, 100%).

L-Fucose 3.18

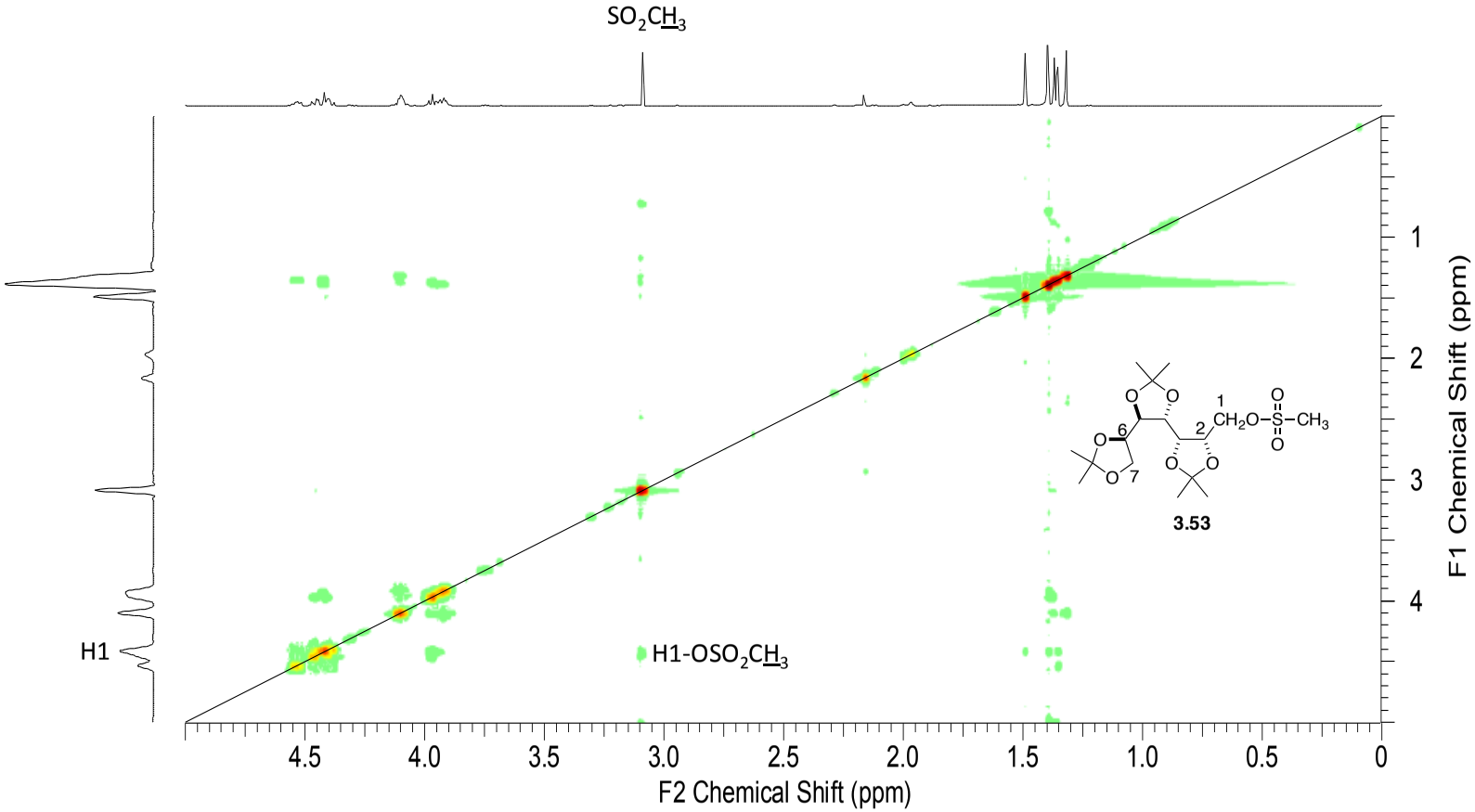
Silica gel-supported $NaIO_4$ (120 g) was added portionwise to a vigorously stirred solution of **3.84** (16.7 g, 60.1 mmol) in dichloromethane (400 mL). After 2 hours, TLC analysis (cyclohexane/ethyl acetate, 1:1) showed no remaining starting material (R_f 0.20) and formation of a single product (R_f 0.43). The mixture was dried ($MgSO_4$), filtered and the silica gel was thoroughly washed with dichloromethane (4 x 100 mL). The solvents were removed *in vacuo* to afford the crude aldehyde **3.85** (11.8 g, 80%) which was suspended in water (100 mL) and treated with DOWEX® 50WX8-200 (~3 g, pre-washed with water). After 24 hours, TLC analysis (ethyl acetate) showed no remaining starting material and formation of a single product (baseline). The resin was filtered off and washed with water. Removal of water *in vacuo* afforded crude **3.18** (7.8 g, 100%; 96% pure by HPLC analysis); recrystallization from ethanol gave pure L-fucose **3.18** as a white solid (5.8 g, 74%; 99.3% pure by HPLC analysis). [The residue from the mother liquor contained 48% of fucose]; m.p.: 130 – 132 °C; $[\alpha]_D^{20}$ = -74.9 (*c* 1.83, water, eq) [commercial sample: m.p. 132 – 134 °C; $[\alpha]_D^{20}$ -76.0 (*c* 1.20, water), lit.[43b] m.p. 140 – 141 °C; $[\alpha]_D^{20}$ -76.0 (*c* 2.0, water)]; ^1H and ^{13}C NMR were identical with those of authentic L-fucose sample (Appendix 7).

3.6 Appendix

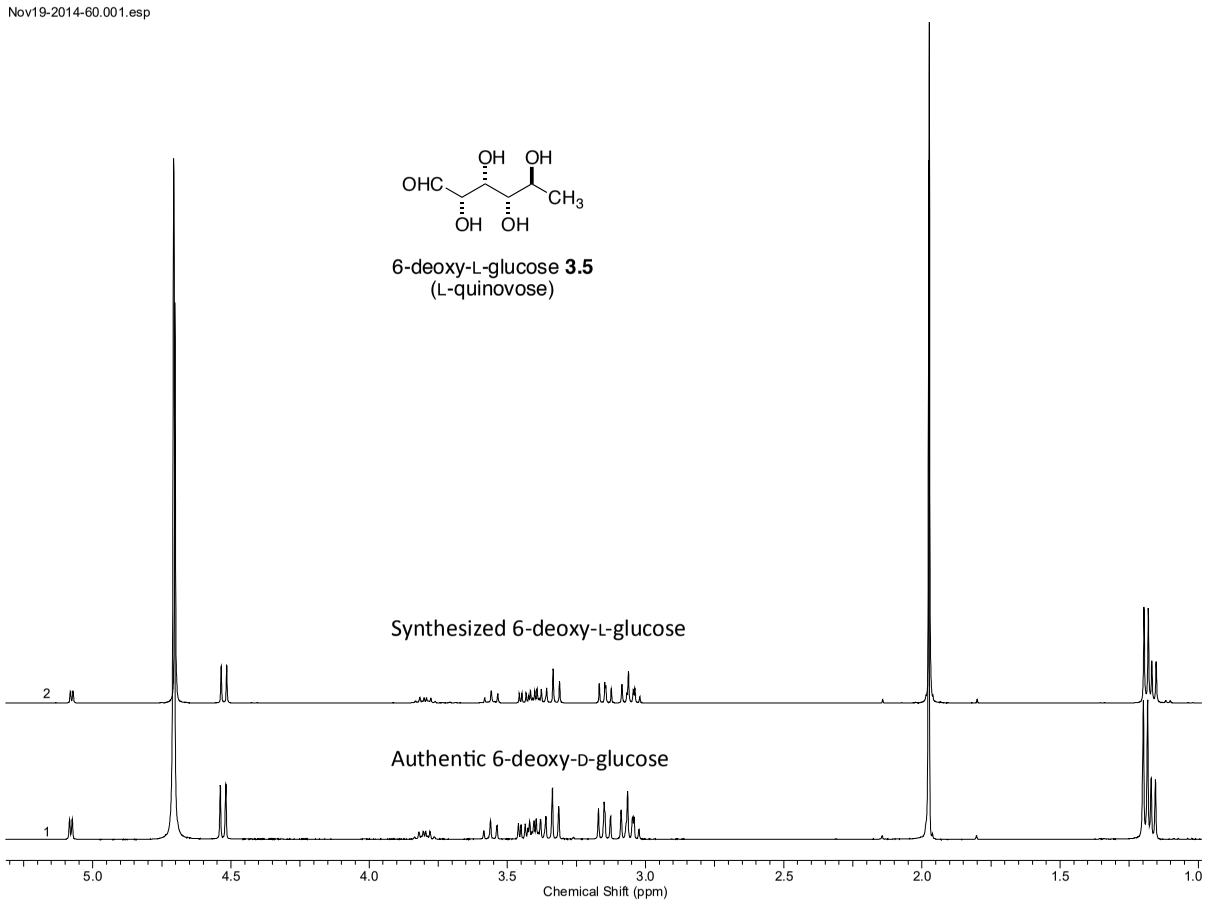
1. ^{1}H NMR spectra of synthesized L-glucose **3.4** and commercial sample of L-glucose



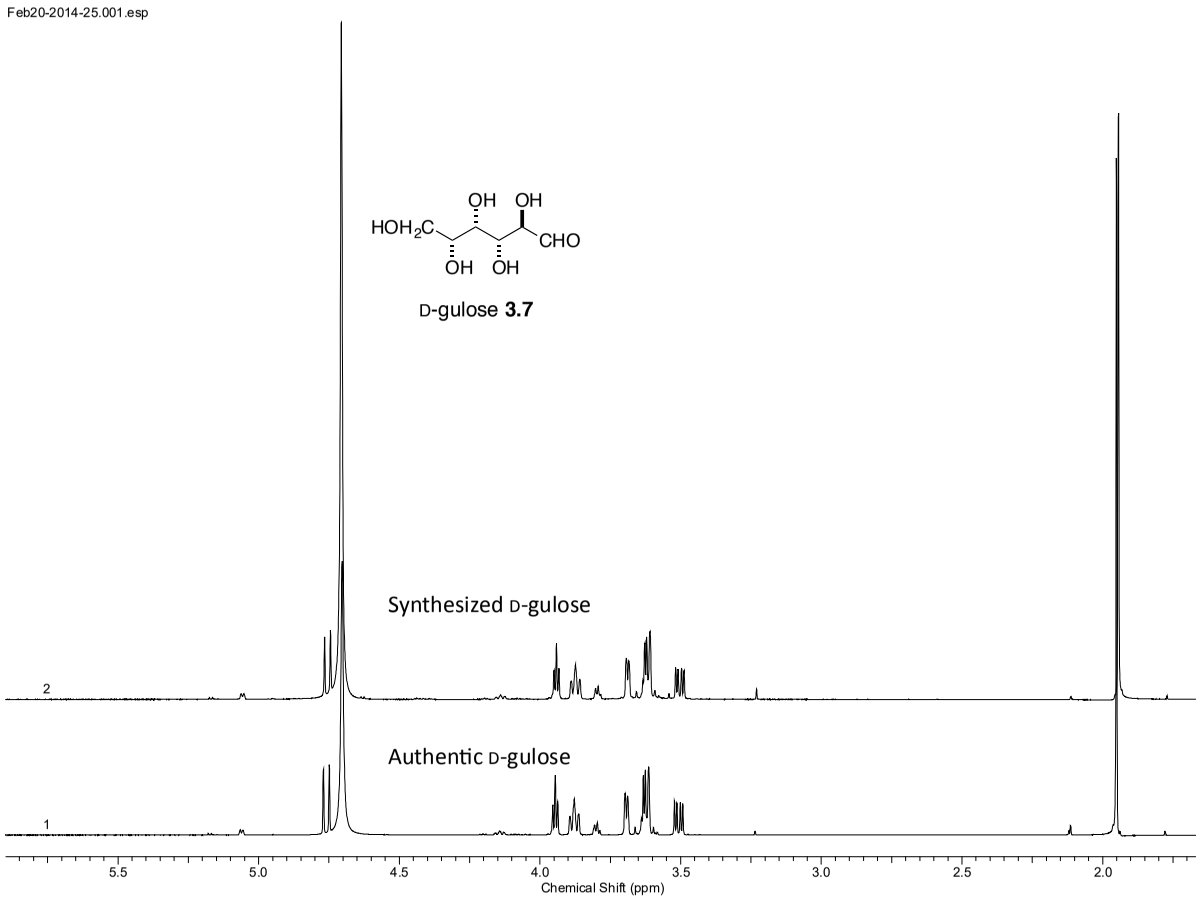
2. TCOSY spectrum of mesylate **3.53**, AVB500 (CD_3CN, 500MHz)



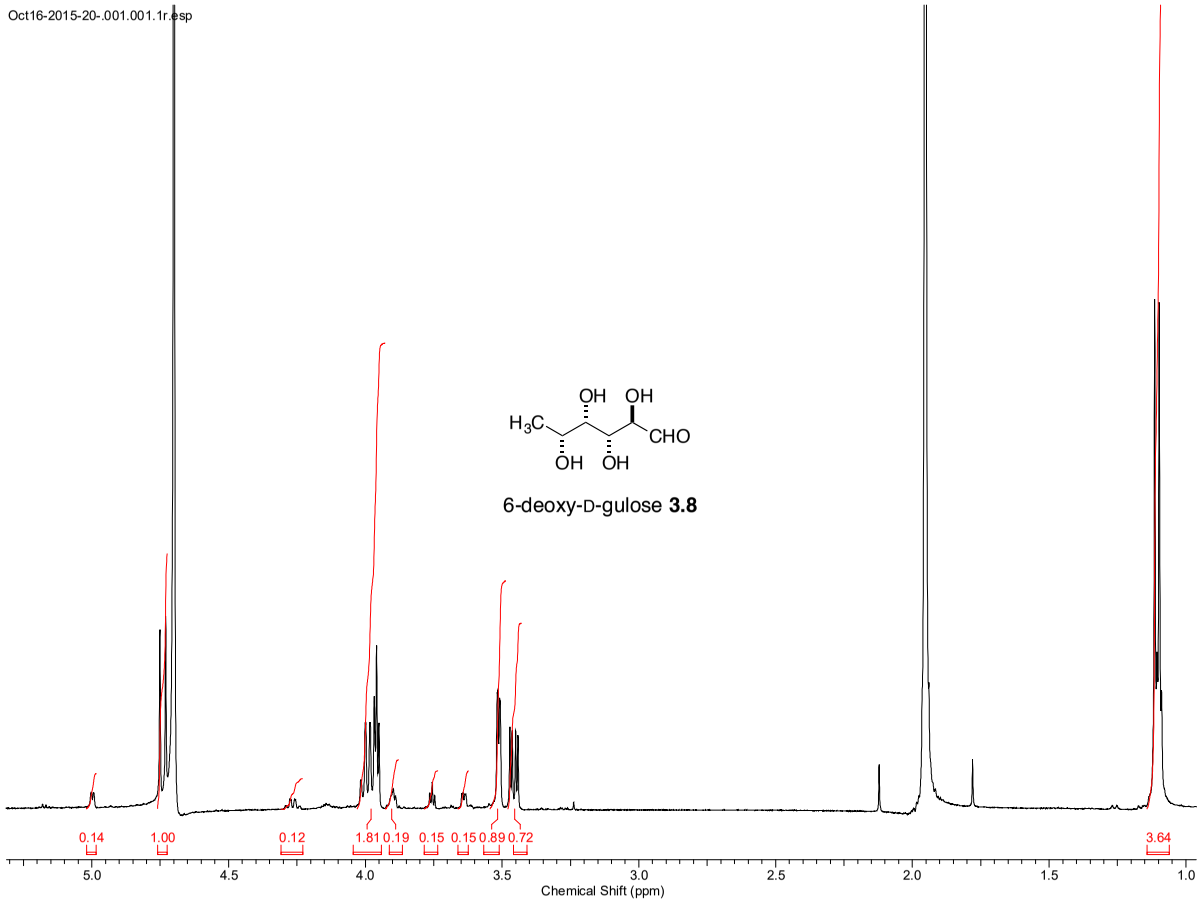
3. ^{1}H NMR spectra (D_2O, 400 MHz) of synthesized 6-deoxy-L-glucose **3.5** and commercial sample of 6-deoxy-D-glucose



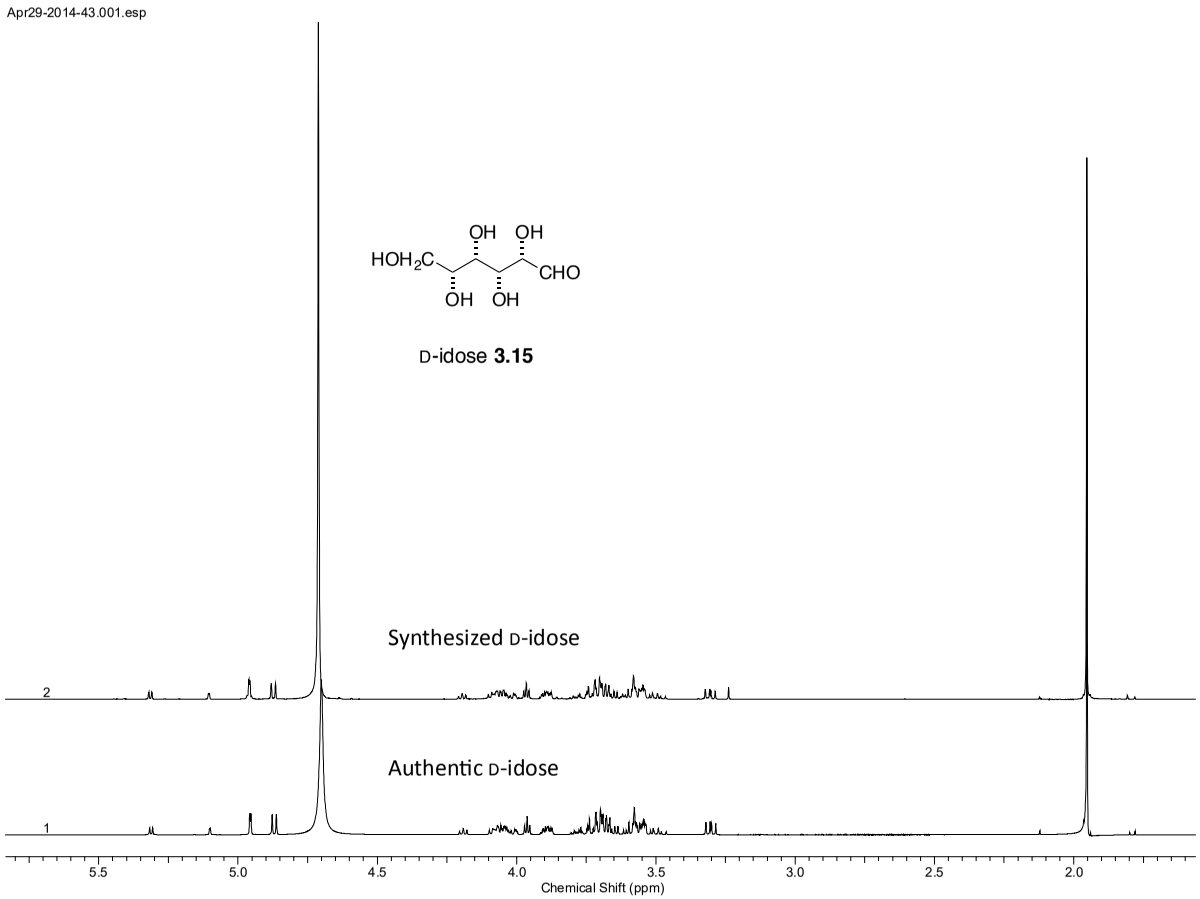
4. ^{1}H NMR spectra (D_2O, 400 MHz) of synthesized D-gulose **3.7** and commercial sample of D-gulose



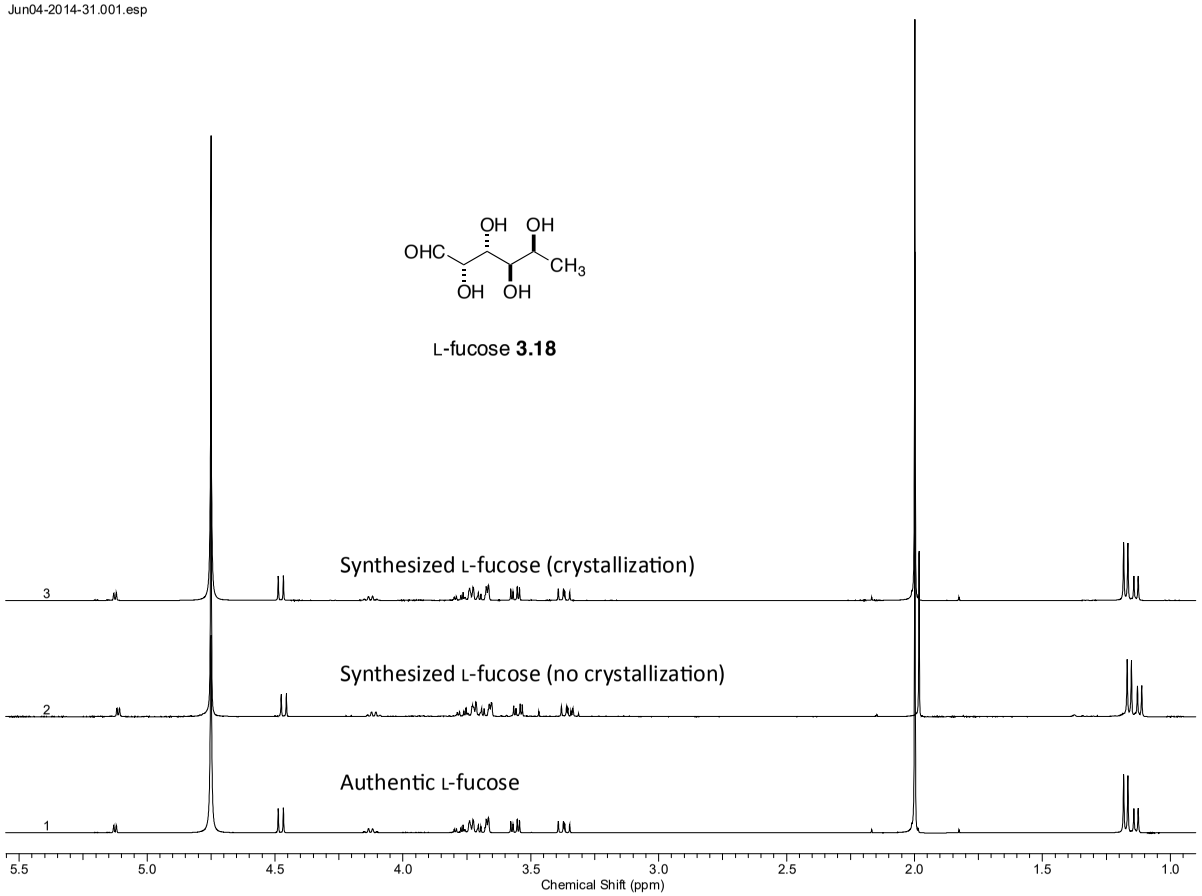
5. ^{1}H NMR spectra (D_2O, 400 MHz) of synthesized 6-deoxy-D-gulose **3.8**



6. ^{1}H NMR spectra (D_2O, 400 MHz) of synthesized D-idose **3.15** and commercial sample of D-idose



7. ^{1}H NMR spectra (D_2O, 400 MHz) of synthesized L-fucose **3.18** (crystallization/no crystallization) and commercial sample of L-fucose



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Chapter 4 Synthesis of 6-deoxy hexoses, 6-deoxy iminosugars as potential inducers for L-rhamnose promoter system and potential inhibitors of L-rhamnosidase, rhamnose isomerase and other glycosidases

4.1 Introduction

As described in Chapter 1, most 6-deoxy-L-hexoses are rare in nature but several have important biological activity. L-Rhamnose (6-deoxy-L-mannose) **4.1** is one of the most abundant 6-deoxy-L-hexoses in nature and it is widely distributed in plants and bacteria. Some L-rhamnose containing natural products are bioactive such as spicamycin, an antitumor nucleoside containing a L-rhamnose moiety.[1] In addition, L-rhamnose is a common component in the *O*-antigen of lipopolysaccharides (LPS) of Gram-negative pathogenic bacteria including *Salmonella enterica*[2], *Shigella flexneri*[3] and *Escherichia coli* (*E. coli*) strain VW187[4]. L-Rhamnose containing molecules are also found in animals: Cammarata *et al*. recently identified a novel rhamnose-binding lectin in sea bass and suggested it plays an important role in the immune recognition of pathogens.[5] However, neither L-rhamnose nor any of its enzymes have been found in mammals.[6] Consequently, its therapeutic potential has been recognized. In synthetic biology, a gene expression system, L-rhamnose operon in *E. coli*., has been frequently used for the production of various proteins and has shown a promising commercial prospect.

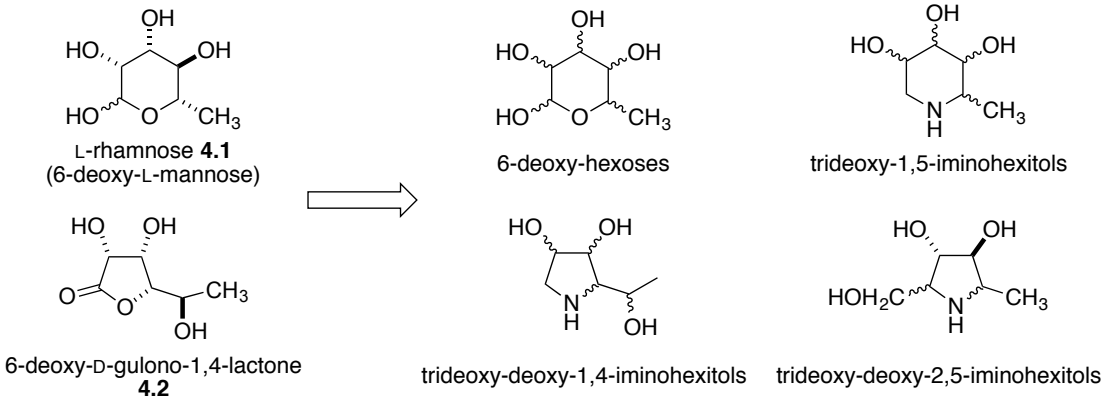


Figure 4.1 Synthetic targets

In this chapter (Figure 4.1), a variety of 6-deoxy hexoses were efficiently synthesized from L-rhamnose **4.1** and 6-deoxy-D-gulono-1,4-lactone **4.2** as potential synthetic inducers of L-rhamnose inducible gene expression system (L-rhamnose operon) in *E. coli*. A number of pyrrolidine and piperidine iminosugars were also synthesized from L-rhamnose **4.1** and 6-deoxy-D-gulono-1,4-lactone **4.2** and subjected to bioassays for inhibition of different glycosidases including L-rhamnosidase and L-rhamnose isomerase and for the induction effects on the L-rhamnose operon. In this introduction section, the biosynthesis and metabolism of L-rhamnose in bacteria, the development of inhibitors targeting L-rhamnose processing enzymes and the L-rhamnose inducible gene expression system are discussed.

4.1.1 Biosynthesis of L-rhamnose

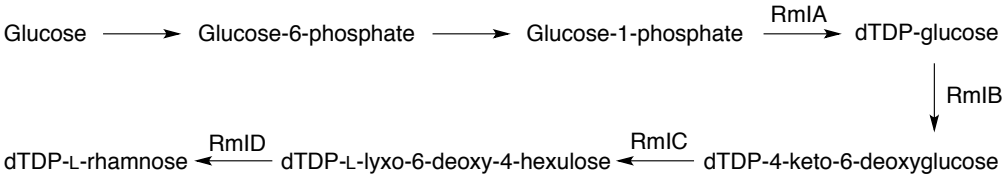


Figure 4.2 Biosynthesis of L-rhamnose in bacteria

As shown in Figure 4.2, the biosynthesis of L-rhamnose in bacteria begins from Glu-1-P (glucose-1-phophate), which is initially transformed to dTDP-glucose by RmlA

(glucose-1-phophate thymidylyltransferase). It is then oxidized and dehydrated to dTDP-4-keto-6-deoxyglucose by RmlB (dTDP-glucose-4,6-dehydratase). The third enzyme, RmlC (dTDP-glucose-D-xylo-hexulose 3,5-epimerase) catalyzes a double epimerization at C3 and C5 to dTDP-L-lyxo-6-deoxy-hexulose which is ultimately reduced by RmlD (dTDP-L-lyxo-6-deoxy-hexulose reductase) to generate dTDP-L-rhamnose, which is then subjected to the further biosynthesis of L-rhamnose containing molecules in cell wall of bacteria. This bioproduction of L-rhamnose from Glu-1-P is named as the 'L-rhamnose pathway'. The structure and mechanism of each enzyme has been well studied.[6]

The therapeutic potential of the L-rhamnose pathway has been recognized. Since there is no biosynthesis of L-rhamnose in humans, the disruption of the enzymes involved in L-rhamnose pathway is unlikely to affect human metabolic pathways. For example, the deletion of the *rmlB* and *rmlD* genes in *vibrio cholerae* severely disabled its intestinal colonization.[7] Yamashita *et al*. reported that the deficiency of any *rml* gene in *Streptococcus mutans* reduced its survival rate in the presence of sucrose.[8] The *mycobacterium tuberculosis* RmlC epimerase has also been studied as a promising target for the design of drugs.[9]

4.1.2 Metabolism of L-rhamnose in bacteria

The catabolism cascade of L-rhamnose in bacteria has also been studied since the 1980s. First, free L-rhamnose is released by hydrolysis of L-rhamnose containing polysaccharides, which is catalyzed by α-L-rhamnosidase, a glycosidase widely occurring not only in microorganisms but also in animals.[10] Three metabolic pathways of L-rhamnose in different fungi and bacteria have

been identified (Figure 4.3): phosphorylated metabolic pathway (PMP), non-phosphorylated metabolic pathway I (NMP-I) and non-phosphorylated metabolic pathway II (NMP-II).[11]

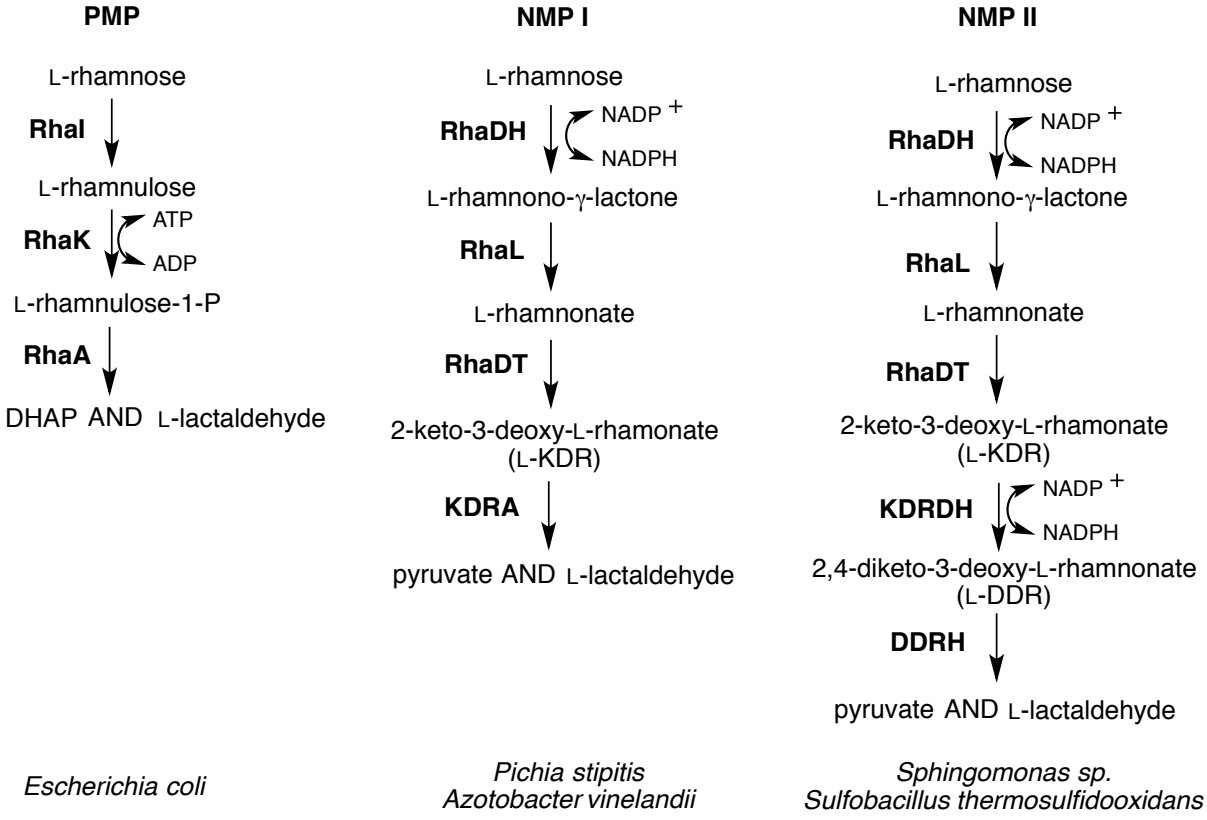


Figure 4.3 Metabolic pathways of L-rhamnose[11]

As shown in Figure 4.3, PMP exists in some bacteria including *E. coli*.: L-rhamnose is degraded to dihydroxyacetone phosphate (DHAP) and L-lactaldehyde. Three enzymes were involved in this process: Rhamnose isomerase (RhaA) catalyzes the isomerization of rhamnose to rhamnulose; Rhamnulose kinase (RhaB) catalyzes the 1-phosphorylation of rhamnulose to L-rhamnulose-1-phosphate; Rhamnulose-1-phosphate aldolase (RhaD) catalyzes the retro-aldol fragmentation of L-rhamnulose-1-phosphate to DHAP and L-lactaldehyde.

In contrast, some fungi and bacteria, such as *Pichia stipites* and *Debaryomyces hansenii*, metabolize L-rhamnose to pyruvate and L-lactaldehyde *via* non-phosphorylated pathway

(NMP-I)[11]: L-rhamnose is oxidized to L-rhamnose-γ-lactone by L-rhamnose-1-dehydrnase (RhaDH); L-rhamnose-γ-lactone is then transferred to L-rhamnonate by L-rhamnose-γ-lactonase (RhaL); L-rhamnonate was dehydrated to 2-keto-3-deoxy-L-rhamnonate by L-rhamnonate dehydrogenase (RhaDT). Eventually 2-keto-3-deoxy-L-rhamnonate is cleaved to pyruvate and L-lactaldehyde in a reverse aldol reaction catalyzed by 2-keto-3-deoxy-L-rhamnonate aldolase (KDRA).[12] An alternative pathway NMP-II occurs in *Sphingomonas sp. SKA58*: before the formation of pyruvate and L-lactaldehyde, 2-keto-3-deoxy-L-rhamnonate is oxidized to 2,4-diketo-3-deoxy-L-rhamnonate by the corresponding dehydrogenase (KDRDH).[13]

4.1.3 Development of iminosugars inhibitors of L-rhamnose processing enzymes

As discussed, enzymes involved in L-rhamnose biosynthesis and metabolism could be a fertile field for the development of novel antibiotics. Both non-sugar and sugar mimics have been identified as good inhibitors of L-rhamnose processing enzymes in bacteria. For instance, L-rhamnose 1C-phosphonate **4.3** was synthesized and was a potent inhibitor of RmlA.[14] Several nano-molar inhibitors **4.4** of *P. aeruginosa* RmlA were recently synthesized and showed inhibitory effects towards *M. tuberculosis*.[15] (Figure 4.4A)

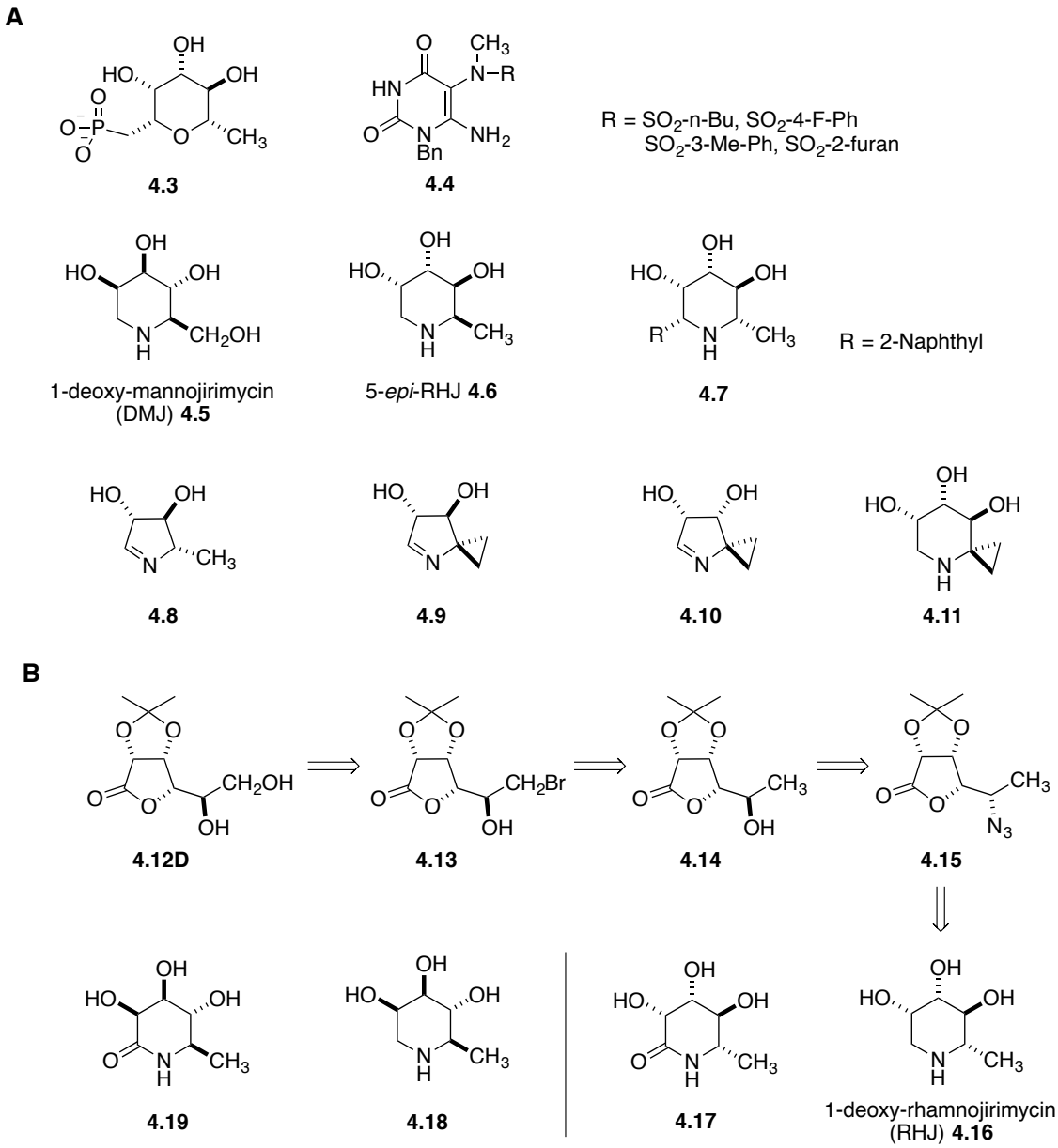


Figure 4.4 **A** Potent inhibitors of L-rhamnose processing enzymes; **B** Synthesis of RHJ **4.16** and its analogues

Numerous iminosugars have been isolated from nature or synthesized as inhibitors of different glycosidases. However, there are only a few reports on the iminosugar inhibitors of L-rhamnosidases. Dexoymannojirimycin (DMJ) **4.5** was a potent α-mannosidase inhibitor but showed no inhibitory activity to any α-rhamnosidase.[16] In the same report, the enantiomers of 1-deoxyrhamnojirimycin (RHJ[17]) (**4.16**, **4.18**) and 1-deoxy-rhamnonolactam (**4.17**, **4.19**) from D- and L-gulonolactones (**4.12D/L**) were synthesized by a strategy involving the deoxygenation

of C-6 of gulonolactones, the introduction of azido group at C-5 and subsequent ring closure of C-1 and C-5 (Figure 4.4B). None of them inhibited naringinase, a commercial available α-L-rhamnosidase from *Penicillium decumbens*. In a later report, RHJ **4.16** was synthesized by an analogous procedure and was found to very weakly inhibit α-L-rhamnosidase (K_i 2.73 mM).[18] Wong *et al*. reported the concise synthesis of a range of 5-membered 1,6-dideoxy iminosugars *via* the formation of imine **4.8** and the enzymatic synthesis of RHJ **4.16** in 1994.[19] Imine **4.8** was a micromolar inhibitor of α-L-rhamnosidase (naringinase) and the reduction of imine **4.8** caused the decrease of inhibitory activity. Interestingly, RHJ **4.16** synthesized in the same report showed a much stronger inhibition (K_i 62 μM) of the same type of α-L-rhamnosidase than that in previous reports. A small amount of 5-*epi*-RHJ **4.6** might be formed during the enzymatic synthesis, suggesting that 5-*epi*-RHJ **4.6** might be a potent inhibitor of α-L-rhamnosidase.[19] To prove this hypothesis, pure 5-*epi*-RHJ **4.6** was synthesized from L-rhamnolactone with the same strategy shown in Figure 4.4B and it was confirmed to be a potent inhibitor of naringinase (IC_{50} 5.0 μM).[20] In 2008, a similar approach was used to access various α- and β-homonojirimycin analogues of L-rhamnopyranose from D-gulonolactone.[21] Several candidates including **4.7** showed specific inhibitory effect towards α-L-rhamnosidase. In the same report, several β-pseudoanomeric substituted L-rhamnose mimics were identified as potent inhibitors of rhamnose processing system in mycobacterial systems. In 2009, spirocyclopropyl iminosugars **4.9** – **4.11** was prepared as analogues of imine **4.8**, using a titanium-mediate aminocyclopropanation of nitriles as the key reaction.[22] Pyrrolidine imines **4.9**, **4.10**, as analogues of **4.8**, and piperidine **4.11** as an analogue of 5-*epi*-RHJ **4.6** and RHJ **4.16**, showed inhibitory activities towards α-L-rhamnosidase (*penicllium decumbens*).

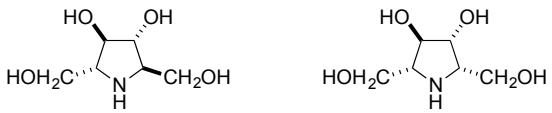


Figure 4.5 DMDP **4.20** and DGDP **4.21**

In contrast, there are no iminosugars that have yet been tested as inhibitors of L-rhamnose isomerase, another key enzyme in the metabolism of L-rhamnose in bacteria. 2,5-Dideoxy-2,5-imino-D-glucitol (DGDP) **4.21** (Figure 4.5) was previously reported to weakly but competitively inhibit D-xylose isomerase (XI), an enzyme converts D-xylose to D-xylulose. XI have been used on a massive industrial scale for the equilibration of D-glucose and D-fructose in the production of corresponding ketoses.[23] However, DMDP **4.20** did not show any inhibitory effect towards to this isomerase. DMDP **4.20** and DGDP **4.21** can be considered iminosugar mimics of α- and β-fructose. Their corresponding rhamnulose analogues might give some clues to the development of novel inhibitors of L-rhamnose isomerase.

4.1.4 L-Rhamnose inducible gene expression system

4.1.4.1 Regulation of L-rhamnose metabolism in *E. coli*.

L-Rhamnose is readily metabolized by *E. coli*. in the absence of a preferred carbon source.[24] As introduced in Figure 4.3, L-rhamnose is metabolized by a PMP pathway in *E. coli*. L-Rhamnose is firstly transported into the cell by RhaT, an L-rhamnose:proton symporter,[25] then isomerized by L-rhamnose isomerase (RhaA) to L-rhamnulose.[26] Subsequent phosphorylation of L-rhamnulose by L-rhamnulose kinase (RhaB)[27] and conversion of the resulting L-rhamnulose-1-phosphate by rhamnulose-1-phosphate aldolase (RhaD) give L-lactaldehyde and dihydroxyacetone phosphate

(DHAP).[28] L-Lactaldehyde can be further metabolized to pyruvate by an aerobic pathway and to propane-1,2-diol by an anaerobic pathway.[29]

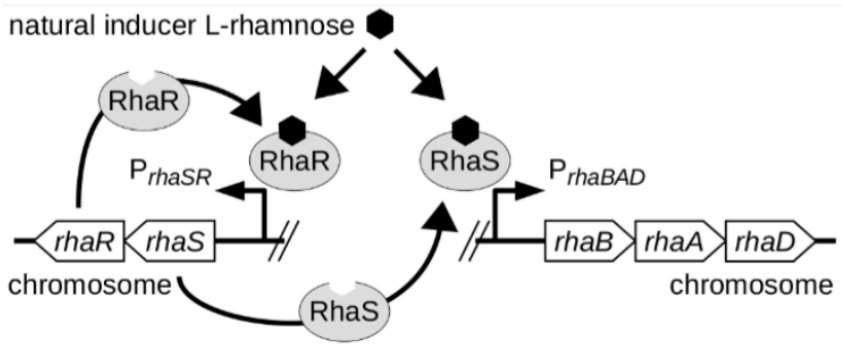


Figure 4.6 Regulation cascade of L-rhamnose inducible P_{rhaSR} and P_{rhaBAD} in *E. coli*.[30]

The metabolism of L-rhamnose in bacteria is precisely controlled. Figure 4.6 shows the regulation cascade of the expression of L-rhamnose processing enzymes in *E. coli*.. The genes (*rhaBAD*) encoding L-rhamnose metabolic enzymes and the genes (*rhaSR*) encoding their regulatory proteins are found in two divergent gene operons,[26a, 31] while the gene encoding RhaT is found at another locus.[32] Expression of both *rhaBAD* and *rhaT* results from the same regulatory cascade (Figure 4.6). In the presence of L-rhamnose, the AraC/XylS transcriptional activator RhaR, which is constitutively expressed at a low basal level, binds with L-rhamnose and introduces the transcription of *rhaSR* operon, resulting in the high level expression of RhaR and RhaS.[33] Then the regulatory protein RhaS binds upstream of the RNA polymerase-binding site of *rhaBAD*[33b] and *rhaT*[34] assisted by L-rhamnose to initiate the transcription of downstream genes.[33c] Autosuppression of the expression of those genes has been reported.[33c] In addition, the binding of L-rhamnose and RhaS also negatively regulates the expression level of RhaS.[35] In addition, although both RhaS and RhaR belongs to the AraC/XylS family, these two proteins share only 36% identity and 54% similarity with each other and L-rhamnose activates RhaS and RhaR in different manners: L-rhamnose is necessary for the effective binding of RhaS to DNA

while L-rhamnose ensures the good contact of RhaR to RNA polymerase (RNAP). In addition, L-rhamnose results in conformational changes in both proteins.[36]

4.1.4.2 Experimental control of gene expression using L-rhamnose inducible operon in *E. coli*.

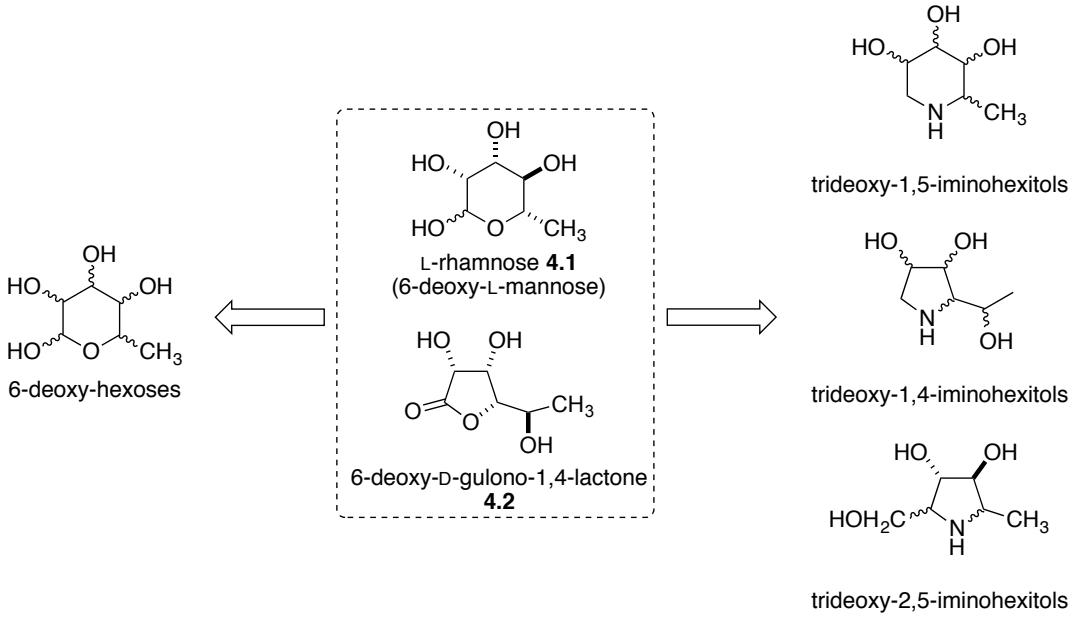
Experimental control of gene expression is required in various studies of biotechnology and synthetic biology. Inducible gene expression systems, where the promoter of gene expression is activated by various stimuli including sugars, antibiotics and metals, have been well developed and frequently reported.[37] For example, tetracycline-inducible promoter systems in *Schizosaccharomyces pombe* and *Streptococcus agalactiae* have been constructed in 1992 and 2014 respectively and strong gene induction was observed after the addition of tetracycline.[38] The applications of tetracycline-inducible promoter system have been intensively reported in different studies on gene therapy and molecular biology.[38a, 39] Other promoter systems induced by lactose (P_{lac}) or arabinose (P_{araBAD}) have been intensively investigated.[40]

The value of L-rhamnose inducible promoter P_{rhaBAD} in the control of gene expression has been recognized for sometime. By the introduction of heterologous genes to the downstream of P_{rhaBAD}, L-rhamnose is used as a 'key' to control the gene expression for different purposes (Figure 4.6).[41] Compared to other promoter system, the L-rhamnose inducible P_{rhaBAD} promoter of the *rhaBAD* operon of *E. coli.* has several advantages. First, P_{rhaBAD} is capable of very high levels of expression.[42] Also, P_{rhaBAD} displays undetectable baseline gene expression in the absence of inducer.[33b, 43] This is particularly crucial for the efficient expression of genes encoding toxic

products; otherwise 'leaky' expression can lead to cellular toxicity, difficulties in generating expression constructs, and selective pressure for mutation of the expressed gene.[44] This system has been successfully applied in the expression of a variety of genes in *E. coli*. and other bacteria[45] and showed commercial value.[46] However, the regulation of L-rhamnose inducible promoters (P_{rhaSR}, P_{rhaBAD}) and metabolism of L-rhamnose give rise to one major limitation of this gene expression system: the level of gene expression decreases quickly and ultimately ceases due to the native metabolism of L-rhamnose and auto-repression.

To overcome this problem, non-metabolized inducers are required. In other promoter systems, several synthetic inducers such as the lactose analogue IPTG[47] and the tetracycline analogue anhydrotetracycline[48] have been utilized to avoid the transient expression. However, to date, no synthetic inducers as L-rhamnose analogues for the *rhaBAD* promoter have been reported.

4.2 Aim



1. Potent inducers for L-rhamnose inducible gene expression system in *E. Coli*
   good induction activity; low toxicity; good water solubility; linear kinetic; non-metabolized;
2. Potent inhibitors of L-rhamnosidase, L-rhamnose isomerase and other glycosidases

Figure 4.7 Synthetic targets for biological assays

i) There are no reports of synthetic L-rhamnose analogues as inducers of the L-rhamnose operon. The efficient synthesis of a series of 6-deoxy-hexoses and their substituted analogues from L-rhamnose **4.1** and 6-deoxy-D-gulonolactone **4.2** were developed to investigate their induction effects on L-rhamnose operon. An ideal inducer should have similar properties to L-rhamnose: powerful induction effect, low toxicity, water solubility and is readily taken up by cells. A linear response to concentrations between low basal to strong maximal expression is also required. More importantly, unlike L-rhamnose, synthetic analogues are required to persist in cells to induce a sustained gene expression. This required inducers that can interact with RhaR and RhaS but are inert to L-rhamnose metabolic enzymes. 6-Deoxy-hexoses and their analogues are structurally related to L-rhamnose are a prospective candidate pool.

ii) At the next stage, a number of trideoxy-1,5-iminohexitols, trideoxy-1,4-iminohexitols, trideoxy-2,5-iminohexitols were prepared from L-rhamnose. They were subjected to the evaluation of their inhibitory effects towards rhamnose isomerase, rhamnosidase and other glycosidases. As mentioned in the introduction, rhamnosidase and rhamnose isomerase catalyze the first two steps in the metabolism of L-rhamnose in pathogenic bacteria. These two enzymes might be potential therapeutic targets. Several piperidine and pyrrolidine iminosugars are well-established inhibitors of L-rhamnosidase but there are no reports of studies on the inhibition of rhamnose isomerase. Therefore the effects of iminosugar mimics of both rhamnose and rhamnulose have been evaluated against rhamnosidase and rhamnose isomerase.

To access deoxy iminosugars, Wong *et al.* reported a general strategy to access six and five ring iminosugars using a distereoselective palladium mediated reductive amination of azido ketoses or aldoses.[49] An efficient route to RHJ **4.16** and related iminosugars involving bromination and deoxygenation of D-gulonolactone, the introduction of azide and ring closure has been described.[16] On the basis of the results in Chapter 3, 6-deoxy-D-gulono-1,4-lactone **4.2** was prepared on a multi-gram scale with only extraction purification. In this chapter, a modified ring closure procedure was developed to access all iminosugar targets from L-rhamnose **4.1** and 6-deoxy-D-gulono-1,4-lactone **4.2**.

4.3 Results and discussion

4.3.1 Chemical synthesis

The chemical synthesis will be introduced in the following three sections: i) synthesis of 6-deoxy hexoses; ii) synthesis of trideoxy-1,5-iminohexitols and trideoxy-1,4-iminohexitols as L-rhamnose analogues; iii) synthesis of trideoxy-2,5-iminohexitols as L-rhamnulose analogues.

4.3.1.1 Synthesis of 6-deoxy hexoses

Figure 4.8 shows the initial plan of accessing various 6-deoxy-hexoses analogues. The treatment of L-rhamnose **4.1** with bromine in water gave L-rhamnonolactone. Subsequent protection of L-rhamnonolactone by benzylidene and acetonide afforded **4.22** and **4.33** respectively. Their scalable syntheses have been previously reported.[20a, 50] From the benzylidene protected lactone **4.22**, L-quinovose **4.23**, L-olivose **4.24** and their 2-substituted analogues (-N_3, -NH_2, -NHAc, -F) **4.25** – **4.32** were synthesized. From acetonide protected lactone **4.33**, inversion and double inversion of hydroxyl groups at C-4 and C-5 gave all 4- and 5-epimers of L-rhamnose **4.1**: 6-deoxy-D-gulose **4.34**, 6-deoxy-D-allose **4.35** and 6-deoxy-L-talose **4.36**. In addition, 6-deoxy-L-altrose **4.37** and 1-deoxy-L-fructose **4.38** were provided by biotechnological approaches. Route B and C were completed by Prof Ramon Estevez's and Prof Ken Izumori's group respectively. The details of those two routes are not included in this thesis.

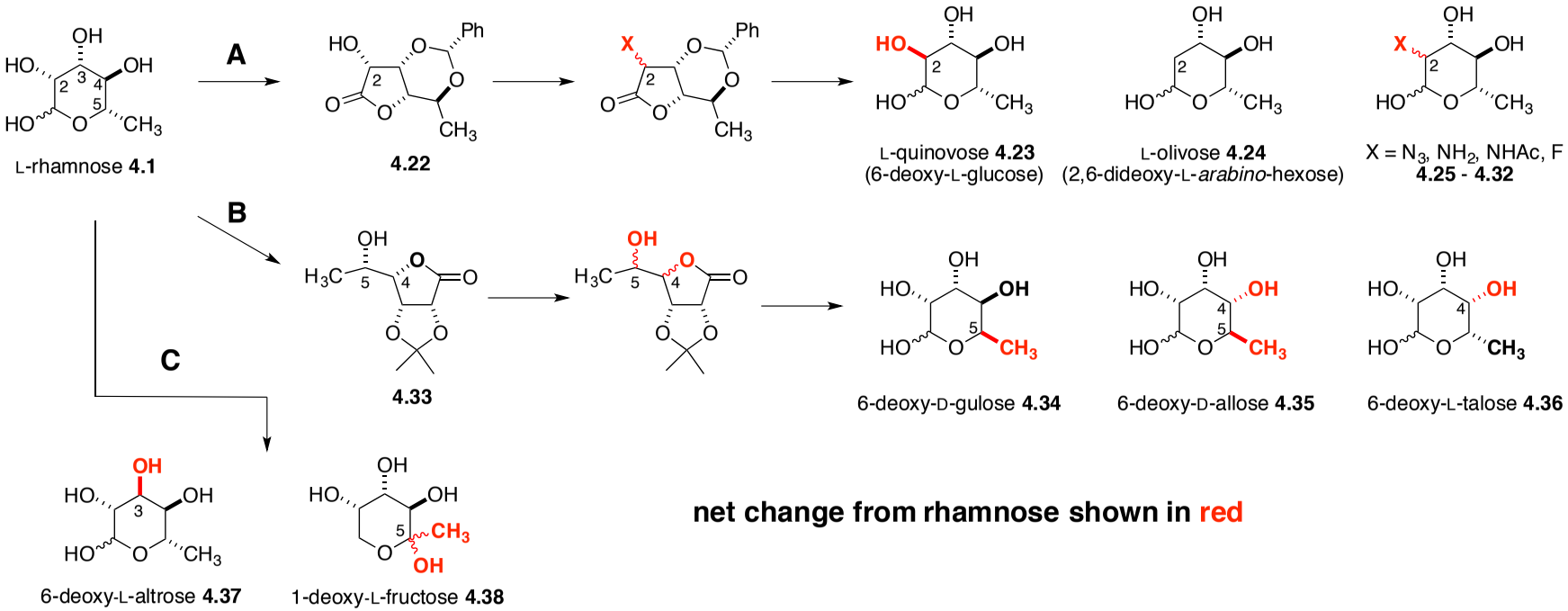
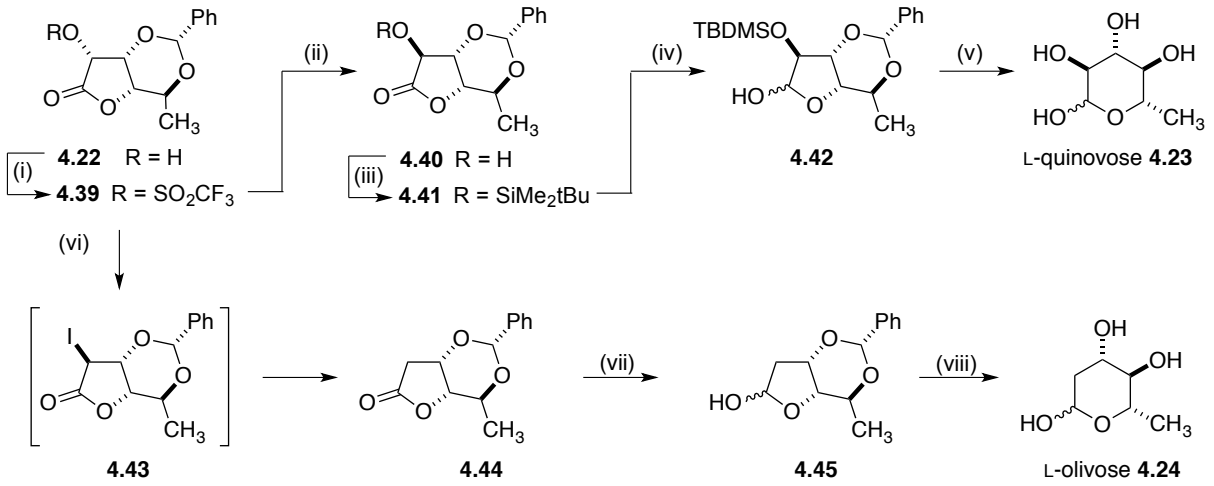


Figure 4.8 Synthesis of 6-deoxy hexoses from L-rhamnose

i) L-Quinovose (6-deoxy-L-glucose) 4.23 and L-olivose (2,6-dideoxy-L-*arabino*-hexose) 4.24

First, the esterification of benzylidene lactone **4.22** (Scheme 4.1) by triflic anhydride in the presence of pyridine gave a stable triflate **4.39**[50b] (95%) which was subjected to the further transformation to other 2-substituted analogues. The treatment of triflate **4.39** with caesium trifluoroacetate with heating in butanone efficiently afforded the *gluco*-lactone **4.40** in a yield of 90%. Reduction of **4.40** to the corresponding lactol by DIBALH in dry THF at -78 °C was not efficient: excess DIBALH (>3 eq) was required and low yields were obtained (44 - 50%); it was necessary to protect the hydroxyl group at C5. Accordingly, *gluco*-lactone **4.40** was treated with tert-butyldimethylsilyl chloride and imidazole to afford fully protected *gluco*-lactone **4.41** (71%). Subsequent reduction of **4.41** by DIBALH in DCM at -78 °C gave the lactol **4.42** in a good yield (88%) with a NMR α:β ratio of 5:4. Subsequent hydrolysis by Dowex resin (H^+ form) in water gave L-quinovose (6-deoxy-L-glucose) **4.23** in an overall yield of 53% (from benzylidene L-rhamnolactone **4.22**). Its NMR spectra were identical with that of L-quinovose synthesized in Chapter 3.



(i) $(CF_3SO_2)_2O$, pyridine, THF, -20 °C, 95%; (ii) CF_3COOCs, butanone, 60 °C, 90%; (iii) TBDMSCl, imidazole, DMF, 71%; (iv) DIBALH, DCM, -78 °C, 88%; (v) DOWEX® 50WX8-200, water, rt, 100%; (vi) LiI:$3H_2O$, butanone, 60 °C, 74%; (vii) DIBALH, DCM, -78 °C, 96%; (viii) DOWEX® 50WX8-200, water, rt, 100%

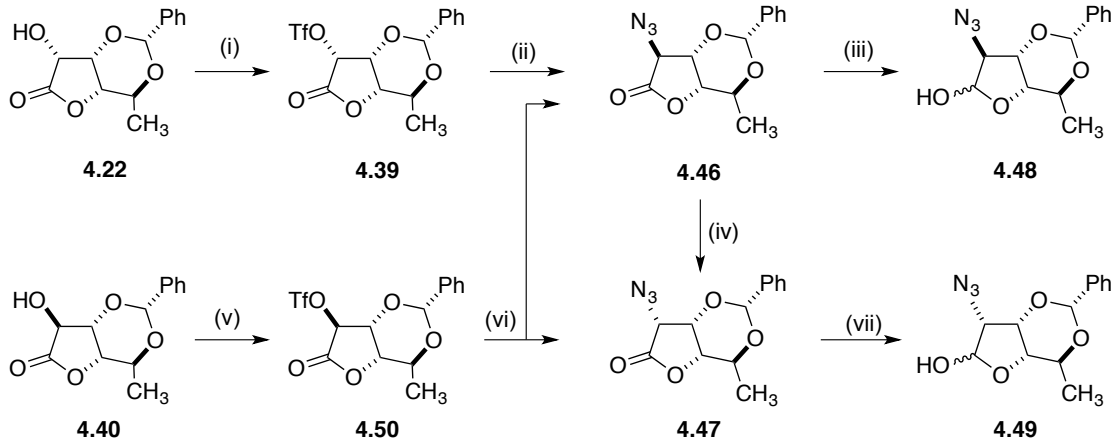
Scheme 4.1 Synthesis of L-quinovose **4.23** and L-olivose **4.24**

The effective deoxygenation of the triflate esters of α-hydroxylactones by lithium iodide trihydrate has been reported.[51] The treatment of triflate **4.39** with lithium iodide hydrate in butanone at 60 °C afforded 2-deoxy lactone **4.44** in a good yield of 74%. Subsequent reduction of **4.44** by DIBALH in DCM at -78 °C gave lactol **4.45** (96%, α:β ratio 10:1). Unprotected L-olivose **4.24** was obtained by the hydrolysis of lactol **4.45** in an overall yield of 67% (from lactone **4.22**) (Scheme 4.1).

ii) 2-Amino/2-acetamido substituted L-rhamnose and L-quinovose

It has been reported that azide displacement of the triflate ester of an α-hydroxy lactone may result in substitution with either retention or inversion of configuration and normally the azide with all *cis* substitutions is the thermodynamic product.[52] Similarly, initial S_N2 substitution of the triflate **4.39** with sodium azide in DMF at -30 °C for 6 hours gave the *gluco*-azide **4.46** as the kinetic product (75%) (Scheme 4.2). In contrast, the treatment of **4.39** with sodium azide in DMF at room temperature for 16 hours gave the *manno*-azide **4.47** isolated as the thermodynamic product in a yield of 85%. The initially formed *gluco*-azide **4.46** could be

converted to the more stable *manno*-azide **4.47** in the presence of sodium azide in DMF at room temperature. The two azides **4.46** and **4.47** were readily identifiable by TLC and easily separable by column chromatography. Their configurations were confirmed by nOe analysis. Additionally, the two azides **4.46** and **4.47** were synthesized by an alternative approach. The *gluco*-triflate **4.50** which was prepared from the triflation of lactone **4.40** was less stable than *manno*-triflate **4.39**.[50b] Subsequent substitution of the triflate **4.50** with sodium azide at -20 °C in DMF gave both *manno*-azide **4.47** (70%) and *gluco*-azide **4.46** (24%). This may be rationalized by the competition between azide displacement to give **4.47** and epimerization of triflate **4.50** to the more stable triflate **4.39** which then underwent substitution to afford the less stable *gluco*-azide **4.46**. Reductions of *gluco*-azide **4.46** and *manno*-azide **4.47** with DIBALH afford the *gluco*-lactol **4.48** (α:β ratio 5:4) and *manno*-lactol **4.49** (α:β ratio 2:1) in yields of 84% and 87% respectively (Scheme 4.2).

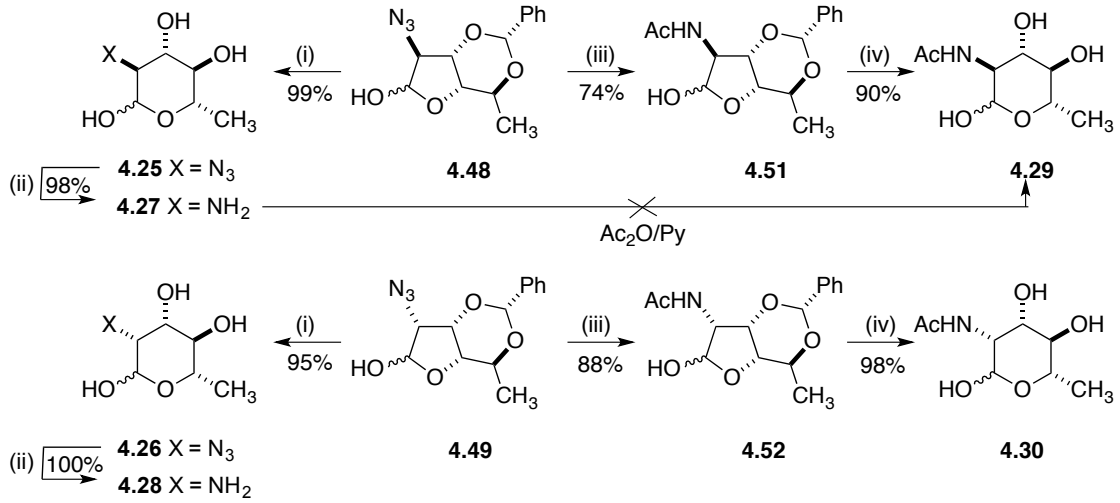


(i) $(CF_3SO_2)_2O$, pyridine, THF, -20 °C, 95%; (ii) NaN_3, DMF, -30 °C, 6 h, 75%; (iii) DIBALH, DCM, -78 °C, 3 h, 84%; (iv) NaN_3, DMF, rt, 16h, 85% from **4.39**; (v) $(CF_3SO_2)_2O$, pyridine, THF; -20 °C (vi) NaN_3, DMF, -40 °C, 70% **4.47** and 24% **4.46** (2 steps); (vii) DIBALH, CH_2Cl_2, -78 °C, 87%

Scheme 4.2 Synthesis of *gluco*-azide **4.46** as kinetic control product and *manno*-azide **4.47** as thermodynamic control product

Direct hydrolysis of azido lactols **4.48** and **4.49** by Dowex resin (H^+ form) in water gave the free 2-azido-2-deoxy-L-quinovose **4.25** (59% from lactone **4.22**) and 2-azido-2-deoxy-L-rhamnose

4.26 (67% from lactone **4.22**). Subsequent hydrogenations of **4.25** and **4.26** under a hydrogen atmosphere gave the crude products with messy NMR spectra. The addition of HCl (2M, aq) during the hydrogenation stabilized the 2-amino sugars as the hydrochloride salts of 2-amino-2-deoxy-L-quinovose **4.27** and 2-amino-2-deoxy-L-rhamnose **4.28** (Scheme 4.3).[53] Several attempts to prepare 2-acetamido targets **4.29** and **4.30** failed. The treatment of unprotected 2-amino sugar **4.27** by a mixture of acetic anhydride/pyridine gave complex products. There were previous reports on the *N*-acetylation of unprotected amino sugars while complex procedures were required.[54] Alternatively, the hydrogenation of azido *gluco*-lactols **4.48** and subsequent treatment of the crude residue with acetic anhydride and pyridine gave an unidentifiable mixture. Pyridine, as a base or nucleophile, can give rise to unwanted reactions e.g. oligomerization of 2-amino lactols. Hydrogenation of 2-azido lactols **4.48** and **4.49** in presence of acetic anhydride (1 eq) in ethyl acetate/1,4-dioxane effectively gave 2-acetamido lactols **4.51** (74%) and **4.52** (88%) without the formation of diacetates. Subsequent hydrolysis by DOWEX® 50WX8-200 resin in water gave 2-acetamido-L-quinovose **4.29** (42% from **4.22**) and 2-acetamido-L-rhamnose **4.30** (64% from **4.22**) (Scheme 4.3).

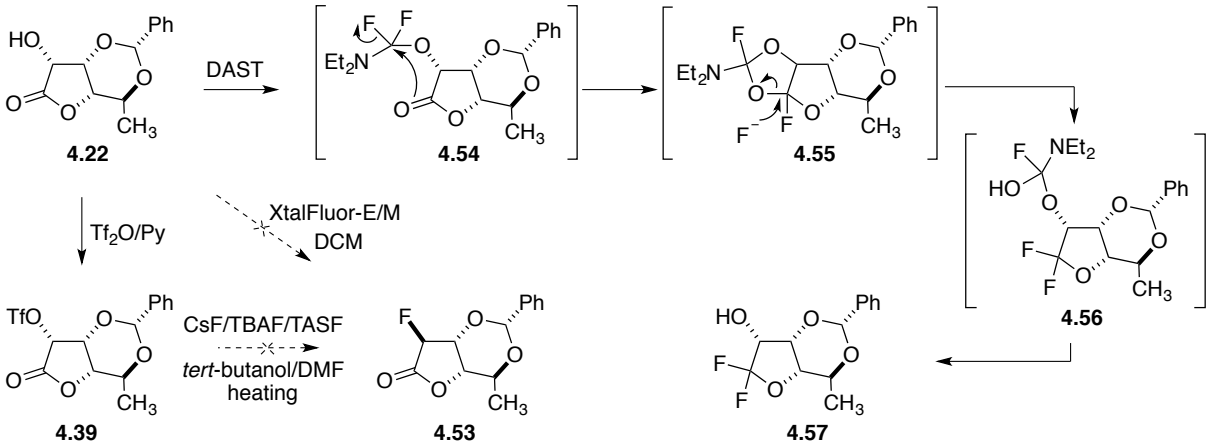


(i) DOWEX® 50WX8-200, water, rt; (ii) H_2, 10% Pd/C, HCl; (iii) H_2, 10% Pd/C, Ac_2O, EtOAc/1,4-dioxane; (iv) DOWEX® 50WX8-200, water

Scheme 4.3 Synthesis of 2-azido, 2-amino-, 2-acetamido-L-quinovose/L-rhamnose **4.25-4.30**

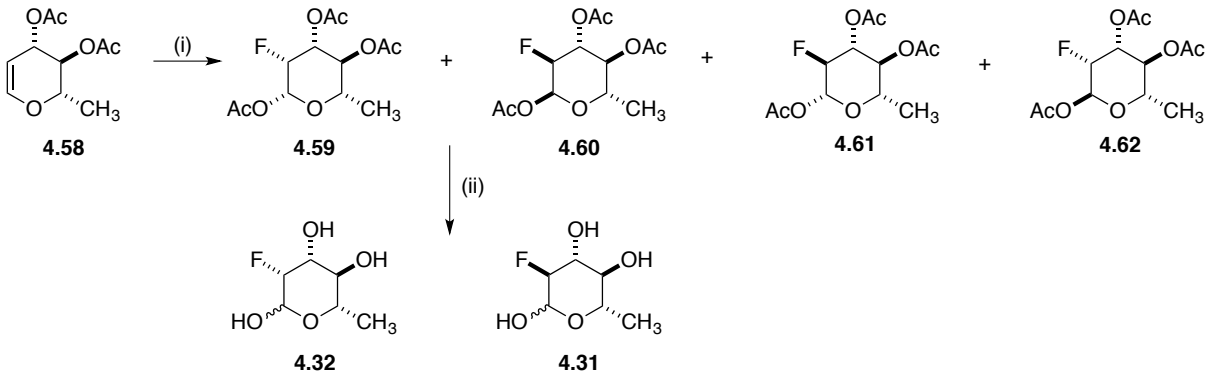
iii) 2-Fluoro substituted L-rhamnose and L-quinovose

Initial attempts of the displacement of **4.39** to 2-fluoro lactone **4.53** by any of a number of nucleophilic sources of fluoride (including TASF, TBAF and CsF) failed. The treatment of *manno*-lactone **4.22** with Xtalfluor-E or Xtalfluor-M (Chapter 2) in DCM gave only complex residues. Alternatively, the treatment of alcohol **4.22** with diethylaminosulfur trifluoride (DAST) afforded difluoride **4.57** in a yield of 50% with inseparable impurities. This observation could be explained a mechanism involving the participation of neighboring carbonyl group (Scheme 4.4).[55]



Scheme 4.4 Attempted synthesis of 2-fluoro lactone **4.53** with various fluorinating reagents

2-deoxy-2-fluoro-D-glucose and 2-deoxy-2-fluoro-D-mannose have been prepared by the reaction of 3,4,6-*O*-triacetyl-D-glucal with SelectFluor.[56] By an analogous strategy, the treatment of commercial available 3,4-*O*-diacetyl-L-rhamnal **4.58** with SelectFluor in acetonitrile and subsequent acetylation of the resulting residue with acetic anhydride/pyridine gave a mixture of the four sugar triacetates **4.59** - **4.62** (Scheme 4.5). A mixture of **4.59** and **4.60** was isolated by flash column chromatography in a yield of 38%. One pure triacetate was recrystallized from the mixture of **4.59** and **4.60** as a pure crystalline solid which was identified as the acetylated 2-deoxy-2-fluoro-L-rhamnose **4.59** by NMR analysis (27% from **4.59**). Hydrolysis of **4.59** in aqueous acid gave a pure sample of previously unknown 2-deoxy-2-fluoro-L-rhamnose **4.32** (100% from **4.59**, 27% from **4.58**) for biological evaluation. Alternatively, hydrolysis of the mixture of **4.59** and **4.60** gave a mixture of fluoro-rhamnose **4.32** and fluoro-quinovose **4.31**; a pure sample of fluoro-quinovose **4.31** was obtained by preparative HPLC.



(i) SelectFluor, water, MeCN; Ac_2O/pyridine, 27% of **4.59** from recrystallization; (ii) CF_3COOH/water 1:1, 100%

Scheme 4.5 Synthesis of **4.11** and **4.12** from 3,4-*O*-diacetal-rhamnal **4.36**

iv) NMR studies of 2-substituted L-rhamnose analogues/L-quinovose

Ten 6-deoxy-hexose targets were effectively synthesized from benzylidene protected L-rhamnonolactone **4.22**. Table 4.1 and Table 4.2 showed the anomeric ^1H, ^{13}C shifts and coupling constant of 6-deoxy-hexose targets. Deuterium oxide was used as the NMR solvent. The pyranose tautomers were the predominant form (>95% NMR ratio) of the ten 6-deoxy-hexoses according to the shifts of the ^{13}C NMR peaks of anomeric carbon (δ = 93 – 97 ppm); very little of furanose anomers, whose anomeric carbon was generally in lower field,[57] were present in any of the derivatives. In the L-*gluco*-series (**4.23**, **4.25**, **4.27**, **4.29**) and L-olivose **4.24**, because of the *trans*-axial-axial configuration (Figure 4.9), the ^1H NMR peaks of β-anomeric protons were at higher field and showed larger coupling constants ($J_{1,2}$ = 8 - 9 Hz) compared with their α counterparts ($J_{1,2}$ = 3 - 4 Hz) (Table 4.1, Table 4.2). In contrast, in the L-*manno*-series, the magnitude of the coupling constants of anomeric protons were similar due to the *trans*-equatorial-equatorial or *cis*-equatorial-axial configurations ($J_{1,2}$ = 0 - 2 Hz) (Table 4.2), while the ^1H peaks of α-anomers were in lower field than their β counterparts. These observations fitted the reports on the NMR analysis of glucose and mannose.[58]

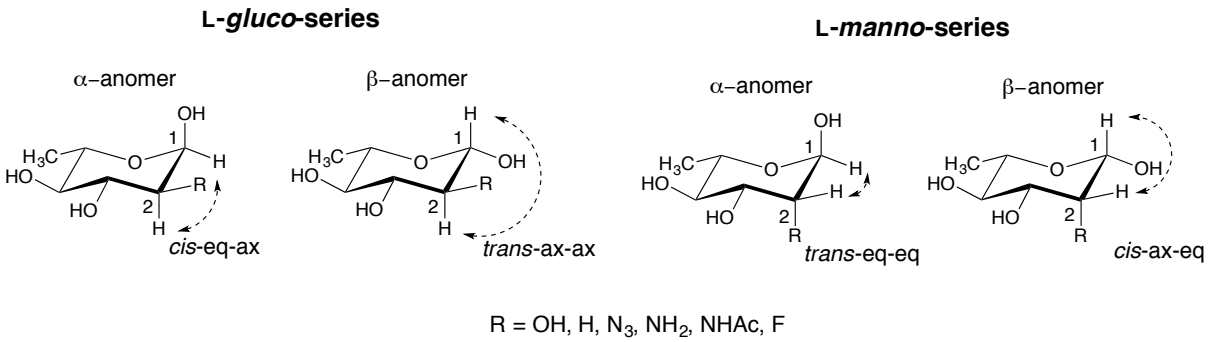
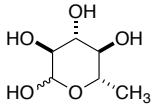
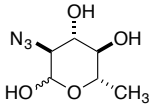
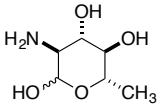
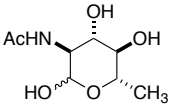
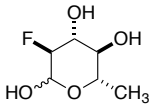


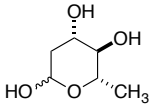
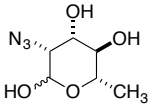
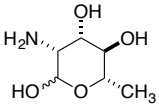
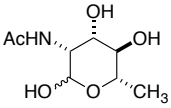
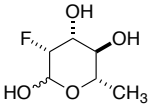
Figure 4.9 Conformations of 2-substituted L-quinovose and L-rhamnose

Table 4.1 NMR comparison of anomeric protons of 6-deoxy-hexoes (1)

	L-*gluco*-series				
					
	4.23	**4.25**	**4.27**	**4.29**	**4.31**
H1α/$J_{1,2}$	5.10[a]/3.8[b]	5.29/3.7	5.38/3.5	5.06/3.5	5.38/4.0
C1α	93.2	91.6	89.5	91.0	90.0
H1β/$J_{1,2}$	4.55/7.9	4.67/8.1	4.91/8.4	4.59/8.5	4.87/7.9
C1β	96.9	95.5	93.1	95.4	93.8

[a]: ppm; [b]: Hz.

Table 4.2 NMR comparison of anomeric protons of 6-deoxy-hexoses (2)

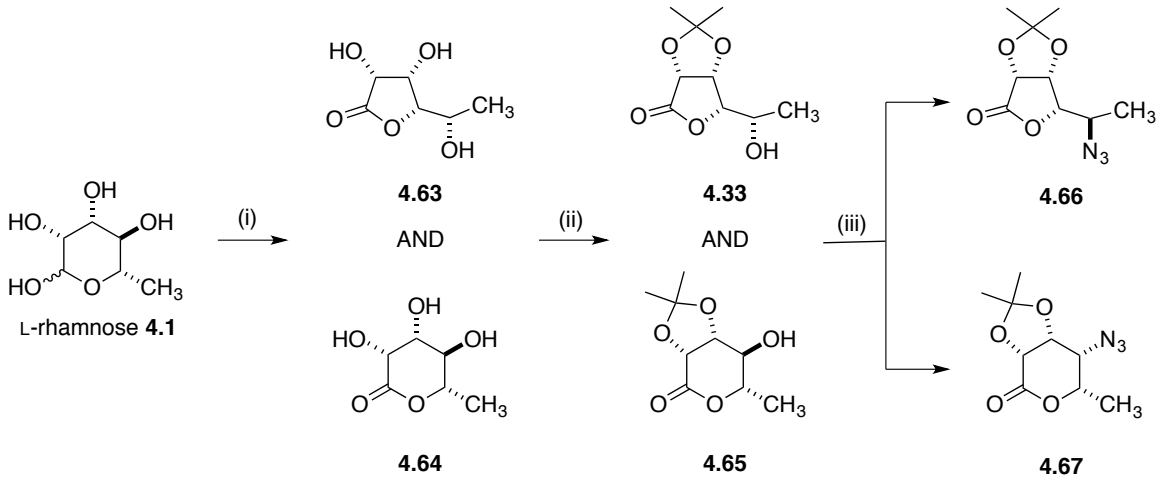
	L-*manno*-series				
					
	4.24	**4.26**	**4.28**	**4.30**	**4.32**
H1α/$J_{1,2}$	5.31[a]/3.7[b]	5.20/1.1	5.32/1.2	4.85/1.1	5.30/1.8
$J_{1,2'}$	1.0	*NA*	*NA*	*NA*	*NA*
C1α	91.7	92.7	90.8	94.8	91.8
H1β/$J_{1,2}$	4.91/9.8	5.01/1.1	5.17/1.5	4.72/1.5	4.98/0
$J_{1,2'}$	2.0	*NA*	*NA*	*NA*	*NA*
C1β	93.8	93.4	91.3	95.0	92.7

[a]: ppm; [b]: Hz.

4.3.1.2 Synthesis of trideoxy-1,5-iminohexitols and trideoxy-1,4-iminohexitols as L-rhamnose analogues

As discussed, a strategy involving the introduction of azide to γ-lactone and ring closure by reductive amination access to 6-membered ring iminosugar targets including RHJ **4.16** and 5-*epi*-RHJ **4.6** has been used previously.[16, 20a] In this project, a modified approach was employed to form different 5- and 6-membered ring iminosugars using L-rhamnose **4.1** and 6-deoxy-D-gulono-1,4-lactone **4.2** as starting materials. Additionally, a ring contraction strategy was successfully employed to transfer piperidine iminosugars to pyrrolidine iminosugars.

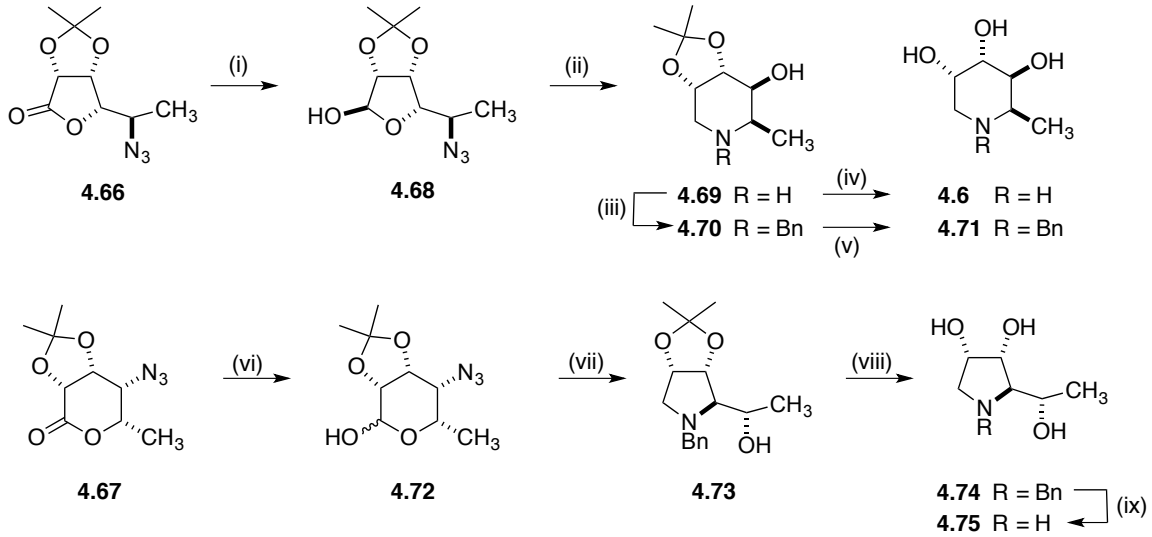
First, the oxidation of L-rhamnose in bromine water gave a mixture of γ-lactone **4.63** and δ-lactone **4.64** (Scheme 4.6). Although pure γ-lactone **4.63** could be obtained as the thermodynamic product by stirring the lactone mixture in aqueous acid at high temperature and recrystallization,[20a, 50a, 50b] the lactone mixture was treated directly with acetone in the presence of H_2SO_4 and anhydrous $CuSO_4$ to afford an inseparable mixture of acetonide protected γ-lactone **4.33** and δ-lactone **4.65** with a NMR ratio of 5:1 (total yield 60%). There was no advantage in using pure γ-lactone **4.63** since this gave the same mixture under the same protection condition. Subsequent triflation of the lactone mixture and S_N2 displacements of the resulting triflates by sodium azide in DMF gave two azides **4.66** and **4.67** with very different polarities. The efficient separation of 5-azido-γ-lactone **4.66** (60% from the mixture of **4.33**/**4.65**) and 4-azido-δ-lactone **4.67** (18% from the mixture of **4.33**/**4.65**) was achieved by simple column chromatography.



(i) Br_2, water; (ii) *conc* H_2SO_4, acetone, $CuSO_4$, 60% crude mixture; (iii) $(CF_3SO_2)_2O$, Pyridine, DCM, -20 °C, then NaN_3, DMF, rt, 60% **4.66** from **4.33**/**4.65** mixture, 18% **4.67** from **4.33**/**4.65** mixture;

Scheme 4.6 Synthesis of 5-azido-γ-lactone **4.66** and 4-azido-δ-lactone **4.67**

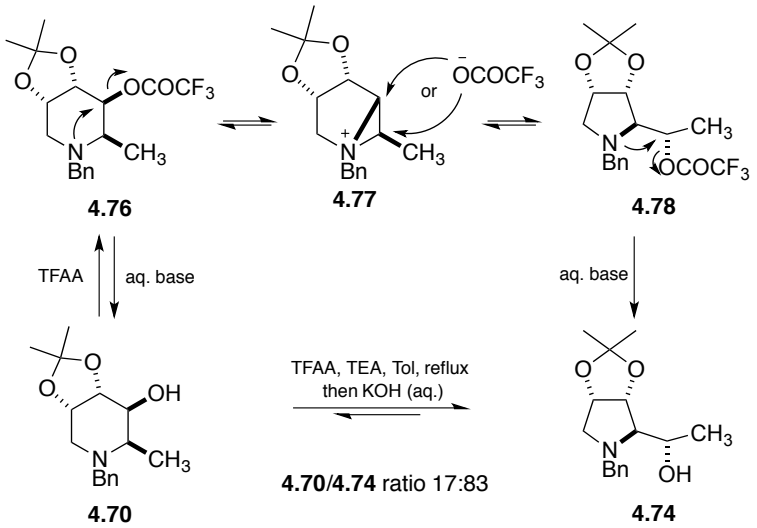
In the next stage, the reduction of 5-azido-γ-lactone **4.66** by DIBALH in DCM gave lactol **4.68** as a single α-anomer (92%, confirmed by nOe analysis). The hydrogenation of **4.68** catalyzed by palladium black afforded acetonide protected 1,5-imino-1,5,6-trideoxy-D-gulitol **4.69** (100%). Subsequent hydrolysis of **4.69** in aqueous acid yielded 1,5-imino-1,5,6-trideoxy-D-gulitol (5-*epi*-RHJ) **4.6** (92% from **4.66**). The reductive amination of **4.69** with benzaldehyde and sodium cyanoborohydride followed by acid hydrolysis generated *N*-benzyl-5-*epi*-RHJ **4.71** (89% from **4.66**). Similarly, 1,4-imino-1,4,6-trideoxy-D-talitol **4.75** and its *N*-benzyl analogue **4.74** were obtained from **4.67** in overall yields of 57% and 58% respectively.



(i) DIBALH, DCM, -78 °C, 92%; (ii) Pd black, H_2, EtOH, 100%; (iii) BnCHO, $NaCNBH_3$, MeOH, 97%; (iv) TFA/water, 100%; (v) TFA/water, 100%; (vi) DIBALH, DCM, -78 °C, 76%; (vii) Pd black, H_2, EtOH, then BnCHO, $NaCNBH_3$, MeOH, 86% (2 steps); (viii) TFA/water, 89%; (ix) Pd/C, H_2, 1,4-dioxane/water, 98%;

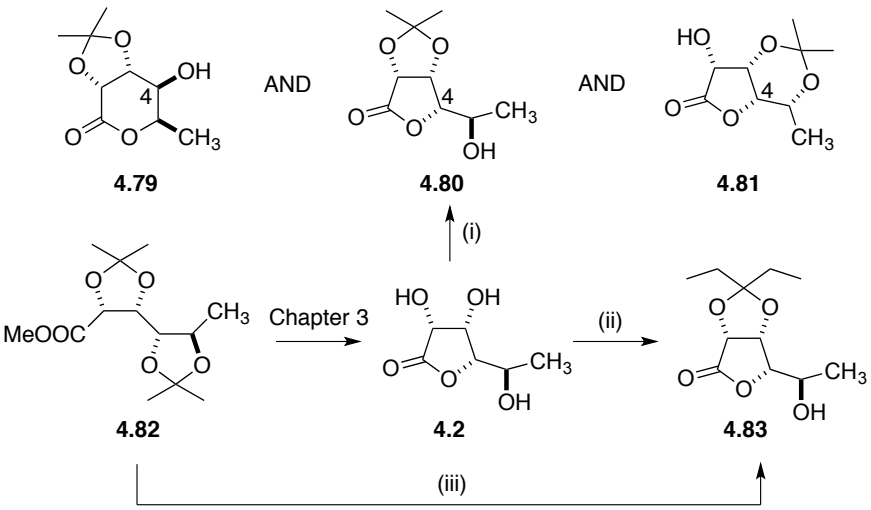
Scheme 4.7 Synthesis of 5-*epi*-RHJ **4.6**, 1,4-imino-1,4,6-trideoxy-D-tallitol **4.75**

Alternatively, a ring contraction route was developed to access pyrrolidine **4.74** from piperidine **4.70** (Scheme 4.8). The treatment of **4.70** with trifluoroacetic anhydride (TFAA) in the presence of triethylamine in toluene at reflux for 3 hours generated an equilibration between piperidine ester **4.76** and pyrrolidine ester **4.78** *via* a bicyclic aziridinium ion **4.77**. Subsequent treatment with aqueous base hydrolyzed the trifluoroacetate mixture to give a mixture of piperidine **4.70** and pyrrolidine **4.74** with a NMR ratio of 17:83. Longer heating did not change the ratio. The ring expansion of pyrrolidine to piperidine using different leaving groups to form aziridinium intermediates has been well studied.[59] However this was a rare case of the equilibrium favoring the five membered ring as the thermodynamically stable product.



Scheme 4.8 A ring contraction strategy generated a mixture of **4.70** and **4.74**

In an analogous strategy, the synthesis of the 5-epimers of **4.6** and **4.75** required 6-deoxy-D-gulono-1,4-lactone **4.2** as starting material. Initial attempts of its protection in acetone with H_2SO_4 and anhydrous $CuSO_4$ at room temperature were not efficient: three acetonides **4.79**, **4.80** and **4.81** were isolated in the yields of 7%, 35% and 24% respectively (Scheme 4.9). The shifts of their quaternary carbons in ^{13}C NMR spectra were 110.8 ppm (**4.79**), 113.4 ppm (**4.80**) and 99.8 ppm (**4.81**). And the shifts of C-4 were 76.5 ppm (**4.79**), 84.1 ppm (**4.80**) and 83.5 ppm (**4.81**), suggesting **4.80** and **4.81** were 1,4-lactones. In addition, the ratio of the three products did not change even when the reaction mixture was refluxed overnight (18 hours), suggesting a more efficient protection strategy was required. The ketals from 3-pentanone were reported to show better 1,2-diol selectivity than that from acetone.[60] Accordingly, the protection of lactone **4.2** in 3-pentanone in the presence of H_2SO_4 and anhydrous $CuSO_4$ at 50 °C gave 2,3-protected γ-lactone **4.83** in a better yield of 65%. Additionally, diacetonide methyl ester **4.82** (Chapter 3), as the precursor of **4.2**, was treated under the same conditions to directly give the **4.83** in a yield of 62%.

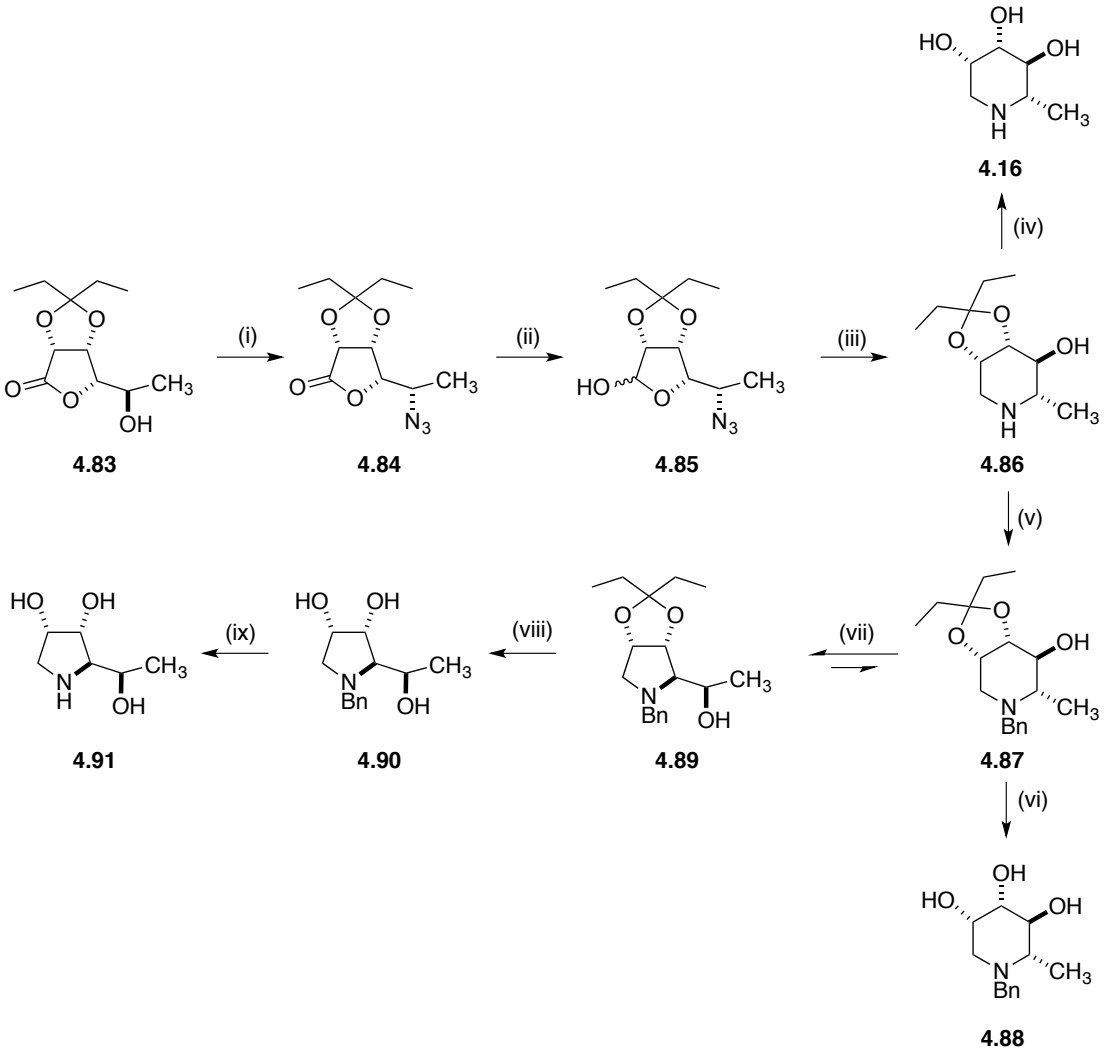


(i) acetone, H_2SO_4, $CuSO_4$, **4.79** 7%, **4.80** 35%, **4.81** 24%; (ii) 3-pentanone, H_2SO_4, $CuSO_4$, 50 °C, 65%; (iii) 3-pentanone, H_2SO_4, $CuSO_4$, 50 °C, 62%

Scheme 4.9 Attempted protection of 6-deoxy-D-gulono-1,4-lactone **4.2**

The esterification of **4.83** by triflic anhydride and pyridine and subsequent treatment of resulting triflate with sodium azide in DMF gave azido lactone **4.84** (Scheme 4.10) in a yield of 59% (2 steps). The reduction of lactone **4.84** by DIBALH afforded lactol **4.85** quantitatively with a α/β ratio of 20:1. Then the hydrogenation of lactol **4.85** led to ring closure to protected piperidine **4.86**; hydrolysis of **4.86** in aqueous acid gave RHJ **4.16** in an overall yield of 38% (from lactone **4.83**). Protected *N*-benzyl-RHJ **4.87** was synthesized by the reductive amination of protected piperidine **4.86** with benzaldehyde and sodium cyanoborohydride in methanol (79%). Unprotected *N*-benzyl-RHJ **4.88** was obtained by the aqueous hydrolysis of **4.87** in an overall yield of 29% (from lactone **4.83**). The *N*-benzyl piperidine **4.87** was subject to the ring contraction procedures (trifluoroacetic anhydride in toluene at reflux, followed by treatment with aqueous NaOH) to generate a separable mixture of piperidine **4.87** and pyrrolidine **4.89** with a NMR ratio of 12:82. An efficient flash column chromatography gave **4.89** in a yield of 70%. Subsequent removal of the ketal protecting group of **4.89** and hydrogenolysis of the benzyl group of resulting **4.90** gave unprotected 1,4-imino-1,4,6-trideoxy-D-allitol **4.91** in a yield of 16%

(from lactone **4.83**).

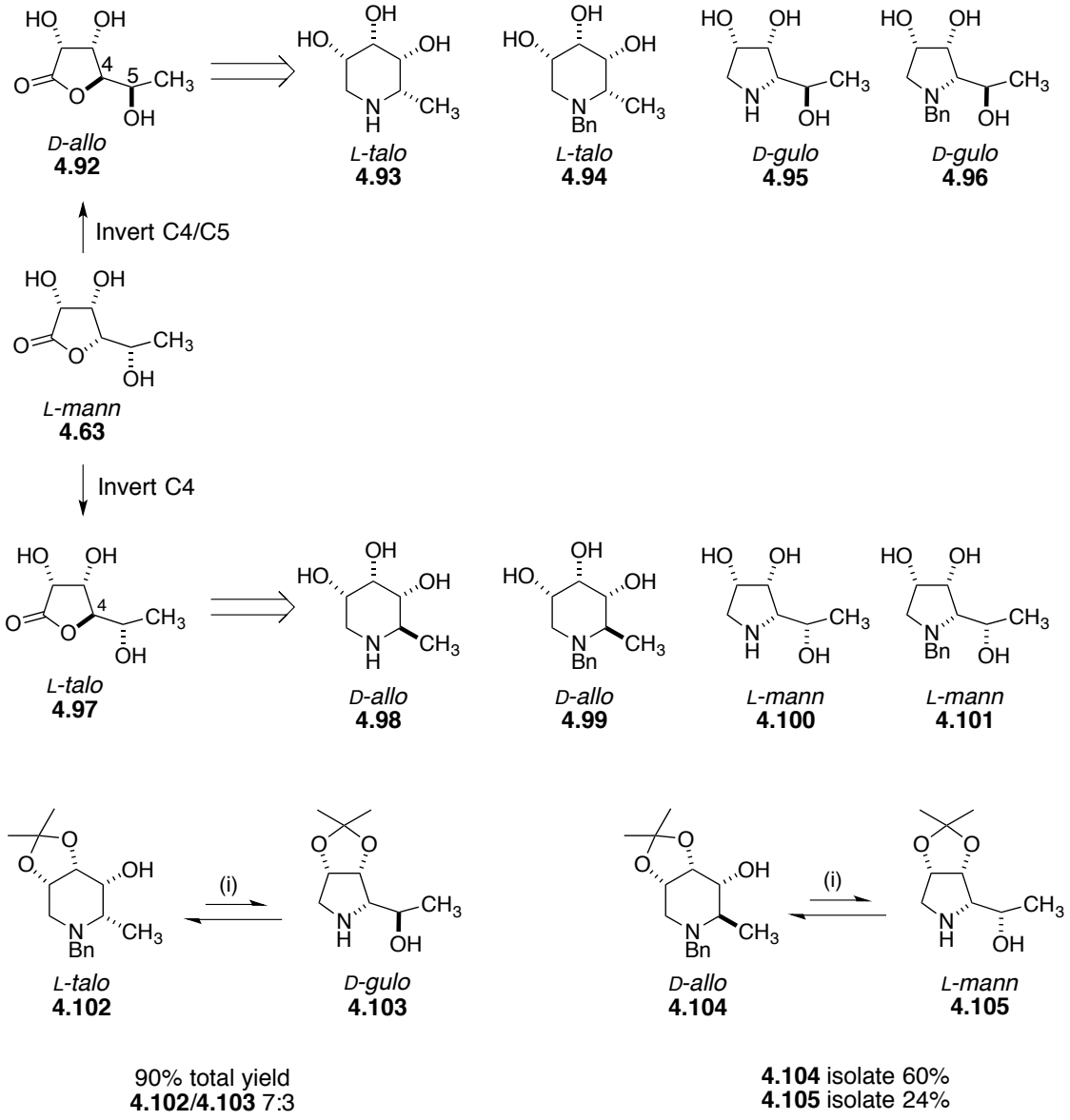


(i) $(CF_3SO_2)_2O$ pyridine, DCM, -20 °C, then NaN_3, DMF, 59% (2 steps); (ii) DIBALH, THF, -78 °C, 100%; (iii) Pd black, H_2, EtOH, 98%; (iv) TFA/water/1,4-dioxane, 98%; (v) BnCHO, $NaCNBH_3$, MeOH, 79%; (vi) TFA/water/1,4-dioxane, 97%; (vii) TFAA, TEA, toluene, relux, then NaOH (aq.), **4.87**/**4.89** NMR ratio 12:82, **4.89** isolate yield 70%; (viii) TFA/water/1,4-dioxane, 82%; (ix) Pd/C, H_2, EtOH, 95%;

Scheme 4.10 Synthesis of RHJ **4.16** and 1,4-imino-1,4,6-trideoxy-D-allitol **4.91**

Our collaborator, Prof Ramon Estevez (University of Santiago de Compostela, Spain), employed an identical strategy in the synthesis of another 9 piperidine/pyrrolidine iminosugar targets from 6-deoxy-D-allono-1,4-lactone **4.92** and 6-deoxy-L-talono-1,4-lactone **4.97** derived from L-rhamnono-1,4-lactone **4.63** (Scheme 4.11). In addition, protected piperidine **4.102** was subjected to the established ring contraction conditions to give an inseparable mixture of

piperidine **4.102** and pyrrolidine **4.103** with a NMR ratio of 7:3 (Scheme 4.11). Similarly, the ring contraction of **4.104** gave **4.104** and **4.105** in the isolate yields of 60% and 24% respectively. In contrast to the ring contractions of **4.70** and **4.87**, piperidine rings were more favorable in these two cases.



(i) TFAA, TEA, toluene, reflux, then NaOH (2M, aq.)

Scheme 4.11 Accessing iminosugar targets **4.93 - 4.101**

4.3.1.3 Synthesis of trideoxy-2,5-iminohexitols as L-rhamnulose analogues

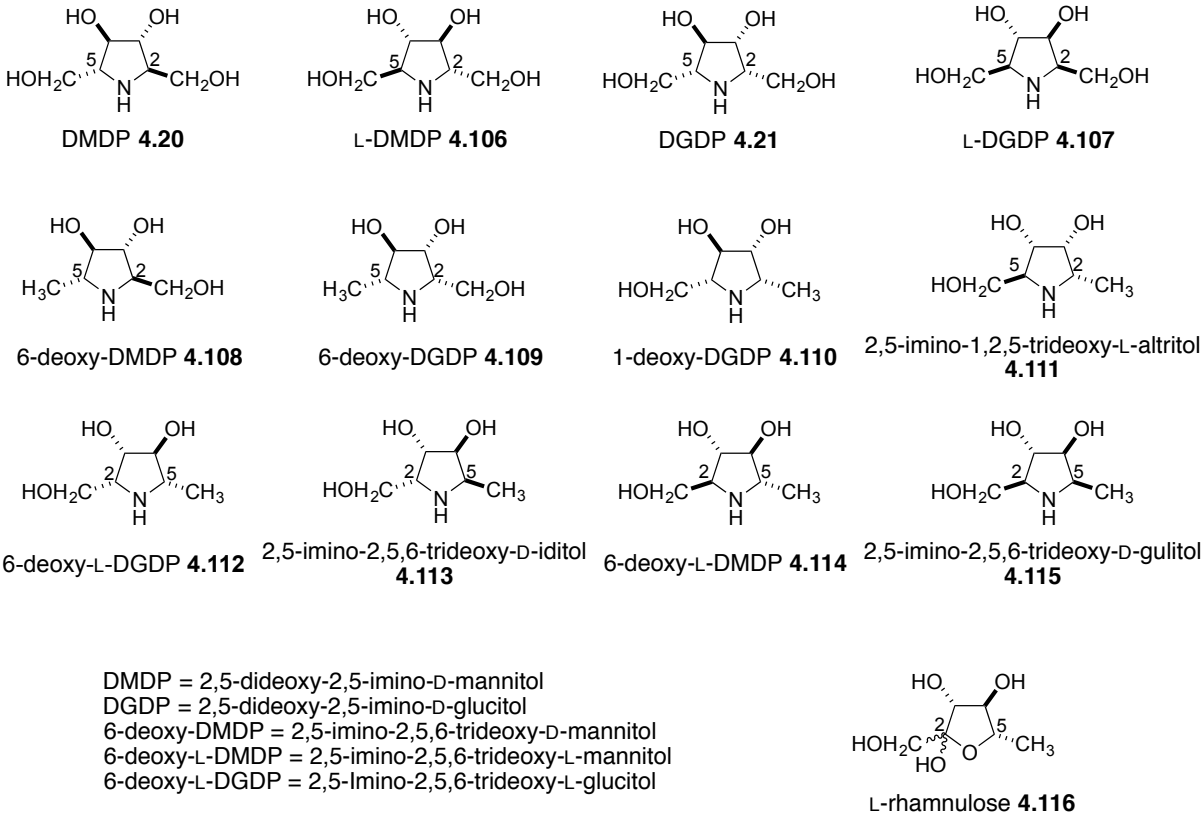


Figure 4.10 Examples of 2,5-iminohexitols[61]

As discussed in the introduction section, many 2,5-iminohexitols, as mimics of corresponding hexuloses, inhibit different glycosidases. Both DMDP **4.20** and DGDP **4.21** are widely distributed in nature[62] and are modest inhibitors of α-glucosidases[63]. In contrast, their unnatural enantiomers L-DMDP[64] **4.106** and L-DGDP[65] **4.107** are stronger sub-micromolar inhibitors of α-glucosidases from various sources.[63] 6-Deoxy-DMDP **4.108** was isolated from the seeds of *Angylocalyx pyraertii* and was a moderate competitive inhibitor of β-mannosidase.[65a] Wong *et al.* reported the synthesis a series trideoxy-2,5-iminohexitols including 6-deoxy-DMDP **4.108**, 6-deoxy-DGDP **4.109**, 1-deoxy-DGDP **4.110** and 1,2,5-trideoxy-2,5-imino-L-altritol **4.111** (Figure 4.10).[49, 66] Compounds **4.109**, **4.110** and **4.111** were potent inhibitors of α-L-fucosidase from bovine kidney with K_i values of 4 μM, 8 μM and 1.4 μM respectively.[65b, 66a, 67] **4.115** also

showed potent inhibition of bovine epididymis α-L-fucosidase.[67a, 68] Their derivatives were also synthesized in the search for potent therapeutic agents.[69] In addition, DGDP **4.21** was previously reported to weakly but competitively inhibit D-xylose isomerase (XI).[23] However, some trideoxy-2,5-iminohexitols such as **4.112**, **4.113** and **4.114** have not been synthesized or studied as glycosidase inhibitors.

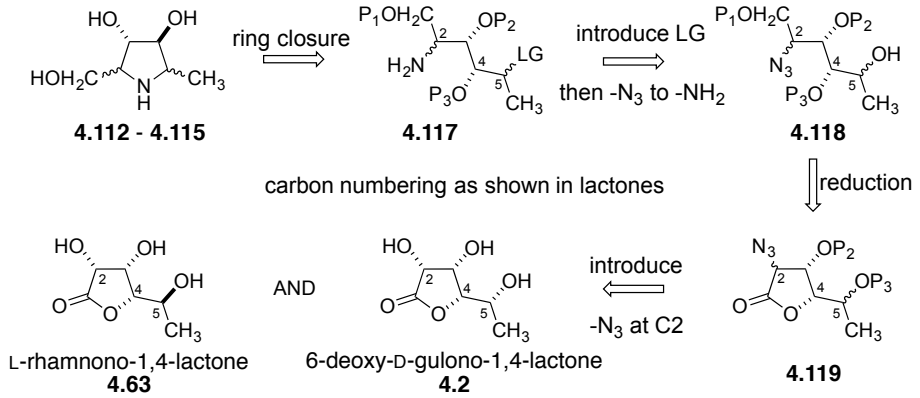
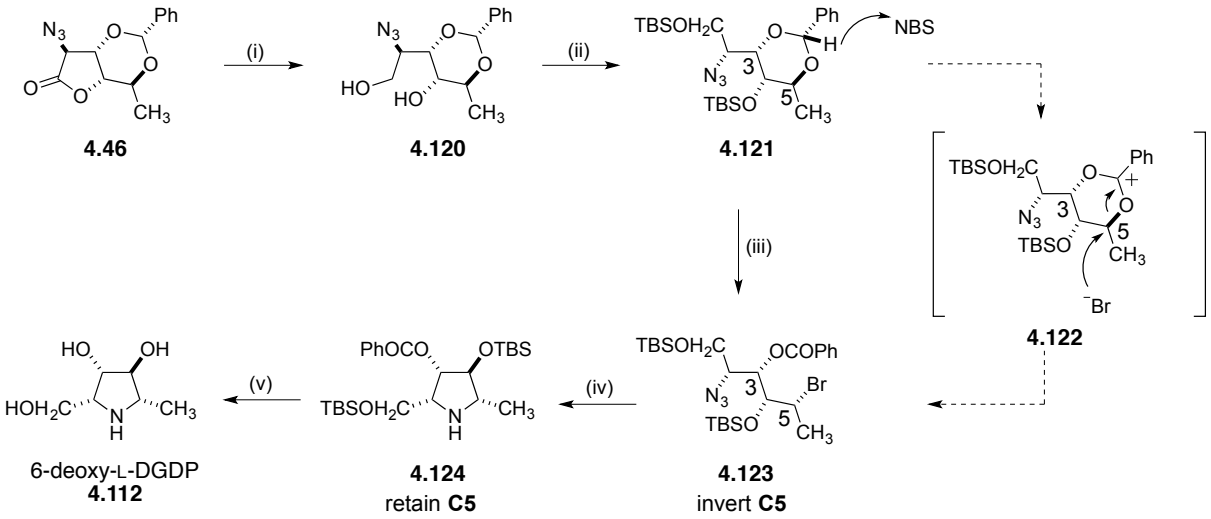


Figure 4.11 Retrosynthetic design of the synthesis of trideoxy-2,5-iminohexitols **4.112** - **4.115**

Trideoxy-2,5-iminohexitol **4.112** - **4.115** could be considered as mimics of α/β-L-rhamnulose **4.116** (Figure 4.10). The strategy adopted for the synthesis of the targets **4.112** - **4.115** from deoxyhexoses involved the introduction of the azido group at C-2 and formation of the pyrrolidine ring by the closure onto C-5. The retrosynthetic route for the four targets is shown in Figure 4.11: i) The formation of the pyrrolidine ring could be achieved by a S_N2 displacement of leaving group at C5 by amine at C-2 in **4.117**; ii) 2-Azido substitution in intermediate **4.118** could be effectively reduced to corresponding 2-amino compound; iii) 2-azido lactone **4.119** could be reduced and subsequent protection of the resulting diol give **4.118**; iv) To access all 2- and 5-epimers of azido lactones **4.119**, L-rhamnono-1,4-lactone **4.63** and 6-deoxy-D-gulono-1,4-lactone **4.2** are required as starting materials.

As described in Scheme 4.2, azido lactones **4.46** and **4.47** have been efficiently synthesized from benzylidene protected L-rhamnonolactone **4.22** by the kinetically/thermodynamically-controlled azide substitutions. First, direct reduction of lactone **4.46** by sodium borohydride in methanol gave no reaction. Alternatively, sequential reduction of **4.46** by DIBALH in DCM and sodium borohydride in methanol afforded diol **4.120** in a yield of 92% (2 steps) (Scheme 4.12). The protection of diol **4.120** by *tert*-butyldimethysilyl chloride (TBSCl) in the presence of imidazole was not efficient. However the treatment of diol **4.120** with *tert*-butyldimethysilyl triflate (TBSOTf) and pyridine in DCM gave the fully protected 2-azido-2,6-dideoxy-L-glucitol **4.121** in a good yield (98%). A Hanessian-Hüllar reaction[70] was performed to generate 2-azido-5-bromo-D-iditol **4.123** with the introduction of bromide with inversion at C-5: the benzylidene acetal **4.121** was treated with *N*-bromosuccinimide (NBS) and barium carbonate in carbon tetrachloride at reflux for 1 hour to form **4.123** in a yield of 86%. The mechanism of Hanessian-Hüllar reaction was still controversial.[70b] In this case, the reaction could be rationalized by a mechanism involving loss of hydride in **4.121** to give an intermediate **4.122** (Scheme 4.12). The C5-regioselectivity of the S_N2 substitution by bromide may be explained since the alternative C-3 attack by bromide in **4.122** was adjacent to both azido and oxygen substituents so that S_N2 reactions at C-3 will be slower than that at C-5. Also, C-5 was less hindered. Hanessian-Hüllar reactions with free diol **4.120** in carbon tetrachloride, or **4.121** with alternative solvents (acetonitrile or toluene) led to complex mixtures. Hydrogenation of azide **4.123** catalysed by palladium on charcoal in the presence of sodium acetate in ethanol gave the corresponding amine which was closed *in situ* to gave pyrrolidine **4.124** (72%). The sequential removal of the silyl groups in aqueous acid and the benzoate in aqueous base afforded

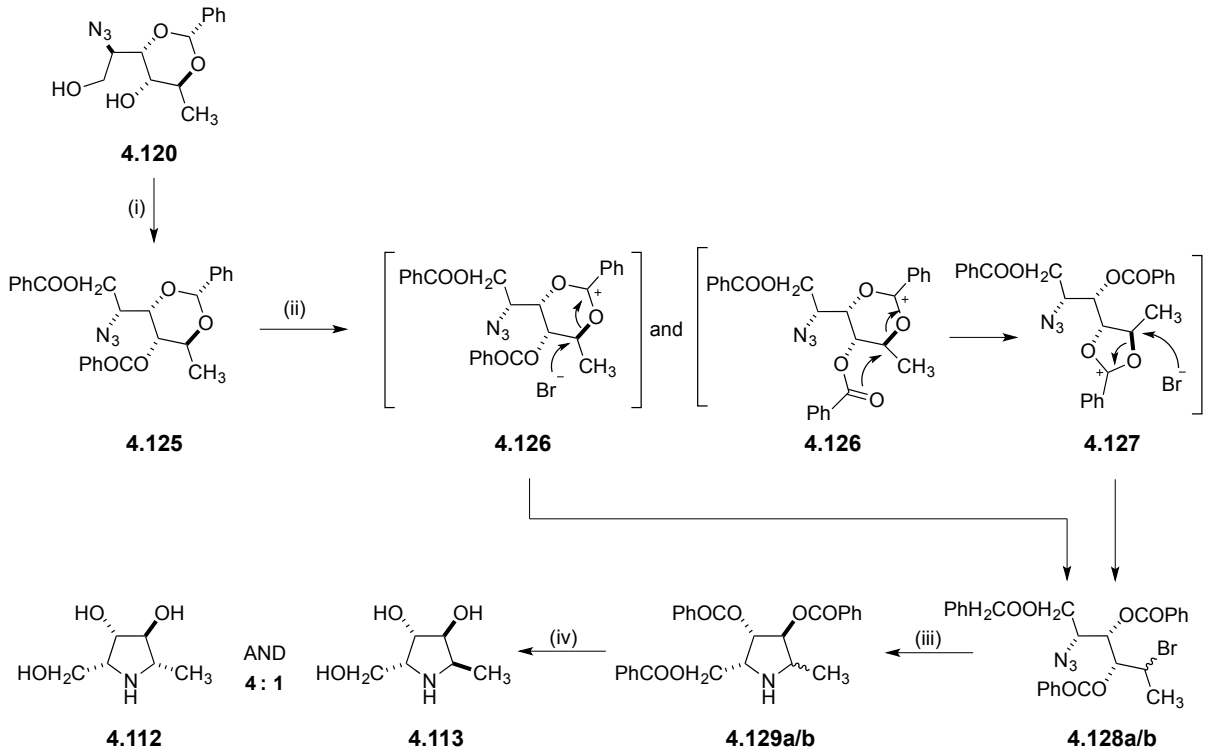
2,5-imino-2,5,6-trideoxy-L-glucitol (6-deoxy-L-DGDP) **4.112** in an overall yield of 45% (from *gluco*-azido lactone **4.46**).



(i) DIBALH, DCM, -78 °C, then $NaBH_4$, MeOH, 92% (2 steps); (ii) TBSOTf, pyridine, DCM, 98%; (iii) NBS, $BaCO_3$, CCl_4, reflux, 86%; (iv) Pd/C, H_2, NaOAc, EtOH, 72%; (v)TFA/water/1,4-dioxane 50 °C, then NaOH (2M, aq), 50 °C, 81% (2 steps)

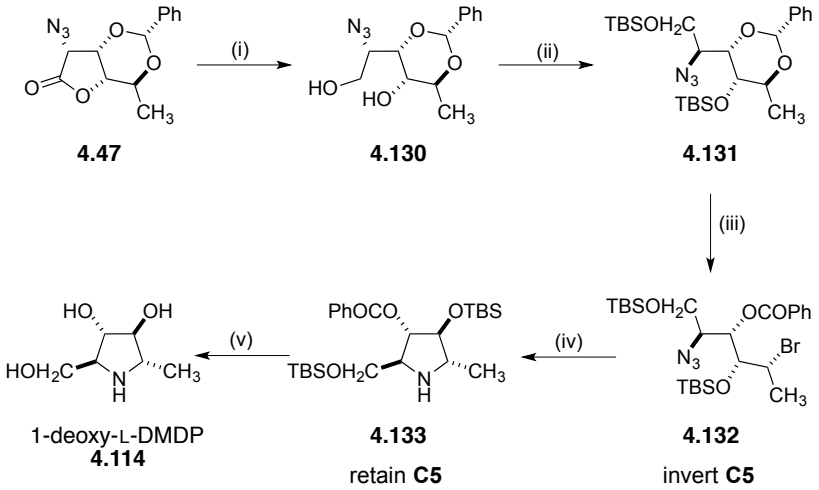
Scheme 4.12 Synthesis of 6-deoxy-L-DGDP **4.112** *via* Hanessian-Hullar reaction

Alternatively, the treatment of diol **4.120** with benzoyl chloride in the presence of pyridine in DCM gave dibenzoate **4.125** (86%). (Scheme 4.13) **4.125** underwent Hanessian-Hüllar reaction to give an inseparable 5-epimeric mixture of **4.128a** and **4.128b** in a ratio of 5:1 according to ^{1}H NMR (yield 91%). This observation could be explained by the neighbouring group participation of 4-*O*-benzoate group in intermediate **4.126**. Subsequent hydrogenation leaded to the ring closure to the epimeric mixture **4.129a** and **4.129b** (4:1, 82%) and the hydrolysis of resulting pyrrolidine mixture **4.129a** and **4.129b** in aqueous base gave an inseparable mixture of 6-deoxy-L-DGDP **4.112** and 2,5-imino-2,5,6-trideoxy-D-iditol **4.113** in a 5:1 ratio according to ^{1}H NMR (43% from *gluco*-azido lactone **4.46**).



(i) BzCl, pyridine, DCM, 86%; (iii) NBS, $BaCO_3$, CCl_4, reflux, 5-epimeric mixture **4.128a/b**, 91%; (iv) Pd/C, H_2, NaOAc, EtOH, 82%; (v)TFA/water/1,4-dioxane 50 °C, then NaOH (2M, aq.), 50 °C, 73% (2 steps), **4.112**/**4.113** NMR ratio 4:1

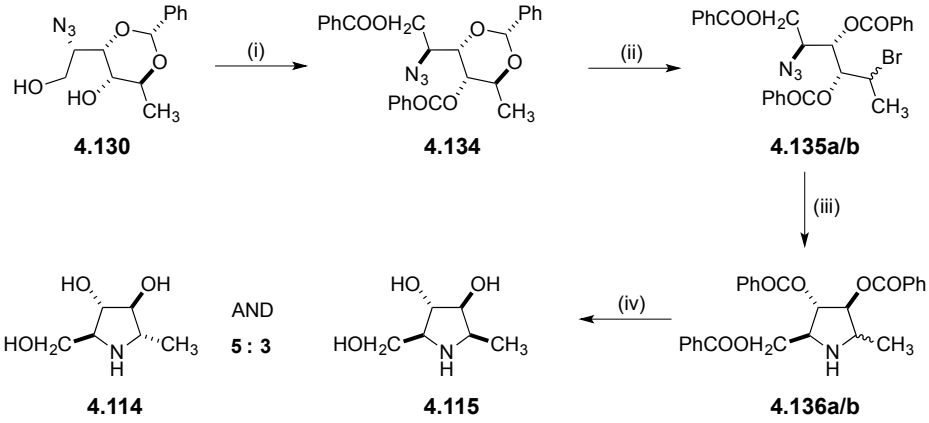
Scheme 4.13 Synthesis of epimeric mixture of **4.112** and **4.113** from benzoate protected **4.125**



(i) DIBALH, DCM, -78 °C, then $NaBH_4$, MeOH, 89% (2 steps); (ii) TBSOTf, pyridine, DCM, 94%; (iii) NBS, $BaCO_3$, CCl_4, reflux, 92%; (iv) Pd/C, H_2, NaOAc, EtOH, 71%; (v)TFA/water/1,4-dioxane 50 °C, then NaOH (2M, aq.), 50 °C, 64% (2 steps)

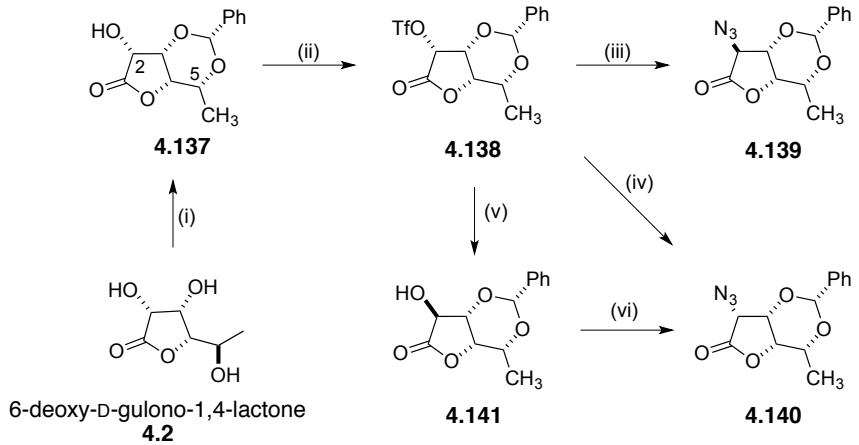
Scheme 4.14 Synthesis of 6-deoxy-L-DMDP **4.114**

An identical strategy provided access to 2,5-imino-2,5,6-trideoxy-L-mannitol (6-deoxy-L-DMDP) **4.114** from *mannono*-azido lactone **4.47** in an overall yield of 35% (Scheme 4.14). An alternative route involving dibenzoate protection of diol **4.130** eventually lead to an epimeric mixture of 1-deoxy-L-DMDP **4.114** and 1-deoxy-L-DGDP **4.115** in a NMR ratio of 5:3 (51% from *mannono*-azido lactone **4.47**). However, the two epimers were not readily separable (Scheme 4.15).



(i) BzCl, pyridine, DCM, 97%; (iii) NBS, $BaCO_3$, CCl_4, reflux, 94%, epimer ratio 4:1; (iv) Pd/C, H_2, NaOAc, EtOH, 82%, epimer ratio 10:7; (v)TFA/water/1,4-dioxane 50 °C, then NaOH (2M, aq.), 50 °C, 73% (2 steps), **4.114**/**4.115** NMR ratio 5:3

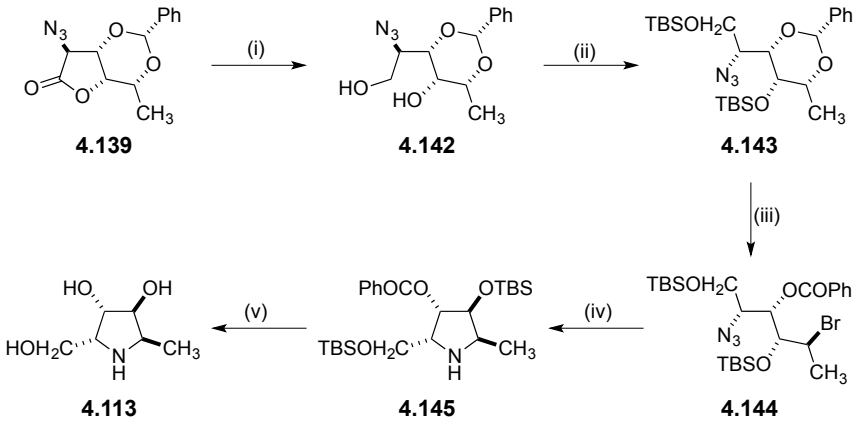
Scheme 4.15 Synthesis **4.114** and **4.115** from dibenzoate **4.134**



(i) PhCHO, *conc* HCl, 92%; (ii) $(CF_3SO_2)_2O$, Pyridine, THF, -20 °C, 98%; (iii) NaN_3, DMF, -10 °C, 20h, 77%; (iv) NaN_3, DMF, rt, 78%, 42h; (v) caesium trifluoroacetate, DMF, 60 °C, 100%; (vi) $(CF_3SO_2)_2O$, Pyridine, THF, -20 °C, then NaN_3, DMF, rt, 30h, 45% (2 steps)

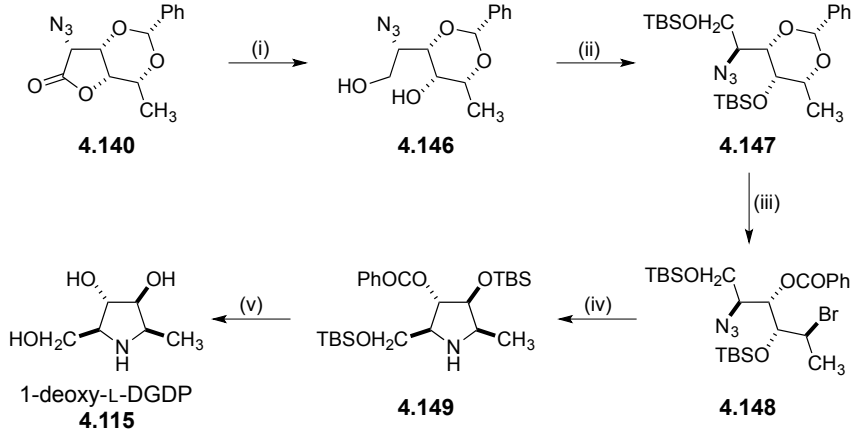
Scheme 4.16 Access to azido lactones **4.139** and **4.140**

6-Deoxy-D-gulono-1,4-lactone **4.2** was used as starting material to access pure 2,5-imino-2,5,6-trideoxy-D-iditol **4.113** and 1-deoxy-DGDP **4.115** using the same strategy. As shown in Scheme 4.16, the treatment of **4.2** with concentrate HCl in benzaldehyde gave the crystalline solid of *(R)*-3,5-*O*-benzylidene-6-deoxy-D-gulono-1,4-lactone **4.137** (92%), the stereochemistry of which was confirmed by nOe analysis. Although the benzylidene lactones derived from L-rhamnose and 6-deoxy-D-gulose are both highly crystalline and formed in good yield, there was significant difference in the subsequent S_N2 displacements on the corresponding triflates. The triflation of lactone **4.137** gave a stable *gulono*-triflate **4.138** (98%) which was then subjected to the azide displacement in DMF at -10 °C for 20 hours to afford *idono*-azido lactone **4.139** in a yield of 77%. The *gulono*-azide **4.140** was formed by two approaches. First, the treatment of *gulono*-triflate **4.138** with sodium azide at room temperature for 42 hours gave the *gulono*-azido lactone **4.140** with all *cis* substitutions (78%). In an analogy with the L-rhamnose series, the transformation of kinetical product, *idono*-azide **4.139** to the thermodynamical product *gulono*-azide **4.140** was observed during the reaction but the rate was much slower. Alternatively, *idono*-lactone **4.141** was obtained by the treatment of **4.138** with caesium trifluoroacetate in anhydrous DMF in a quantitative yield. Subsequent triflation of **4.141** and azide displacement gave *gulono*-azido **4.140** in a low yield of 45% (2 steps).



(i) DIBALH, DCM, -78 °C, then $NaBH_4$, MeOH, 66% (2 steps); (ii) TBSOTf, pyridine, DCM, 74%; (iii) NBS, $BaCO_3$, CCl_4, reflux, 99%; (iv) Pd/C, H_2, NaOAc, EtOH; (v)TFA/water/1,4-dioxane 50 °C, then NaOH (2M, aq), 50 °C, 74% (3 steps from **4.144**)

Scheme 4.17 Synthesis of **4.113** from *idono*-azido lactone **4.139**



(i) $LiBH_4$, THF, -20 °C, 90%; (ii) TBSOTf, pyridine, DCM, 84%; (iii) NBS, $BaCO_3$, CCl_4, reflux, 76%; (iv) Pd/C, H_2, NaOAc, EtOH; (v)TFA/water/1,4-dioxane 50 °C, then NaOH (2M, aq), 50 °C, 51% (3 steps from **4.148**)

Scheme 4.18 Synthesis **4.115** from *gulono*-azido lactone **4.140**

The 'reduction-TBS protection-Hanessian-ring closure' strategy was performed to access 2,5-imino-2,5,6-trideoxy-D-iditol **4.113** in an overall yield of 36% from *idono*-azido lactone **4.139** (Scheme 4.17). However, a modification was required for the synthesis of 2,5-imino-2,5,6-trideoxy-D-gulitol **4.115** since the DIBALH reduction (>10 eq) of *gulono*-azido lactone **4.140** only gave complex mixture. Alternatively, *gulono*-azido lactone **4.140** was treated with lithium borohydride in anhydrous THF at -20 °C to afford diol **4.146** in a good yield of 90%.

Hanessian reaction, ring closure and deprotection gave 2,5-imino-2,5,6-trideoxy-D-gulitol **4.115** in an overall yield of 29% (from **4.140**). As shown in Figure 4.12, the stereochemistries of the 4 rhamnulose mimics **4.112** - **4.115** could be distinguished by nOe analysis. These studies confirmed not only the structures of the target pyrrolidines but also the structures of the proposed bromide intermediates.

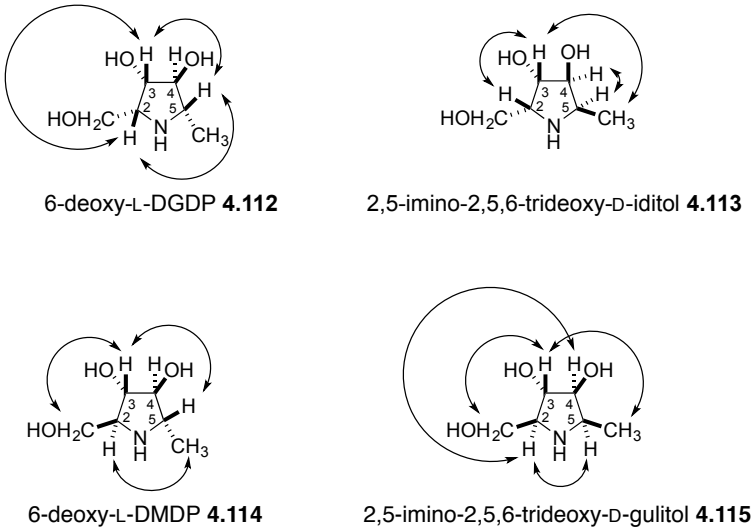


Figure 4.12 Key nOe correlations for **4.112** - **4.115**

4.3.2 Biological assay

4.3.2.1 6-Deoxy-hexoses and 6-deoxy-iminohexitols as potent inducers L-rhamnose operon

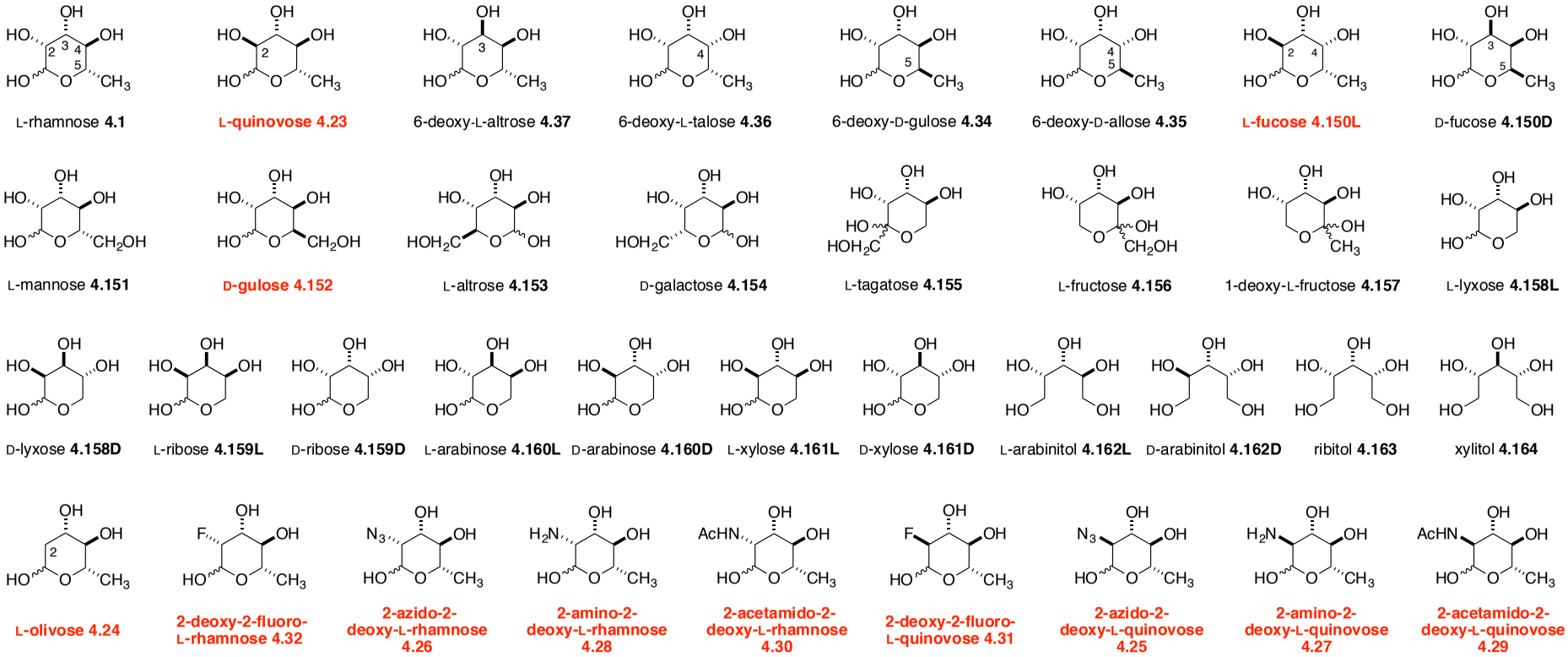


Figure 4.13 Sugar candidates as potent inducers for L-rhamnose inducible gene expression system
(The names of candidates synthesized in this thesis were shown in red)

35 sugar candidates (Figure 4.13) (some candidates were from our collaborators, Prof Ramon Estevez and Prof Ken Izumori) including hexoses, 6-deoxy-hexoses, pentoses and pentitols were subjected to the biological assays for searching potential inducers for L-rhamnose inducible gene expression system. Our collaborator, Prof. John Heap (Imperial College London) performed all biological assays. The biological assays and results are introduced in this section.

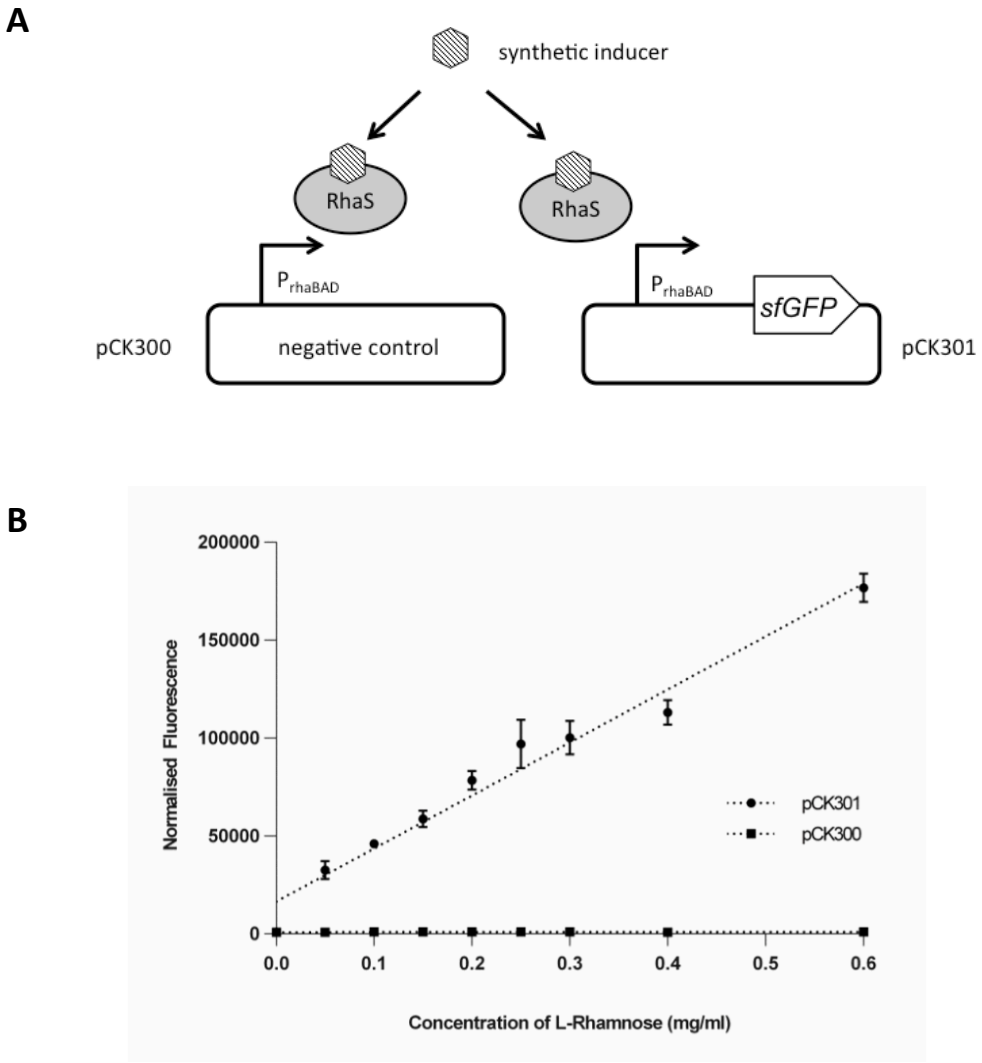


Figure 4.14 **A** The reporter plasmid pCK301 for the evaluation of synthetic inducers; **B** Validation of plasmid pCK301 using L-rhamnose in *E. coli*.[30]

A reporter plasmid (pCK301) was constructed to evaluate the induction abilities of candidates for the *rha*BAD promoter (Figure 4.14A). This vector contains a transcriptional fusion between the *rha*BAD promoter of *E. coli*. MG1655 and a gene encoding the highly stable and widely used

'superfolder' variant of green fluorescent protein (sfGFP).[71] By this method, the transcription level of sfGFP gene could be readily evaluated by the levels of fluorescence. A plasmid lacking the reporter construct, pCK300, was used a negative control. *E. coli*. MG1655 was used as the host for induction assays since it has been shown that the levels of RhaS and RhaR expressed from the native *rha*SR operon in this strain are sufficient for full induction by the native inducer L-rhamnose.[41a] This reporter system was validated using the natural inducer L-rhamnose (Figure 4.14B). A good linear correlation (R^2 value = 0.97) between fluorescence and L-rhamnose concentration was confirmed in vector pCK301. In contrast, there was only very low level of basal expression in control vector pCK300.

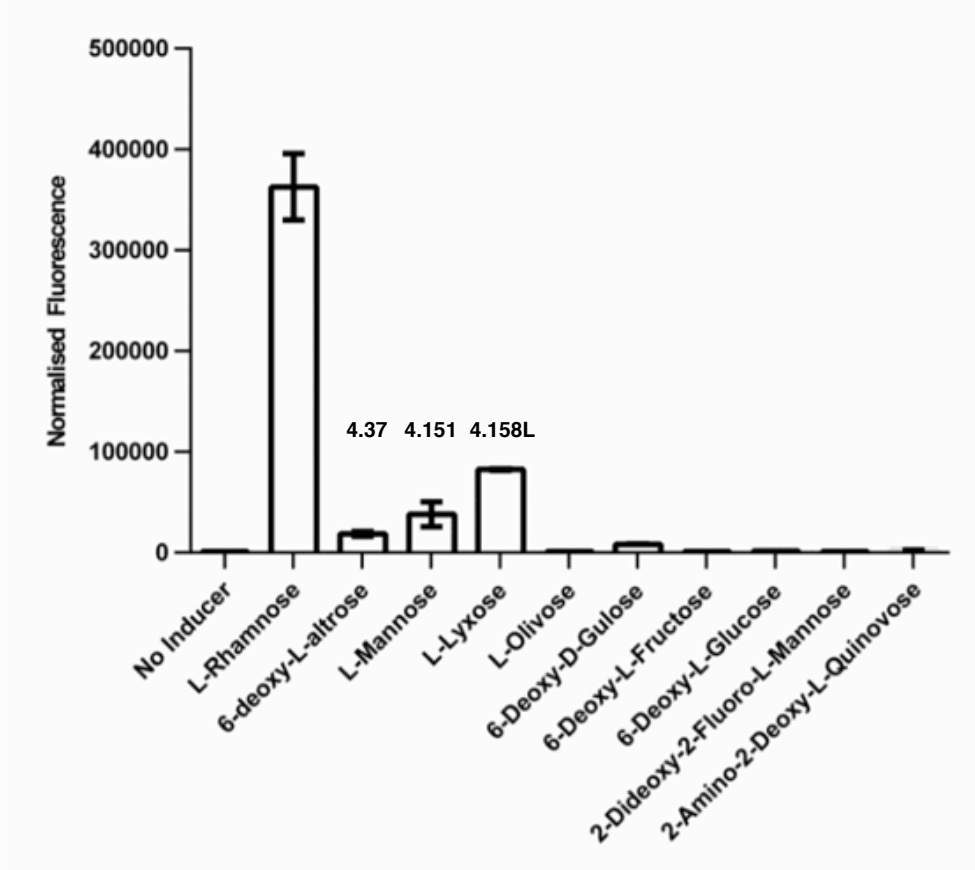


Figure 4.15 Several weak inducers using the reporter pCK301[30]

The 35 sugar candidates were screened for potential induction of the *rha*BAD promoter, using the constructed reporter system as described above (Figure 4.15). To maximize the induction effect and identify the weakest inducers, all candidates were used at a high concentration 1 mg/mL. L-Rhamnose was used a positive control. After the growth in rich media for 16 hours, most compounds showed extremely poor induction of the *rha*BAD promoter system. Some inducers, in particular 6-deoxy-L-altrose **4.37**, L-mannose **4.151** and L-lyxose **4.158L** showed weak inductions.

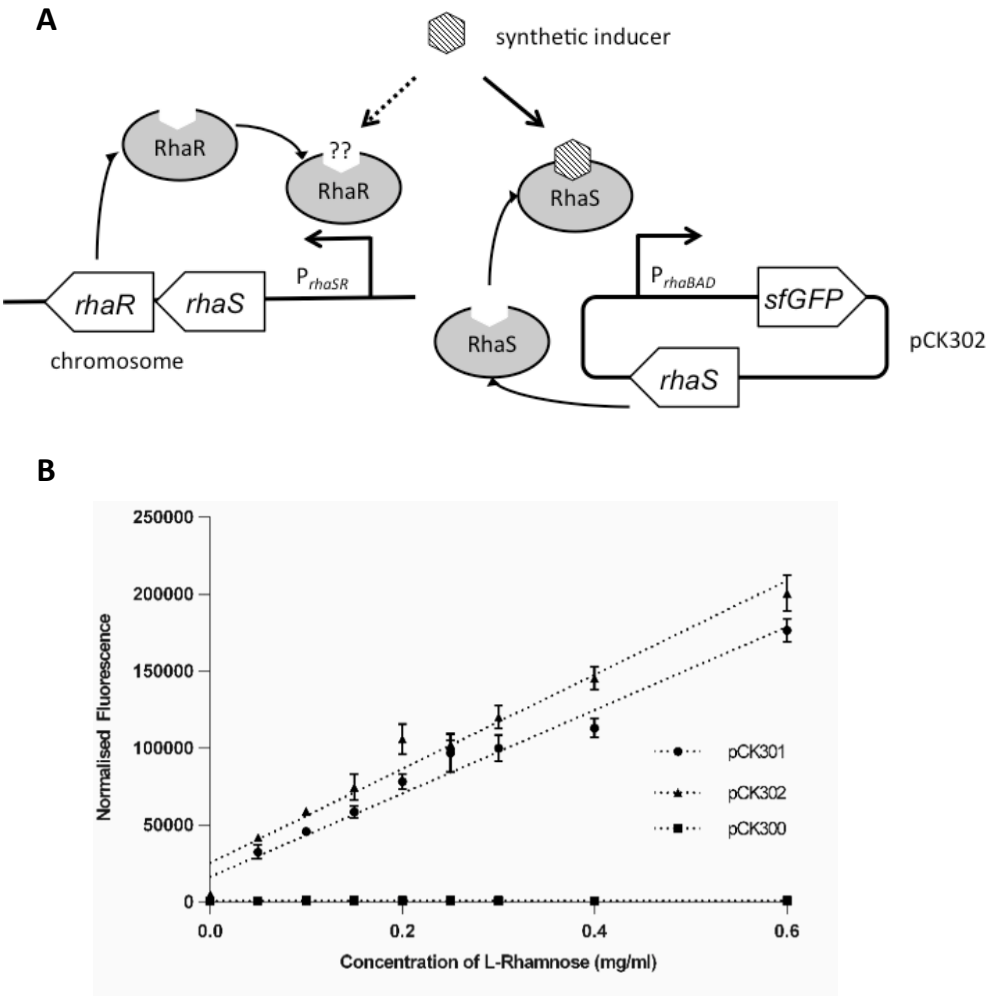


Figure 4.16 Construction of decoupled a reporter pCK302

As mentioned in the introduction section, in the native regulatory cascade of the L-rhamnose operon, two regulatory proteins, RhaS and RhaR, are activated by L-rhamnose in different

ways.[41a] An effective inducer must interact with both RhaS and RhaR properly (Figure 4.6). It is reasonable that synthetic candidates may only induce the right conformational changes in RhaS, but not RhaR, to induce poor transcription of genes or *vice versa*. Such compounds would be expected to give weak or negative results in the initial screen described above, which is dependent on the native *rha*SR regulatory cascade. To investigate this, a new reporter vector pCK302, which was able to expression of *rha*S gene, was constructed (Figure 4.16A). This new reporter system was validated by the treatment of various concentrations of L-rhamnose to the end-point of fluorescence induction (Figure 4.16B). A good linear dose-response relationship (R^2 = 0.9671) was identified. As expected, compared to pCK301, this system could induce a higher expression level in all concentrations.

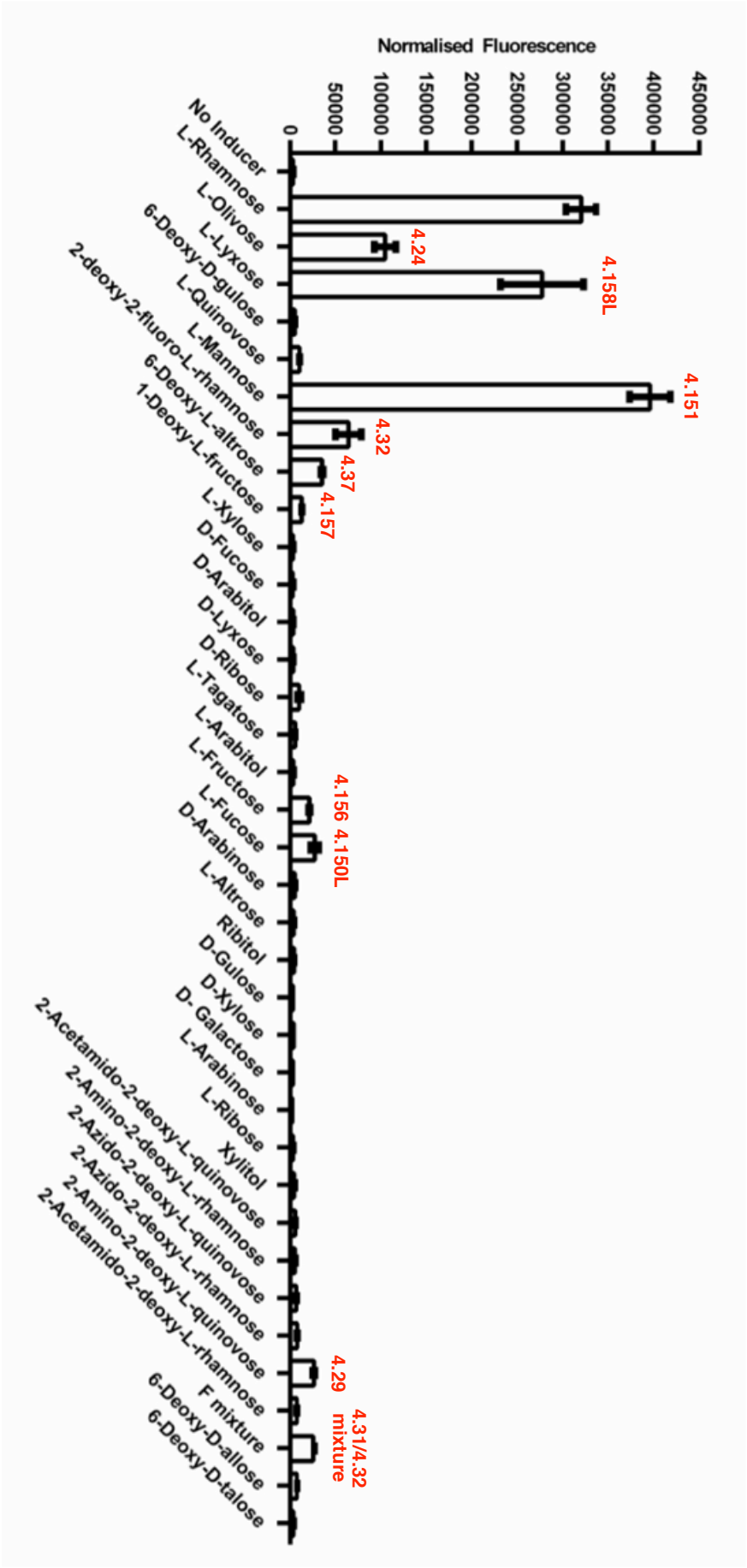
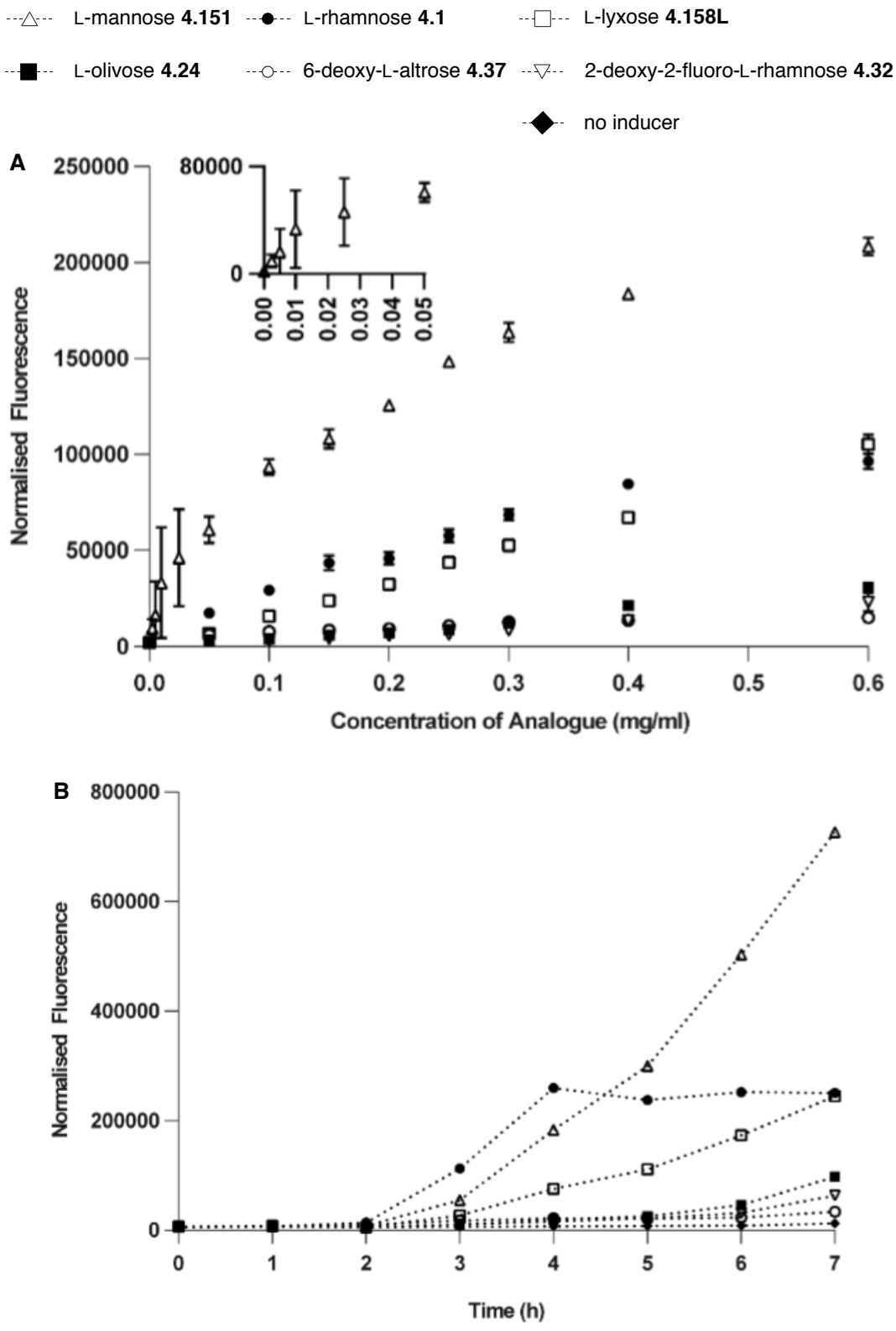


Figure 4.17 Induction effects of all 35 candidates on the reporter pCK302

All 35 candidates were tested using this new reporter system and their induction effects were shown in Figure 4.17. After the incubation in rich media for 16 hours, L-lyxose **4.158L** showed almost equal induction activity with L-rhamnose **4.1**. L-Mannose **4.151** showed an even stronger induction that L-rhamnose **4.1**. Several 2-substituted derivatives of L-rhamnose were moderate inducers: L-olivose **4.24,** 2,6-dideoxy-2-fluoro-L-mannose **4.32**, 2-amino-2-deoxy-L-quinovose **4.29** and the mixture of 2-epimeric fluoro hexoses **4.31** and **4.32**. Additionally, 6-deoxy-L-altrose **4.37**, 6-deoxy-L-fructose **4.157**, L-fructose **4.156** and L-fucose **4.150L** showed weaker but constant inductions.


L-mannose 4.151
L-rhamnose 4.1
L-lyxose 4.158L
L-olivose 4.24
6-deoxy-L-altrose 4.37
2-deoxy-2-fluoro-L-rhamnose 4.32
no inducer
A
Normalised Fluorescence
Concentration of Analogue (mg/ml)
B
Normalised Fluorescence
Time (h)

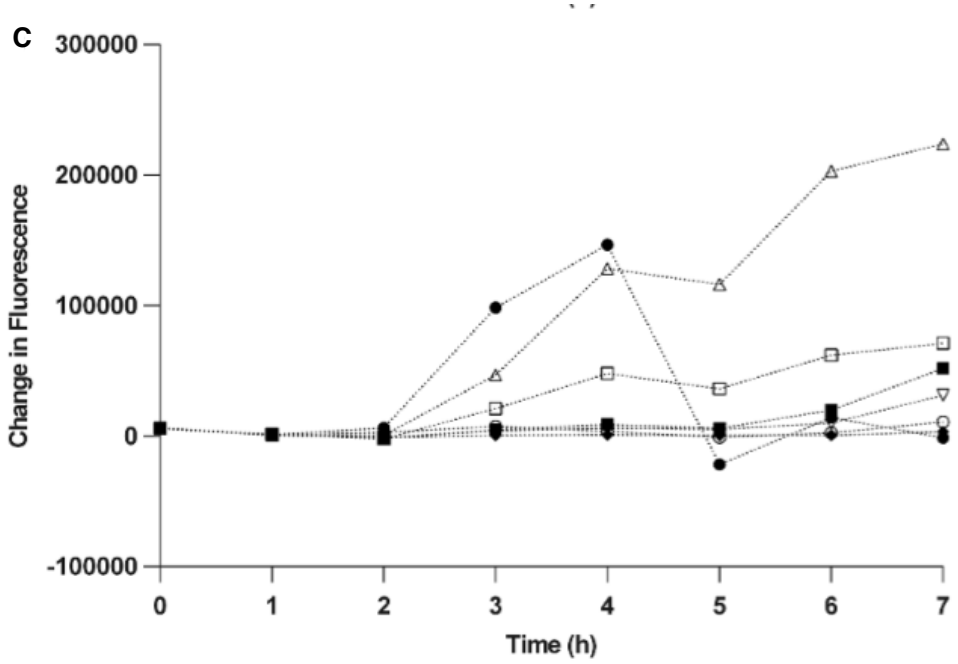


Figure 4.18 **A** Inductions by candidates in various concentrations (inserted chart showed low concentrations); **B** Accumulation of fluorescence inductions by candidates (0.4 mg/ml) in a period of 400 min; **C** Changes of fluoresces by candidates (0.4 mg/ml) per unite time

In the next stage, 5 promising inducers (L-mannose **4.151**, L-lyxose **4.158L**, L-olivose **4.24**, 2-deoxy-2-fluoro-L-rhamnose **4.32** and 6-deoxy-L-altrose **4.37**) were subjected to further studies with L-rhamnose **4.1** as a positive control. First, similar linear responses (R^2 value 0.86 – 0.99) to concentrations were observed in all analogues (Figure 4.18A). Next, the kinetics of induction of each of the five analogues was studied by monitoring the fluorescence of cultures (of *E. coli*. MG1655 cells containing pCK302 in LB medium, as before) containing 0.4 mg/ml of inducer over the course of 7 hours following inoculation. The high stability of sGFP protein[71] allowed the further interpretation of the kinetics of those inducers since the degradation of sGFP protein could be considered as negligible. Figure 4.18B showed the accumulation of fluorescence induction by different analogues in a period of 400 min. And the changes of fluorescence per unit time, which indicated the rate of sGFP production, are shown in Figure 4.18C. First, the induction by L-rhamnose **4.1** was detectable after 2 hours which was earlier that the other analogues

(P<0.05) (Figure 4.18B). Second and most importantly, the rate of induction by L-rhamnose **4.1** dropped to zero after 240 min (Figure 4.18C), which meant L-rhamnose **4.1** could only induce a transient expression of gene. In contrast, the induction by the other analogues did not cease during the entire experiment. After 400 min, L-mannose **4.151** induced a much higher level of sGFP than L-rhamnose **4.1**; L-lyxose **4.158L** induced a similar level of sGFP to L-rhamnose (Figure 4.18B). The patterns of induction rate changes by L-mannose **4.151** and L-lyxose **4.158L** were similar (Figure 4.18C). In addition, the expression rates by L-mannose **4.151** and L-lyxose **4.158L** increased earlier (240 min) than that by the rest of analogues **4.24**, **4.32** and **4.37** (300 min) (Figure 4.18C).

The sustained induction kinetics suggested that the 5 analogues are unlikely to be metabolized in *E. coli*. To study this, a cell growth assay[30] were conducted by measuring the growth of *E. coli* MG1655 containing the reporter plasmid pCK302 in M9 minimal medium agar plates, each supplemented with 0.4 % (w/v) of one of the analogues as the sole carbon source. In the meantime, a plate using glycerol was used as positive control and a plate without and carbon sources was used as negative control. Good growth of cells was observed in the plates using glycerol, L-rhamnose **4.1** and L-lyxose **4.158L**. In contrast, poor growth of cells were observed in the plates using the other analogues, suggesting four promising analogues (L-mannose **4.151**, L-olivose **4.24**, 6-deoxy-L-altrose **4.37** and 2-deoxy-2-fluoro-L-rhamnose **4.32**) were poorly metabolized in *E. coli*..

In addition, according to preliminary assays, 5-*epi*-RHJ **4.6**, a potent α-L-rhamnosidase inhibitor,

showed 9 times of basal induction on plasmid pCK302. Currently the kinetics of its induction activity is under investigation.

4.3.2.2 6-Deoxy-iminohexitols as inhibitors of L-rhamnosidase, L-rhamnose isomerase and other glycosidases

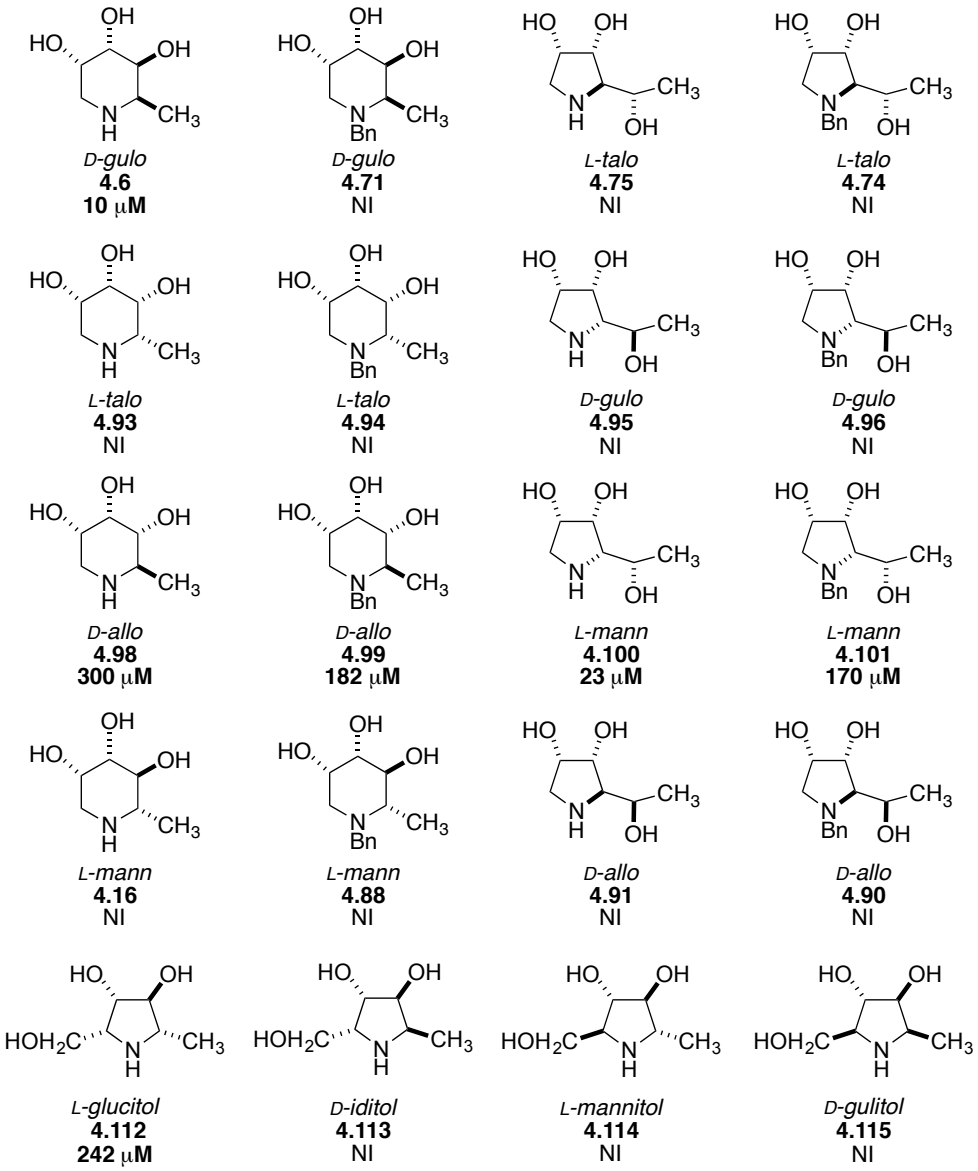


Figure 4.19 Synthesized iminohexitols and IC_{50} of inhibitors of α-L-rhamnosidases (*Penicillium decumbens*) (NI = no inhibition)

In this project, 8 trideoxy-1,5-iminohexitols, 8 trideoxy-1,4-iminohexitols and 4 trideoxy-2,5-iminohexitols were synthesized from L-rhamnono-1,4-lactone **4.63** and

6-deoxy-D-gulonolactone **4.2** (Figure 4.19). First, they were tested as potential inhibitors of α-L-rhamnosidases (*Penicillium decumbens*).[72] Consistent with previous reports,[18, 20a, 73] RHJ **4.16** showed no inhibition although it has the same stereochemistry as L-rhamnose **4.1**. It also failed to inhibit several bacterial strains including *P. aeruginosa* and *Staphylococcus aureus*.[74] In contrast, 5-*epi*-RHJ **4.6** showed very potent inhibition (IC_{50} 10 μM) of α-L-rhamnosidase. 5-*epi*-RHJ **4.6** also inhibited the biosynthesis of dTDP-L-rhamnose which was a key intermediate of bioproduction of L-rhamnose in bacteria.[73] However, *N*-benzyl-5-*epi*-RHJ **4.71** did not inhibit any α-L-rhamnosidase. Several other candidates have also been synthesized and studied as glycosidase inhibitors. **4.100** was reported as a potent inhibitor of α-L-rhamnosidase (IC_{50} 4.0 μM and K_i 1.0 μM)[20b, 75] In this project, it also showed potent inhibition of α-L-rhamnosidase (IC_{50} 23 μM). It is worth noting that **4.100** can be considered as a pyrrolidine analogue of L-swainsonine **4.162** (Figure 4.20), a very potent inhibitor of α-L-rhamnosidase (IC_{50} 0.3 μM and K_i 0.45 μM).[76] **4.101**, as the *N*-benzylated analogue of **4.100**, showed a weaker inhibitory activity (IC_{50} 170 μM). **4.98** and **4.99**, as 4,5-diepimeric analogues of RHJ **4.16**, showed weak (IC_{50} 300 μM) and moderate (IC_{50} 182 μM) inhibitions of α-L-rhamnosidase respectively. Generally, *N*-benzyl analogues showed stronger inhibitions, which might be explained by *N*-benzylation facilitating molecules entering cells. Preliminary tests indicated that **4.101** showed weak inhibitory effect towards L-rhamnose isomerase (RI) (*Pseudomonas stutzeri*): this enzyme showed 75% activity in 0.1mM of **4.101**.[77]

4.100 **4.162**

Figure 4.20 **4.100** as an analogue of L-swainsonine **4.162**

In addition, 2,5-imino-2,5,6-trideoxy-L-glucitol **4.112** (Figure 4.19) showed weak inhibition of α-L-rhamnosidases (IC_{50} 242 μM). As indicated above, a number of trideoxy-2,5-iminohexitols such as 6-deoxy-DMDP **4.108**, 6-deoxy-DGDP **4.109**, 1-deoxy-DGDP **4.110** (Figure 4.10) are natural products or have been synthesized previously.[49, 66] 6-Deoxy-DGDP **4.109** and 1-deoxy-DGDP **4.110** were potent inhibitors of α-L-fucosidase from bovine kidney with K_i values of 4 μM and 8 μM respectively.[104,106] In addition, DGDP **4.90**, has been identified as an inhibitor of xylose isomerase.[23] Their enantiomers or analogues **4.112**, **4.113** and **4.114** were synthesized in this project as mimics of rhamnulose, and for their potential as rhamnose isomerase inhibitors. However, none of the compounds showed any significant inhibition of RI. The trideoxy-2,5-iminohexitols **4.112** - **4.115** were tested as potential inhibitors of a range of other glycosidases. As shown in Table 4.3, 6-deoxy-L-DMDP **4.114** showed good inhibition of different α-glucosidases from rice (IC_{50} 3.1 μM), rat intestinal maltase (IC_{50} 8.1 μM) and rat intestinal sucrose (IC_{50} 0.84 μM). It is worth noting that **4.114** is closely related to two potent α-glucosidases inhibitors L-DMDP **4.106** and LAB **4.165** (Table 4.3).[63] In addition, a number of analogues of LAB **4.165** with long alkyl chains were potent inhibitors of α-glucosidases. For instance, brousonetine I **4.166** and J_2 **4.167** (Figure 4.21) were sub-micromolar inhibitors of α-glucosidase (rat intestinal maltase) with IC_{50} of 0.33 μM and 0.53 μM respectively.[78] Another two α-glucosidases inhibitors **4.168** and **4.169** (Figure 4.21) were studied as pharmacological chaperons for Gaucher disease.[79] Last but not the least, **4.112** and **4.115** showed weak inhibitions (IC_{50} 115 – 300 μM) and moderate inhibitions (IC_{50} 65 – 137 μM) of α-glucosidases respectively. None of these iminosugars inhibited any other glycosidases including β-glucosidases, α/β-glucosidases, α/β-mannosidases, α-L-fucosidases and amyloglucosidases.

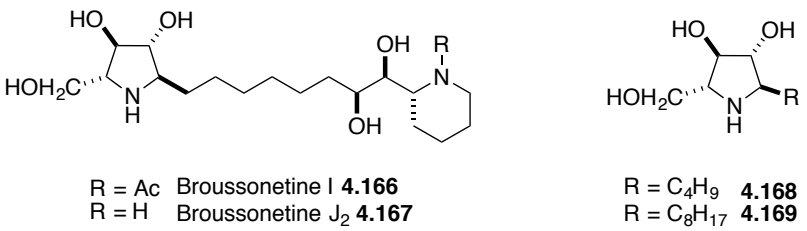
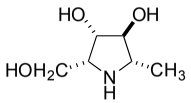
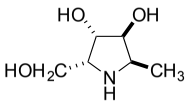
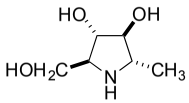
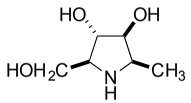
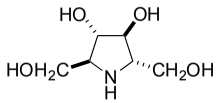
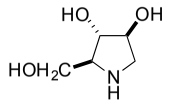


Figure 4.21 Examples of LAB analogues with long alkyl side chains

	IC$_{50}$ (μM)					
						
	4.112	**4.113**	**4.114**	**4.115**	**4.106**	**4.165**
α-glucosidases						
yeast	NI (11.3%)	NI (1.6%)	NI (2.4%)	NI (0.8%)	NI (34.9%)	70
rice	300	NI (5.9%)	3.1	137	5.8	3.2
rat intestinal maltase	116	NI (27.4%)	8.1	65	1.2	0.93
rat intestinal sucrase	115	NI (32.0%)	0.84	68.9	1.4	1.0

NI: no inhibition

Data of **4.106** and **4.165** is from previous report[63]

Table 4.3 IC$_{50}$ of 2,5,6-trideoxy-2,5-iminohexitols **4.112 - 4.115**, L-DMDP **4.106** and LAB **4.165** on α-glucosidases

4.4 Conclusions

In collaboration with Prof Ramon Esteve and Prof Ken Izumori's groups, 13 6-deoxy-hexoses, 8 trideoxy-1,5-iminohexitols, 8 trideoxy-1,4-iminohexitols and 4 trideoxy-2,5-iminohexitols were efficiently synthesized from easily accessible L-rhamnono-1,4-lactone **4.63** and 6-deoxy-D-gulono-1,4-lactone **4.2**. One ring contraction strategy was applied to efficiently transform piperidine iminosugars to pyrrolidine iminosugars. In addition, in the synthesis of trideoxy-2,5-iminohexitols, a Hanessian-Hullar reaction was used to open the benzylidene ring to access the corresponding bromides in excellent yields. This methodology is likely to be used in other carbohydrate syntheses.

In the biological aspect, 35 6-deoxy-hexoses were screened as potential inducers of L-rhamnose inducible gene expression system in *E. coli.* Five non-metabolizable inducers with strong (**4.151**), moderate (**4.24**) and weak (**4.32**, **4.37**) induction effects were identified. It is worth noting that they have different induction kinetics and could be used for different purposes. It is the first report on synthetic inducers of L-rhamnose operon. Co-treatments of 6-deoxy-hexoses and iminosugars were currently under investigation. In addition, glycosidase inhibition studies identified several potent inhibitors of α-L-rhamnosidases (**4.6**, **4.100**, **4.101**), L-rhamnose isomerase (**4.101**) and α-glucosidases (**4.112**, **4.113** and **4.114**).

4.5 Experimental

4.5.1 Synthesis of 2-Substituted rhamnose analogues

3,5-*O*-Benzylidene-2-*O*-trifluoromethanesulfonyl-L-rhamnono-1,4-lactone 4.39

Triflic anhydride (2.70 mL, 16.0 mmol) was added dropwise to a solution of the benzylidene lactone **4.22** (2.0 g, 8.0 mmol) and anhydrous pyridine (1.93 mL, 24.0 mmol) in anhydrous THF (30 mL) at -20 °C. After 2 h, TLC (cyclohexane/ethyl acetate, 1:1) indicated the consumption of

the starting material (R_f 0.54) and the formation of one major product (R_f 0.61). The reaction mixture was diluted with dichloromethane (10 mL) and washed with HCl (2 M, aq., 3 x 40 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed *in vacuo* to give the residue which was purified by flash chromatography (cyclohexane/ethyl acetate, 5:1 to 3:1) to obtain pure triflate **4.39** (2.9 g, 95%) as a white solid.

HRMS (ESI+ve): found 405.0226 $[M + Na]^+$; $C_{14}H_{13}F_3NaO_7S^+$ requires 405.0226; m.p.: 115 - 117 °C; $[\alpha]_D^{20}$ -78 (*c* 0.69, $CHCl_3$) [lit.[50b] m.p.: 139-140 °C; $[\alpha]_D^{20}$ -63.9 (*c* 0.69, CH_3CN)]; ν_{max} (thin film): 1780 (s, C=O); δ_H (CD_3CN, 400 MHz): 1.55 (3H, d, H6, $J_{6,5}$ 7.3), 4.38 (1H, br-s, H4), 4.60 (1H, dq, H5, $J_{5,4}$ 0.8, $J_{5,6}$ 7.3), 5.23 (1H, dd, H3, $J_{3,4}$ 1.7, $J_{3,2}$ 3.9), 5.85 (1H, d, H2, $J_{2,3}$ 3.9), 5.97 (1H, s, H7), 7.42 – 7.46 (5H, m, -Ar); δ_C (CD_3CN, 100 MHz): 14.4 (C6), 69.9 (C5), 71.0 (C3), 73.8 (C4), 81.1 (C2), 92.1 (C7), 126.7, 128.9, 129.8, 137.9 (-Ar), 168.6 (C=O); *m/z* (ESI+ve): 405 ($[M + Na]^+$, 100%).

3,5-*O*-Benzylidene-6-deoxy-L-glucono-1,4-lactone 4.40

Caesium trifluoroacetate (4.45 g, 18.2 mmol) was added to a solution of **4.39** (2.9 g, 7.6 mmol) in butanone (30 mL). The reaction mixture was then stirred at 60 °C for 4 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the disappearance of starting material (R_f 0.61) and the formation of one major product (R_f 0.57). Solvent was removed *in vacuo* to give a residue that was purified by flash chromatography (cyclohexane/ethyl acetate, 5:1 to 3:1) to obtain the *glucono*-lactone **4.40** (1.8 g, 90%) as a white solid.

HRMS (ESI+ve): found 273.0726 $[M + Na]^+$; $C_{13}H_{14}NaO_5^+$ requires 273.0733; m.p.: 146 - 148 °C; $[\alpha]_D^{20}$ -80 (*c* 0.90, MeCN) [lit.[50b] m.p.: 145 - 148 °C; $[\alpha]_D^{20}$ -84.1 (*c* 1.15, CH_3CN)]; ν_{max} (thin film): 1785 (s, C=O); δ_H (CD_3CN, 400 MHz): 1.54 (3H, d, H6, $J_{6,5}$ 7.2), 4.14 (1H, br-s, H2), 4.47 (1H, br-s, H4), 4.57 (1H, q, H5, $J_{5,6}$ 7.2), 4.66 (1H, d, H3, $J_{3,4}$ 2.0), 5.92(1H, s, H7), 7.39 – 7.46 (5H, m, -Ar); δ_C (CD_3CN, 100 MHz): 15.3 (C6), 70.1 (C5), 73.8 (C2), 75.5 (C3), 77.5 (C4), 92.8 (C7), 127.1, 129.2, 129.6, 139.1 (-Ar), 175.6 (C=O); *m/z* (ESI+ve): 405 ($[M + Na]^+$, 100%).

3,5-*O*-Benzylidene-2-*O*-*tert*-butyldimethylsilyl-6-deoxy-L-glucono-1,4-lactone 4.41

tert-Butyldimethylsilyl chloride (111 mg, 0.74 mmol) and imidazole (78 mg, 1.14 mmol) were added to a solution of **4.40** (143 mg, 0.57 mmol) in anhydrous DMF (3 mL). The reaction mixture was stirred at rt for 15 h until TLC (cyclohexane/ethyl acetate, 1:1) showed the formation of one major product (R_f 0.8). Then the mixture was diluted with ethyl acetate (10 mL) and washed with half saturated brine (10 mL). The organic phase was dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give a residue that was purified by flash chromatography (cyclohexane/ethyl acetate, 7:1 to 5:1) to yield the silyl ether **4.41** (148 mg, 71%) as a white solid.

HRMS (ESI+ve): found 387.1597 $[M + Na]^+$; $C_{19}H_{28}NaO_5Si^+$ requires 387.1598; m.p.: 58 - 60 °C; $[\alpha]_D^{20}$ -70.9 (*c* 1.17, $CHCl_3$); ν_{max} (thin film): 1793 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 0.18 (3H, s, CH_3), 0.20 (3H, s, CH_3), 0.92 (9H, s, 3 x CH_3), 1.56 (3H, d, H6, $J_{6,5}$ 7.1), 4.23 (1H, s, H2), 4.39 (1H, dd, H4, $J_{4,5}$ 0.8, $J_{4,3}$ 2.3), 4.10 (1H, d, H3, $J_{3,4}$ 2.3), 4.59 (1H, dq, H5, $J_{5,4}$ 0.8, $J_{5,6}$ 7.1), 5.8 (1H, s, H7), 7.37 – 7.47 (5H, m, -Ar); δ_C ($CDCl_3$, 100 MHz): -4.9 (CH_3), -5.2 (CH_3), 15.5 (C6), 25.5 (CH_3), 68.8 (C5), 73.9 (C2), 75.4 (C3), 77.2 (C4), 92.7 (C7), 126.3, 128.4, 129.4, 137.3 (-Ar), 173.8 (C=O); *m/z* (ESI+ve): 387 ($[M + Na]^+$, 100%).

3,5-*O*-Benzylidene-2-*O*-*tert*-butyldimethylsilyl-6-deoxy-L-glucose 4.42

Diisobutylaluminium hydride (25% w/v in toluene, 0.30 mL, 0.53 mmol) was added dropwise to a solution of **4.41** (148 mg, 0.41 mmol) in anhydrous dichloromethane (3 mL) at -78 °C. The reaction mixture was stirred at -78°C for 1 h until mass spectrometry showed the formation of desired product peak ($[M + H]^+$ 367) and disappearance of starting material peak ($[M + H]^+$ 365). Then the mixture was diluted with ethyl acetate (10 mL) and potassium sodium tartrate (sat. aq., ~0.5 mL) was added. After stirring for 8 h, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a residue that was further purified *via* flash chromatography (cyclohexane/ethyl acetate, 7:1 to 5:1) to yield the lactols **4.42** as a syrup (α:β ratio 5:4) (130 mg, 88%).

HRMS (ESI+ve): found 389.1755 $[M + Na]^+$; $C_{19}H_{30}NaO_5Si^+$ requires 389.1755; $[\alpha]_D^{20}$ -100 (*c* 1.38, MeOH); ν_{max} (thin film): 3400 (br, OH); δ_H (CD_3CN, 400 MHz): -0.08 (6H, s, 2 x CH_3), -0.06 (3H, s, CH_3), -0.04 (3H, s, CH_3), 0.69 (9H, s, 3 x CH_3 (β)), 0.72 (9H, s, 3 x CH_3 (α)), 1.19 (3H, d, H6α, $J_{6,5}$ 7.2), 1.23 (3H, d, H6β, $J_{6,5}$ 7.2), 3.59 (1H, d, OHβ, $J_{OH,1}$ 10.7), 3.64 (2H, br-s, H4β, H4β), 3.80 (1H, d, OHα, $J_{OH,1}$ 11.1), 3.88 (1H, br-s, H2β), 3.90 (1H, d, H2α, $J_{2,1}$ 3.5), 4.07 (1H, a-q, H5α, $J_{5,6}$ 7.2), 4.15-4.18 (3H, m, H3α, H3β, H5β), 4.78 (1H, H1β, $J_{1,OH}$ 10.7), 5.24 (1H, H1α, $J_{1,2}$ 3.6, $J_{1,OH}$ 11.3), 5.54 (1H, s, H7α), 5.54 (1H, s, H7β), 7.14 - 7.24 (10H, m, -Arα, -Arβ); δ_C (CD_3CN, 100 MHz): -4.9 (CH_3), -4.8 (2 x CH_3), -4.7 (CH_3), 16.3 (C6α), 16.6 (C6β), 26.1, 26.2 (3 x CH_3 (α), 3 x CH_3 (β)), 71.2 (C5α), 71.5 (C5β), 75.5, 78.4 (C4α, C4β), 77.2 (C2α), 78.9, 79.6 (C3α, C3β), 81.7 (C2β), 93.1 (C7α), 93.4 (C7β), 98.9 (C1α), 105.3 (C1β), 127.2, 127.3, 129.2, 129.4, 129.8, 130.0, 139.8, 140.0 (-Arα, -Arβ); *m/z* (ESI+ve): 389 ($[M + Na]^+$, 100%).

L-Quinovose (6-Deoxy-L-glucose) 4.23

DOWEX® 50WX8-200 (100 mg) was added to a solution of lactol **4.42** (130 mg, 0.36 mmol) in water (5 mL). After stirring at rt for 15 h mass spectrometry indicated the completion of reaction, the resin was filtered off and water was removed *in vacuo* to yield 6-deoxy-L-glucose **4.23** as a syrup (α:β pyranoside ratio 3:5) (58 mg, 100%). The spectra were identical with the 6-deoxy-L-glucose synthesized in Chapter 3.

HRMS (ESI+ve): found 165.0757 $[M + H]^+$; $C_6H_{13}O_5^+$ requires 165.0757; $[\alpha]_D^{20}$ -33 (*c* 0.32, water); ν_{max} (thin film): finger print region only; δ_H (D_2O, 400 MHz): 1.19 (3H, d, H6α, $J_{6\alpha,5\alpha}$ 6.3), 1.21 (3H, d, H6β, $J_{6,5}$ 6.1), 3.07 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.5), 3.08 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 9.3), 3.17 (1H, dd, H2β, $J_{2,1}$ 7.9, $J_{2,3}$ 9.3), 3.36 (1H, t, H3β, $J_{3,2} = J_{3,4}$ 9.3), 3.42 (1H, dq, H5β, $J_{5,6}$ 6.2, $J_{5,4}$ 9.5), 3.47 (1H, dd, H2α, $J_{2,1}$ 3.8, $J_{2,3}$ 9.9), 3.58 (1H, t, H3α, $J_{3,2} = J_{3,4}$ 9.5), 3.85 (1H, dq, H5α, $J_{5,6}$ 6.3, $J_{5,4}$ 9.6), 4.55 (1H, d, H1β, $J_{1,2}$ 7.9), 5.10 (1H, d, H1α, $J_{1,2}$ 3.8); δ_C (D_2O, 100 MHz): 17.9 (C6α, C6β), 68.6 (C5α), 72.9 (C2α), 73.1 (C5β), 73.6 (C3α), 75.6 (C2β), 76.1 (C4β), 76.4 (C4α), 76.7 (C3β), 93.2 (C1α), 96.9 (C1β); *m/z* (ESI+ve): 165 ($[M + H]^+$, 100%).

3,5-*O*-Benzylidene-2,6-dideoxy-L-*arabino*-hexono-1,4-lactone 4.44

Lithium iodide hydrate (917 mg, 4.91 mmol) was added to a solution of triflate **4.39** (300 mg, 0.79 mmol) in butanone (10 mL). The reaction mixture was stirred at 60 °C for 16 h until TLC (cyclohexane/ethyl acetate, 1:1) showed the disappearance of starting material (R_f 0.57) and formation of one major product (R_f 0.39). Then sodium thiosulfate (sat. aq., sat, 10 mL) was added. After stirring at rt for 10 min, the reaction mixture was extracted with ethyl acetate (3 x 10 mL), the organic phase was dried ($MgSO_4$) and the solvent was removed in *vacuo* to give a residue which was purified by flash chromatography (cyclohexane/ethyl acetate, 4:1 to 1:1) to obtain the deoxylactone **4.44** (137 mg, 74%) as a white solid.

HRMS (ESI+ve): found 298.0798 $[M + Na]^+$; $C_{13}H_{13}N_3NaO_4^+$ requires 298.0798; m.p.: 100 - 102 °C; $[\alpha]_D^{20}$ -61.6 (*c* 0.25, MeOH); ν_{max} (thin film): 1780 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.53 (3H, d, H6, $J_{6,5}$ 7.0), 2.74 (1H, d, H2, J_{gem} 17.6), 2.82 (1H, dd, H2', $J_{2',3}$ 4.6, J_{gem} 17.7), 4.16 (1H, br-dd, H4, $J_{4,3}$ 3.7, $J_{4,5}$ 2.4), 4.48 (1H, dq, H5, $J_{5,4}$ 2.4, $J_{5,6}$ 7.0), 4.78 (1H, br-dd, H3, $J_{3,4}$ 3.7, $J_{3,2'}$ 4.6), 5.8 (1H, s, H7), 7.38 – 7.49 (5H, m, -Ar); δ_C ($CDCl_3$, 100 MHz): 16.7 (C6), 37.5 (C2), 68.5 (C5), 70.7 (C3), 79.1 (C4), 93.8 (C7), 126.5, 128.7, 129.6, 137.8 (-Ar), 175.2 (C=O); *m/z* (ESI+ve): 235 ($[M + H]^+$, 100%).

3,5-*O*-Benzylidene-2,6-dideoxy-L-*arabino*-hexose 4.45

Diisobutylaluminium hydride (25% wt. in toluene, 0.32 mL, 0.56 mmol) was added dropwise to a solution of **4.44** (100 mg, 0.43 mmol) in anhydrous dichloromethane (4 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 3 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the disappearance of starting material (R_f 0.42) and the formation of one product (R_f 0.49). Also mass spectrometry showed the formation of desired product peak ($[M + H]^+$ 259). Then the mixture was diluted with ethyl acetate (5 mL) and potassium sodium tartrate (sat. aq., ~0.5 mL) was added. After stirring for 5 h, the mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a residue that was further purified *via* flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1) to yield the benzylidene lactols **4.45** as a

white solid (α:β ratio 10:1) (96 mg, 96%).

HRMS (ESI+ve): found 237.1118 $[M + H]^+$; $C_{13}H_{17}O_4^+$ requires 237.1120; m.p.: 90 - 92 °C; $[\alpha]_D^{20}$ -34 (*c* 0.27, MeOH); ν_{max} (thin film): finger print region only; δ_H ($CDCl_3$, 400 MHz): 1.49 (3H, d, H6α, $J_{6,5}$ 7.0), 1.50 (3H, d, H6β, $J_{6,5}$ 7.0), 2.12 (1H, dt, H2α, $J_{2,1} = J_{2,3}$ 4.5, J_{gem} 14.3), 2.19 (1H, dt, H2β, $J_{2,1} = J_{2,3}$ 4.7, J_{gem} 14.0), 2.28 (1H, d, H2β', J_{gem} 14.0), 2.47 (1H, dd, H2α', $J_{2',3}$ 5.7, J_{gem} 14.5), 3.7 (1H, br-s, H4β), 3.9 (1H, br-s, H4α), 4.41 (1H, a-q, H5α, $J_{5,6}$ 7.0), 4.54 (1H, a-q, H5β, $J_{5,6}$ 7.0), 4.61 (2H, br-s, H3α, H3β), 5.46 (1H, d, H1β, $J_{1,2}$ 5.1), 5.73 (1H, s, H7α), 5.78 (1H, s, H7β), 5.83 (1H, t, H1α, $J_{1,2} = J_{1,2'}$ 4.7); 7.34 - 7.54 (10H, m, -Arα, -Arβ); δ_C ($CDCl_3$, 100 MHz): 17.0 (C6β), 17.2 (C6α), 41.5 (C2β), 41.9 (C2α), 69.7 (C5α), 70.8 (C5β), 73.8 (C3β), 74.8 (C3α), 77.5 (C4α), 79.5 (C4β), 93.3 (C7α, C7β), 98.7 (C1α), 99.6 (C1β), 126.4, 126.5, 128.6, 128.7, 128.8, 129.2, 129.5, 129.6 (-Arα, -Arβ); *m/z* (ESI+ve): 237 ($[M + H]^+$, 100%).

L-Olivose (2,6-Dideoxy-L-*arabino*-hexose) 4.24

Dowex® 50WX8-200 (100 mg) was added into a solution of lactol **4.45** (90 mg, 0.38 mmol) in water (5 mL). After stirring at rt for 18 h and mass spectrum indicated the completion of reaction, the resin was filtered off and water was removed *in vacuo* to yield L-olivose **4.24** as a syrup (α:β pyranoside ratio 1:1) (56 mg, 100%).

HRMS (ESI+ve): found 149.0808 $[M + H]^+$; $C_6H_{13}O_4^+$ requires 149.0808; $[\alpha]_D^{20}$ -13 (*c* 0.49, water) [lit.[80] $[\alpha]_D^{20}$ -20 (*c* 0.80, water)]; ν_{max} (thin film): finger print region only; δ_H (D_2O, 400 MHz): 1.25 (3H, d, H6α, $J_{6,5}$ 6.4), 1.28 (3H, d, H6β, $J_{6,5}$ 6.3), 1.51 (1H, dt, H2β, $J_{2,1}$ 9.9, $J_{2,3} = J_{gem}$ 12.0), 1.71 (1H, ddd, H2α, $J_{2,1}$ 3.7, $J_{2,3}$ 11.9, J_{gem} 13.3), 2.13 (1H, ddd, H2α', $J_{2',1}$ 1.0, $J_{2',3}$ 5.1, J_{gem} 13.4), 2.25 (1H, ddd, H2β', $J_{2',1}$ 2.0, $J_{2',3}$ 5.0, J_{gem} 12.3), 3.05 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 9.3), 3.10 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.3), 3.41 (1H, dq, H5β, $J_{5,6}$ 6.3, $J_{5,4}$ 9.5), 3.66 (1H, ddd, H3β, $J_{3,2'}$ 5.0, $J_{3,4}$ 9.0, $J_{3,2}$ 12.0), 3.83 - 3.91 (2H, m, H3α, H5α), 4.91 (1H, dd, H1β, $J_{1,2'}$ 2.0, $J_{1,2}$ 9.8), 5.31 (1H, a-dd, H1α, $J_{1,2'}$ 1.0, $J_{1,2}$ 3.7); δ_C (D_2O, 100 MHz): 17.5 (C6α), 17.5 (C6β), 38.2 (C2α), 40.3 (C2β), 68.3, 68.5 (C3α, C5α), 70.7 (C3β), 72.5 (C5β), 76.8 (C4β), 77.4 (C4α), 91.7 (C1α), 93.8 (C1β); *m/z* (ESI+ve): 171 ($[M + Na]^+$, 100%).

3,5-*O*-Benzylidene-2-azido-2,6-dideoxy-L-glucono-1,4-lactone 4.46

Sodium azide (16.6 mg, 0.26 mmol) was added to a solution of triflate **4.39** (100 mg, 0.26 mmol) in anhydrous DMF. The mixture was stirred at -30 °C for 6 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the formation of a single product (R_f 0.68). After being diluted with ethyl acetate (5 mL), the mixture was washed with half saturated brine (10 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a crude solid that was purified *via* flash chromatography (cyclohexane/ethyl acetate 5:1) to yield the azido lactone **4.46** as a white solid (53.3 mg, 75%).

HRMS (ESI+ve): found 298.0796 $[M + Na]^+$; $C_{12}H_{19}FNaO_5^+$ requires 298.0798; m.p.: 70 - 74 °C; $[\alpha]_D^{20}$ -150 (c 0.89, $CHCl_3$); ν_{max} (thin film): 2112 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.54 (3H, d, H6, $J_{6,5}$ 7.2), 4.24 (1H, s, H2), 4.31 (1H, t, H4, $J_{4,3} = J_{4,5}$ 2.4), 4.51 (1H, d, H3, $J_{3,4}$ 2.9), 4.57 (1H, dq, H5, $J_{5,4}$ 1.8, $J_{5,6}$ 7.2), 5.8 (1H, s, H7), 7.39 – 7.46 (5H, m, -Ar); δ_C ($CDCl_3$, 100 MHz): 16.0 (C6), 63.0 (C2), 68.7 (C5), 73.7 (C3), 77.5 (C4), 93.4 (C7), 126.5, 128.8, 129.6, 137.2 (-Ar) 171.3 (C=O); *m/z* (ESI+ve): 298 ($[M + Na]^+$, 100%).

3,5-*O*-Benzylidene-2-azido-2,6-dideoxy-L-glucose 4.48

Diisobutylaluminium hydride (25% w/v in toluene, 0.34 mL, 0.60 mmol) was added dropwise to a solution of **4.46** (150 mg, 0.55 mmol) in anhydrous dichloromethane (5 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 2 h until mass spectrometry showed the formation of desired product peak ($[M + Na]^+$ 300) and disappearance of starting material peak ($[M + Na]^+$ 298). The mixture was diluted with ethyl acetate (5 mL) and potassium sodium tartrate (sat. aq., ~0.5 mL) was added. After stirring for 5 h, the mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain a crude product that was purified *via* flash chromatography (cyclohexane/ethyl acetate 7:1) to yield the benzylidene lactol **4.48** as a colourless oil (α:β ratio 5:4) (126 mg, 84%).

HRMS (ESI+ve): found 300.0905 $[M + Na]^+$; $C_{19}H_{30}NaO_5Si^+$ requires 300.0905; $[\alpha]_D^{20}$ -142 (c 1.70,

MeOH); ν_{max} (thin film): 2110 (s, N_3); δ_H (CD_3CN, 400 MHz): 1.17 (3H, d, H6β, $J_{6,5}$ 7.2), 1.21 (3H, d, H6α, $J_{6,5}$ 7.0), 3.59 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 2.6), 3.67 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 2.7), 3.81-3.84 (2H, m, H2α, H2β), 3.96 (1H, d, OHβ, $J_{OH,1}$ 9.5), 4.00 (1H, dq, H5β, $J_{5,4}$ 2.6, $J_{5,6}$ 7.0), 4.14 (1H, dq, H5α, $J_{5,4}$ 2.3, $J_{5,6}$ 7.0), 4.24 (1H, t, H3β, $J_{3,4} = J_{3,2}$ 2.5), 4.32 (1H, br-d, H3α, $J_{3,2} = J_{3,4}$ 2.7), 4.50 (1H, d, OHα, $J_{OH,1}$ 6.9), 4.94 (1H, d, H1β, $J_{1,OH}$ 9.5), 5.46 (1H, dd, H1α, $J_{1,2}$ 4.1, $J_{1,OH}$ 6.7), 5.52 (1H, s, H7β), 5.59 (1H, s, H7α), 7.14 - 7.25 (10H, m, -Arα, -Arβ); δ_C (CD_3CN, 100 MHz): 15.6, 15.8 (C6α, C6β), 66.8, 70.2 (C2α, C2β), 69.7 (C5β), 70.1 (C5α), 75.6 (C4β), 76.2 (C3α), 77.1 (C3β), 77.7 (C4α), 92.8 (C7β), 93.0 (C7α), 98.1 (C1α), 101.5 (C1β), 126.2, 126.3, 128.3, 128.4, 128.9, 129.1, 138.5, 138.8 (-Arα, -Arβ); *m/z* (ESI+ve): 300 ($[M + Na]^+$, 100%).

3,5-*O*-Benzylidene-2-azido-2,6-dideoxy-L-mannono-1,4-lactone 4.47

Method 1: From the *manno*-triflate **4.39**

Sodium azide (260 mg, 4.0 mmol) was added to a solution of triflate **4.39** (1.5 g, 4.0 mmol) in anhydrous DMF at 0 °C. The mixture was stirred at 0 °C for 1.5 h after which it was stirred at rt overnight. After 12 hours TLC analysis (cyclohexane/ethyl acetate, 1:1) indicated the formation of the only product (R_f 0.38). The mixture was diluted with ethyl acetate (50 mL), washed with brine (50 mL), half saturated brine (50 mL) and then brine (50 mL) again. The organic phase was dried ($MgSO_4$), filtered and reduced *in vacuo* to obtain a crude solid which was purified by flash column chromatography (cyclohexane/ethyl acetate 2:1 to 1:2) to afford the *manno*-azido **4.47** as a colourless solid (932 mg, 85%); none of the epimeric *gluco*-azide **4.46** was isolated under these conditions.

Method 2: From the *gluco*-triflate **4.50**

Triflic anhydride (1.11 mL, 6.5 mmol) was added dropwise to a solution of benzylidene lactone **4.40** (800 mg, 3.2 mmol) and anhydrous pyridine (1.25 mL, 15.5 mmol) in anhydrous THF (10 mL) at -20 °C. After stirring at -20 °C for 8 h, TLC (cyclohexane/ethyl acetate, 1:1) indicated the formation of one major product (R_f 0.70). The reaction mixture was diluted with DCM (10 mL) and washed with HCl (2 M, aq. 3 x 10 mL). The organic layer was dried ($MgSO_4$) and the solvent

was removed *in vacuo* to give the crude triflate **4.50** (1.3 g) which was not stable to chromatography and was used without further purification.

Sodium azide (204.8 mg, 3.2 mmol) was added into a solution of the crude triflate (1.3 g) in anhydrous DMF (20 mL). The mixture was stirred at -40 °C for 18 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the formation of one major product (R_f 0.42) and one minor product (R_f 0.68). After being diluted with ethyl acetate (20 mL), the mixture was washed with half saturated brine (20 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a crude that was further purified *via* flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1) to yield the azido lactone **4.47** as a white solid (616 mg, 70%) and **4.46** (211 mg, 24%).

HRMS (ESI+ve): found 298.0798 $[M + Na]^+$; $C_{12}H_{19}FNaO_5^+$ requires 298.0798; m.p.: 148 - 152 °C; $[\alpha]_D^{20}$ -70 (*c* 0.52, $CHCl_3$); ν_{max} (thin film): 2109 (s, N_3), 1789 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.56 (3H, d, H6, $J_{6,5}$ 7.3), 4.04 (1H, d, H2, $J_{2,3}$ 4.1), 4.12 (1H, t, H4, $J_{4,3} = J_{4,5}$ 1.7), 4.57 (1H, br-dq, H5, $J_{5,4}$ 1.7, $J_{5,6}$ 7.3), 4.87 (1H, dd, H3, $J_{3,4}$ 2.1, $J_{3,2}$ 4.0), 5.9 (1H, s, H7), 7.38 – 7.49 (5H, m, -Ar); d_C ($CDCl_3$, 100 MHz): 15.7 (C6), 62.0 (C2), 69.3 (C5), 72.5 (C3), 75.0 (C4), 93.0 (C7), 126.5, 128.7, 129.7, 137.0 (-Ar), 170.8 (C=O); *m/z* (ESI+ve): 298 ($[M + H]^+$, 100%).

3,5-*O*-Benzylidene-2-azido-2, 6-dideoxy-L-mannose 4.49

Diisobutylaluminium hydride (25% w/v in toluene, 0.83 mL, 1.46 mmol) was added dropwise to a solution of **4.47** (310 mg, 1.13 mmol) in anhydrous dichloromethane (10 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 3 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the consumption of starting material (R_f 0.42) and the formation of one product (R_f 0.50). Then the mixture was diluted with ethyl acetate (10 mL) and potassium sodium tartrate (sat. aq., ~1.0 mL) was added. After stirring for 5 h, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). Organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a crude that was further purified *via* flash chromatography (cyclohexane/ethyl acetate 3:1 to 1:1) to yield the lactol **4.49** as a white solid

(α:β ratio 2:1) (270 mg, 87%).

HRMS (ESI+ve): found 300.0901 $[M + Na]^+$; $C_{19}H_{30}NaO_5Si^+$ requires 300.0905; $[\alpha]_D^{20}$ -84 (*c* 0.75, $CHCl_3$); ν_{max} (thin film): 2110 (s, N_3); δ_H ($CDCl_3$, 500 MHz): 1.46 (3H, d, H6β, $J_{6,5}$ 7.3), 1.49 (3H, d, H6α, $J_{6,5}$ 7.3), 3.64 (1H, dd, H2α, $J_{2,3}$ 3.2, $J_{2,1}$ 4.7), 3.72 (1H, br-t, H4α, $J_{4,3} = J_{4,5}$ 1.8), 3.74 (1H, dd, H2β, $J_{2,1}$ 4.1, $J_{2,1}$ 5.0), 4.03 (1H, br-t, H4β, $J_{4,3} = J_{4,5}$ 1.8), 4.35 (1H, dq, H5β, $J_{5,4}$ 1.6, $J_{5,6}$ 7.3), 4.46 (1H, dq, H5α, $J_{5,4}$ 1.3, $J_{5,6}$ 7.3), 4.62 (1H, dd, H3β, $J_{3,4}$ 2.5, $J_{3,2}$ 4.1), 4.69 (1H, t, H3α, $J_{3,2} = J_{3,4}$ 3.2), 5.38 (1H, d, H1α, $J_{1,2}$ 4.7), 5.71 (1H, d, H1β, $J_{1,2}$ 5.0), 5.78 (1H, s, H7β), 5.80 (1H, s, H7α), 7.34-7.51 (10H, m, -Arα, -Arβ); δ_C ($CDCl_3$, 100 MHz): 16.6 (C6β), 16.7 (C6α), 63.5 (C2α), 68.7 (C2β), 70.2 (C5β), 71.1 (C5α), 74.1 (C3α), 74.8 (C3β), 77.0 (C4α, C4β), 93.2 (C7α, C7β), 98.3 (C1α), 100.6 (C1β), 126.2, 126.5, 128.6, 128.8, 129.3, 129.5, 137.5, 138.0 (-Arα, -Arβ); *m/z* (ESI+ve): 300 ($[M + Na]^+$, 100%).

2-Azido-2,6-dideoxy-L-glucose 4.25

DOWEX® 50WX8-200 resin (100 mg) was added to a solution of the protected lactol **4.48** (100 mg, 0.36 mmol) in water/1,4-dioxane (1:1, 5 mL). After stirring at rt for 21 h, the mass spectrum indicated the completion of reaction; the resin was filtered off and water was removed *in vacuo* to yield the azide **4.25** as a light yellow syrup (α:β pyranoside ratio 2:5) (67 mg, 99%).

HRMS (ESI+ve): found 190.0820 $[M + H]^+$; $C_6H_{13}O_5^+$ requires 190.0822; $[\alpha]_D^{20}$ +1.6 (*c* 0.80, water); ν_{max} (thin film): 2110 (s, N_3); δ_H (D_2O, 400 MHz): 1.26 (3H, d, H6α, $J_{6,5}$ 6.3), 1.28 (3H, d, H6β, $J_{6,5}$ 6.1), 3.20 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 9.3), 3.21 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.5), 3.27 (1H, dd, H2β, $J_{2,1}$ 8.1, $J_{2,3}$ 9.9), 3.43 (1H, t, H3β, $J_{3,2} = J_{3,4}$ 9.3), 3.46 (1H, dd, H2α, $J_{2,1}$ 3.4, $J_{2,3}$ 10.3), 3.47 (1H, dq, H5β, $J_{5,6}$ 6.2, $J_{5,4}$ 9.5), 3.79 (1H, dd, H3α, $J_{3,4}$ 9.3, $J_{3,2}$ 10.2), 3.90 (1H, dq, H5α, $J_{5,6}$ 6.3, $J_{5,4}$ 9.6), 4.67 (1H, d, H1β, $J_{1,2}$ 8.1), 5.29 (1H, d, H1α, $J_{1,2}$ 3.7); δ_C (D_2O, 100 MHz): 17.3 (C6α, C6β), 64.4 (C2α), 67.7 (C2β), 68.2 (C5α), 71.6 (C3α), 72.6 (C5β), 74.6 (C3β), 75.4 (C4β), 75.9 (C4α), 91.6 (C1α), 95.5 (C1β); *m/z* (ESI+ve): 212 ($[M + Na]^+$, 100%).

2-Amino-2,6-dideoxy-L-glucose hydrochloride 4.27

10% Palladium on charcoal (10 % wt., 15 mg) and aqueous HCl (2M, 0.1 mL) was added to a solution of **4.25** (67 mg, 0.36 mmol) in water (3 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 2 h at rt under hydrogen atmosphere until the completion of reaction was confirmed by mass spectrometry (*m/z* (ESI+ve): 164 $[M+H]^+$). After filtration, the solvent was removed *in vacuo* to afford the HCl salt of **4.27** as a brown gum (α:β pyranoside ratio 1:1) (70 mg, 98%).

HRMS (ESI+ve): found 164.0916 $[M + H]^+$; $C_6H_{14}NO_4^+$ requires 164.0917; $[\alpha]_D^{20}$ -49.6 (*c* 0.29, water) [lit.[53] $[\alpha]_D^{20}$ -50 (*c* 1.1, water)]; ν_{max} (thin film): finger print region only; δ_H (D_2O, 400 MHz): 1.27 (3H, d, H6α, $J_{6,5}$ 6.3), 1.30 (3H, d, H6β, $J_{6,5}$ 6.1), 3.00 (1H, dd, H2β, $J_{2,1}$ 8.7, $J_{2,3}$ 10.4), 3.21 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.3), 3.22 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 9.2), 3.30 (1H, dd, H2α, $J_{2,1}$ 3.5, $J_{2,3}$ 10.7), 3.54 (1H, dq, H5β, $J_{5,6}$ 6.2, $J_{5,4}$ 9.3), 3.62 (1H, t, H3β, $J_{3,2} = J_{3,4}$ 9.3), 3.82 (1H, t, H3α, $J_{3,2} = J_{3,4}$ 9.7), 3.95 (1H, dq, H5α, $J_{5,6}$ 6.3, $J_{5,4}$ 9.6), 4.91 (1H, d, H1β, $J_{1,2}$ 8.4), 5.38 (1H, d, H1α, $J_{1,2}$ 3.5); δ_C (D_2O, 100 MHz): 17.1, 17.2 (C6α, C6β), 55.1 (C2α), 57.5 (C2β), 68.2 (C5α), 70.0 (C3α), 72.3 (C3β), 72.8 (C5β), 75.5, 75.6 (C4α, C4β), 89.5 (C1α), 93.0 (C1β); *m/z* (ESI+ve): 164 ($[M + H]^+$, 100%).

3,5-*O*-Benzylidene-2-acetamido-2, 6-dideoxy-L-glucose 4.51

10% Palladium on charcoal (10 % wt., 20 mg) and acetic anhydride (0.050 mL, 0.51 mmol) were added in a solution of lactol **4.48** (140 mg, 0.51 mmol) in ethyl acetate/1,4-dioxane (2:1, 6 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 4 h at rt under hydrogen atmosphere until TLC (ethyl acetate/methanol, 9:1) showed the disappearance of starting material (R_f 0.98) and the formation of the product (R_f 0.71). After filtration, the solvent was removed *in vacuo* to afford a residue that was purified *via* flash chromatography (ethyl acetate/ethanol 15:1) to yield the protected azide **4.51** as a white solid (α:β ratio 3:1) (110.7 mg, 74%).

HRMS (ESI+ve): found 294.1335 $[M + H]^+$; $C_{15}H_{20}NO_5^+$ requires 294.1336; m.p.: 131 - 133 °C; $[\alpha]_D^{20}$ +2.3 (*c* 0.86, MeOH); ν_{max} (thin film): 1673 (s, C=O); δ_H (CD_3CN, 400 MHz): 1.41 (3H, d, H6α, $J_{6,5}$ 7.0), 1.45 (3H, d, H6β, $J_{6,5}$ 7.2), 1.91 (3H, s, CH_3(β)), 1.91 (3H, s, CH_3(α)), 3.85 (1H, t, H4β, $J_{4,3}$ =

$J_{4,5}$ 2.4), 3.88 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 3.1), 4.11 (1H, br-dd, H2β, $J_{2,3}$ 3.0, $J_{2,1}$ 9.9), 4.26 (1H, dq, H5α, $J_{5,4}$ 2.9, $J_{5,6}$ 7.0), 4.26 - 4.29 (1H, m, H2α), 4.39 (1H, dq, H5β, $J_{5,4}$ 1.7, $J_{5,6}$ 7.1), 4.41 (1H, m, H3α), 4.44 (1H, br-d, H3β, $J_{3,2}$ 2.9), 4.84 (2H, br-s, OHα and OHβ), 5.11 (1H, d, H1β, $J_{1,2}$ 9.8), 5.58 (1H, br-d, H1α, $J_{1,2}$ 3.8), 5.75 (1H, s, H7α), 5.82 (1H, s, H7β), 6.67 (2H, br-s, NHα and NHβ), 7.38 - 7.50 (10H, m, -Arα, -Arβ); δ_C (CD_3CN, 100 MHz): 16.9 (C6β), 17.1 (C6α), 23.0 (CH_3(β)), 23.2 (CH_3(α)), 59.3 (C2α), 63.0 (C2β), 70.2 (C5α), 71.2 (C5β), 76.3 (C4α), 77.6 (C4β), 78.5 (C3β), 79.5 (C3α), 93.8 (C7β), 94.1 (C7α), 96.2 (C1α), 103.2 (C1β), 127.1, 127.2, 129.2, 129.3, 129.8, 130.0, 140.1 (-Arα, -Arβ), 171.1 (C=O(α), C=O(β)); *m/z* (ESI+ve): 294 ($[M + H]^+$, 100%).

2-Acetamido-2,6-dideoxy-L-glucose 4.29

DOWEX® 50WX8-200 (100 mg) was added into a solution of **4.51** (110 mg, 0.38 mmol) in water / ethanol (1:1, 5 mL). After stirring at rt for 15 h, TLC (ethyl acetate/methanol, 9:1) indicated the disappearance of **4.51** (R_f 0.71) and the formation of a new spot (R_f 0.17). The resin was filtered off and solvent was removed *in vacuo* to yield **4.29** as a white solid (α:β pyranoside ratio 10:3) (69 mg, 90%).

HRMS (ESI+ve): found 228.0838 $[M + Na]^+$; $C_8H_{15}NNaO_5^+$ requires 228.0842; m.p.: 180 - 184 °C; $[\alpha]_D^{20}$ -53 (*c* 0.22, MeOH), $[\alpha]_D^{20}$ -48 (*c* 1.0, water, eq) [lit.[53] m.p.: 201 – 204 °C, $[\alpha]_D^{20}$ -54 to -15 (*c* 1.0, water)]; ν_{max} (thin film): 1633 (s, C=O); δ_H (CD_3OD, 500 MHz): 1.26 (3H, d, H6α, $J_{6,5}$ 6.3), 1.32 (3H, d, H6β, $J_{6,5}$ 6.3), 2.02 (3H, s, CH_3α), 2.02 (3H, s, CH_3β), 3.06 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.0), 3.07 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 8.8), 3.34 - 3.38 (1H, m, H5β), 3.42 (1H, dd, H3β, $J_{3,4}$ 8.8, $J_{3,2}$ 10.4), 3.62 (1H, dd, H2β, $J_{2,1}$ 8.5, $J_{2,3}$ 10.4), 3.68 (1H, dd, H3α, $J_{3,4}$ 8.9, $J_{3,2}$ 10.7), 3.87 (1H, dd, H2α, $J_{2,1}$ 3.5, $J_{2,3}$ 10.7), 3.91 (1H, dq, H5α, $J_{5,6}$ 6.3, $J_{5,4}$ 9.1), 4.59 (1H, d, H1β, $J_{1,2}$ 8.5), 5.06 (1H, d, H1α, $J_{1,2}$ 3.5); δ_C (CD_3OD, 100 MHz): 16.8 (C6α), 16.9 (C6β), 21.3 (CH_3β), 21.5 (CH_3α), 54.8 (C2α), 57.6 (C2β), 66.8 (C5α), 71.4 (C3α), 71.9 (C5β), 74.4 (C3β), 76.1 (C4β), 76.8 (C4α), 91.0 (C1α), 95.4 (C1β), 172.3 (C=O(β)), 172.8 (C=O(α)); *m/z* (ESI+ve): 228 ($[M + Na]^+$, 100%).

2-Azido-2,6-dideoxy-L-mannose 4.26

DOWEX@ 50WX8-200 (90 mg) was added into a solution of the protected *rhamnono*-azide **4.49** (47 mg, 0.17 mmol) in water/1,4-dioxane (1:1, 3 mL). After stirring at rt for 18 h, TLC (ethyl acetate/methanol 9:1) indicated the disappearance of **4.49** (Rf 0.50). Then resin was filtered off and solvent was removed *in vacuo* to yield the azide **4.26** as a light yellow gum (α:β pyranoside ratio 7:10) (30 mg, 95%).

HRMS (ESI+ve): found 212.0641 $[M + Na]^+$; $C_6H_{11}NaO_4^+$ requires 212.0642; $[\alpha]_D^{20}$ +9.4 (*c* 0.30, water, eq); ν_{max} (thin film): 2106 (s, N_3); δ_H (D_2O, 400 MHz): 1.25 (3H, d, H6α, $J_{6,5}$ 6.8), 1.27 (3H, d, H6β, $J_{6,5}$ 6.4), 3.30 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 9.5), 3.40 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.6), 3.41 (1H, dq, H5β, $J_{5,6}$ 6.3, $J_{5,4}$ 9.5), 3.81 (1H, dd, H3β, $J_{3,2}$ 3.8, $J_{3,4}$ 9.5), 3.87 (1H, dq, H5α, $J_{5,6}$ 6.4, $J_{5,4}$ 9.6), 3.99 - 4.02 (2H, m, H2α, H3α), 4.04 (1H, a-dq, H2β, $J_{2,1}$ 1.1, $J_{2,3}$ 3.7), 5.01 (1H, d, H1β, $J_{1,2}$ 1.1), 5.20 (1H, d, H1α, $J_{1,2}$ 1.1); δ_C (D_2O, 100 MHz): 17.2, 17.3 (C6α, C6β), 65.2 (C2α), 66.7 (C2β), 68.9 (C5α), 70.5 (C3α), 72.4 (C4β), 72.9 (C4α, C3β, C5β), 92.7 (C1α), 93.4 (C1β); *m/z* (ESI+ve): 212 ($[M + Na]^+$, 100%).

2-Amino-2,6-dideoxy-L-mannose hydrochloride salt 4.28

10% Palladium on charcoal (10 % wt., 10 mg) and aqueous HCl (2 M, 0.1 mL) was added to a solution of the azide **4.26** (71 mg, 0.38 mmol) in water (5 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 2 h at rt under hydrogen atmosphere until the completion of reaction was confirmed by mass spectrometry ($[M+H]^+$ 164). After filtration, the solvent was removed *in vacuo* to afford the HCl salt of **4.28** as an off white solid (α:β pyranoside ratio 1:2) (75 mg, 100%).

HRMS (ESI+ve): found 164.0917 $[M + H]^+$; $C_6H_{14}NO_4^+$ requires 164.0917; m.p.: 186-190 °C; $[\alpha]_D^{20}$ +21 (*c* 0.26, water, eq) [lit.[53] m.p.: 170 – 175 °C (decomp), $[\alpha]_D^{20}$ +25.5 (*c* 0.4, water)]; ν_{max} (thin film): finger print region only; δ_H (D_2O, 400 MHz): 1.28 (3H, d, H6α, $J_{6,5}$ 6.3), 1.30 (3H, d, H6β, $J_{6,5}$ 6.1), 3.31 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 9.6), 3.36 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.6), 3.48 (1H, dq, H5β, $J_{5,6}$ 6.1, $J_{5,4}$ 9.6), 3.63 (1H, dd, H2α, $J_{2,1}$ 1.3, $J_{4,5}$ 4.6), 3.69 (1H, dd, H2β, $J_{2,1}$ 1.2, $J_{2,3}$ 4.6), 3.92 (1H, dd, H3β, $J_{3,2}$ 4.6, $J_{3,4}$ 9.6), 3.97 (1H, dq, H5α, $J_{5,6}$ 6.3, $J_{5,4}$ 9.6), 4.10 (1H, dd, H3α, $J_{3,2}$ 4.7, $J_{3,4}$ 9.6), 5.17 (1H, d, H1β, $J_{1,2}$ 1.5), 5.32 (1H, d, H1α, $J_{1,2}$ 1.2); δ_C (D_2O, 100 MHz): 17.1, 17.2 (C6α, C6β), 55.1 (C2α), 56.3 (C2β),

67.1 (C3α), 68.5 (C5α), 69.7 (C3β), 71.9 (C4β), 72.1 (C4α), 72.8 (C5β), 90.8 (C1α), 91.3 (C1β); *m/z* (ESI+ve): 164 ([M + H]$^+$, 100%).

3,5-*O*-Benzylidene-2-acetamido-2,6-dideoxy-L-mannose 4.52

10% Palladium on charcoal (10 % wt., 20 mg) and acetic anhydride (0.043 mL, 0.43 mmol) were added in a solution of lactol **4.49** (120 mg, 0.43 mmol) in ethyl acetate/1,4-dioxane (2:1, 6 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 5 h at rt under hydrogen atmosphere until TLC (ethyl acetate/methanol, 9:1) showed the disappearance of starting material (R_f 0.6) and the formation of the product (R_f 0.86). After filtration, the solvent was removed *in vacuo* to afford a residue that was purified *via* flash chromatography (ethyl acetate/ethanol 20:1) to yield the **4.52** as a white solid (α:β ratio 1:10) (110 mg, 88%).

HRMS (ESI+ve): found 294.1330 [M + H]$^+$; $C_{15}H_{20}NO_5^+$ requires 294.1336; m.p.: 178 - 180 °C; $[\alpha]_D^{20}$ -26 (*c* 0.82, MeOH); ν_{max} (thin film): 1653 (s, C=O); δ_H (CD_3OD, 400 MHz): 1.46 (3H, d, H6α, $J_{6,5}$ 7.0), 1.49 (3H, d, H6β, $J_{6,5}$ 7.2), 2.00 (3H, s, CH_3(β)), 2.03 (3H, s, CH_3(α)), 3.82 (1H, t, H4α, $J_{4,3}$ = $J_{4,5}$ 3.0), 4.07 (1H, br-s, H4β), 4.32 (1H, q, H5β, $J_{5,6}$ 7.0), 4.39 - 4.35 (1H, m, H5α), 4.43 (1H, H2β, $J_{2,3}$ 4.3, $J_{2,1}$ 6.0), 4.53 - 4.56 (2H, m, H2α, H3α), 4.58 (1H, dd, H3β, $J_{3,4}$ 1.8, $J_{3,2}$ 4.1), 5.33 (1H, d, H1α, $J_{1,2}$ 5.2), 5.46 (1H, d, H1β, $J_{1,2}$ 6.0), 5.82 (1H, s, H7β), 5.85 (1H, s, H7α), 7.36 - 7.58 (10H, m, -Arα, -Arβ); δ_C (CD_3OD, 100 MHz): 17.0 (C6β), 17.8 (C6α), 23.2 (CH_3(α), CH_3(β)), 57.2 (C2α), 62.4 (C2β), 72.2 (C5α), 72.7 (C5β), 74.5 (C3α), 75.4 (C3β), 78.2 (C4β), 79.6 (C4α), 94.3 (C7β), 95.9 (C7α), 97.6 (C1α), 102.8 (C1β), 128.4, 128.5, 129.9, 130.1, 130.7, 130.8, 140.7 (-Arα, -Arβ), 174.3 (C=O(α)), 174.6 (C=O(β)); *m/z* (ESI+ve): 294 ([M + H]$^+$, 100%).

2-Acetamido-2,6-dideoxy-L-mannose 4.30

DOWEX® 50WX8-200 (90 mg) was added into a solution of **4.52** (90 mg, 0.31 mmol) in water/ethanol (1:1, 5 mL). After stirring at rt for 15 h, TLC (ethyl acetate/methanol 9:1) indicated the disappearance of **4.52** (R_f 0.86) and the formation of a new spot (Rf 0.17). The

resin was filtered off and the solvent was removed *in vacuo* to yield **4.30** as a white solid (α:β pyranoside ratio 5:2) (60 mg, 98%).

HRMS (ESI+ve): found 228.0840 $[M + H]^+$; $C_8H_{15}NNaO_5^+$ requires 228.0842; m.p.: 144 - 146 °C; $[\alpha]_D^{20}$ +8.9 (*c* 0.36, MeOH); ν_{max} (thin film): 1636 (s, C=O); δ_H (CD_3OD, 400 MHz): 1.17 (3H, d, H6α, $J_{6,5}$ 6.3), 1.22 (3H, d, H6β, $J_{6,5}$ 6.0), 1.91 (3H, s, CH_3β), 1.96 (3H, s, CH_3α), 3.11 (1H, t, H4β, $J_{4,3} = J_{4,5}$ 9.3), 3.14-3.18 (1H, m, H5α), 3.22 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.6), 3.50 (1H, dd, H3β, $J_{3,2}$ 4.6, $J_{3,4}$ 9.2), 3.75 (1H, dq, H5β, $J_{5,6}$ 6.3, $J_{5,4}$ 9.3), 3.86 (1H, dd, H3α, $J_{3,2}$ 4.7, $J_{3,4}$ 9.6), 4.15 (1H, dd, H2α, $J_{2,1}$ 1.4, $J_{4,5}$ 4.7), 4.31 (1H, dd, H2β, $J_{2,1}$ 1.3, $J_{2,3}$ 4.4), 4.72 (1H, d, H1β, $J_{1,2}$ 1.5), 4.85 (1H, d, H1α, $J_{1,2}$ 1.1); δ_C (CD_3OD, 100 MHz): 18.1 (C6β), 18.2 (C6α), 22.7 (CH_3α), 22.9 (CH_3β), 50.0 (C5α), 55.6 (C2α), 56.1 (C2β), 69.1 (C5β), 70.2 (C3α), 74.0 (C3β), 74.2 (C4β), 74.4 (C4α), 94.8 (C1α), 95.0 (C1β), 174.3 (C=O(α)), 175.5 (C=O(β)); *m/z* (ESI+ve): 228 ($[M + Na]^+$, 100%).

3,5-*O*-Benzylidene-1-difluoro-1,6-dideoxy-L-mannofuranose 4.57

Diethylaminosulfur trifluoride (DAST) (0.03 ml, 0.24 mmol) was added to a solution of lactone **4.22** (50 mg, 0.2 mmol) in DCM (2 mL) at -78 °C under argon atmosphere. The reaction mixture was warmed to rt and stirred for 4 h until mass spectrometry showed the formation of **4.57** ($[M + Na]^+$ 295) and the consumption of starting material ($[M + Na]^+$ 275). After sodium bicarbonate (sat., aq., 1 mL) was slowly added to the reaction mixture, organic layer was separated and dried ($MgSO_4$). After removing solvent *in vacuo*, the resulting residue was purified by flash chromatography (cyclohexane/ethyl acetate, 3:1) to obtain **4.57** with minor amount of inseparable impurities (27 mg, 50%).

NMR data of **4.57**: δ_H ($CDCl_3$, 400 MHz): 1.37 (3H, d, H6, $J_{6,5}$ 7.3), 3.90 (1H, ddd, H4, $J_{4,3}$ 2.0, *J* 2.7, *J* 6.6), 4.28 – 4.36 (2H, m, H2, H5), 4.44 (1H, ddd, H3, $J_{3,4}$ 2.0, *J* 2.5, *J* 4.4), 5.7 (1H, s, H7), 7.28 – 7.38 (5H, m, Ar); δ_C ($CDCl_3$, 100 MHz): 15.7 (C6), 68.9 (C5), 70.7 (C3, d, *J* 4.8), 74.9 (C2, dd, *J* 25.4, *J* 35.0), 75.6 (C4), 92.9 (C7), 128.6 (C1, dd, *J* 178.0, *J* 257.5), 126.4, 128.4, 129.5, 137.3 (Ar); δ_F ($CDCl_3$, 376 MHz): -78.0 (dd, *J* 6.9, *J* 144), -74.6 (ddd, *J* 6.9, *J* 8.7, *J* 144); *m/z* (ESI+ve): 273 ($[M + H]^+$, 22%), 295 ($[M + Na]^+$, 100%).

1, 3, 4-Tri-*O*-acetyl-2-fluoro-2, 6-dideoxy-β-L-mannose 4.59

Selectfluor (2.16 g, 6.1 mmol) was added to a solution of diacetyl rhamnal **4.58** (1.0 g, 4.6 mmol) in acetonitrile/water (40 mL, 3:1) at 0 °C. The reaction mixture was stirred at rt for 16 h until TLC (cyclohexane/ethyl acetate, 1:1) showed the disappearance of starting material (R_f 0.81). After removing the solvent *in vacuo*, the mixture was dissolved into ethyl acetate (20 mL) and was washed with water (20 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a residue (900 mg) which was dissolved into acetic anhydride/pyridine (20 mL, 1:1). The mixture was stirred for 6 h until TLC (hexane/ethyl acetate, 1:1) showed the formation of two major products (R_f 0.80, R_f 0.78). After removing the solvent *in vacuo*, the residue was purified *via* flash chromatography (cyclohexane/ethyl acetate, 7:1) to yield a fluoro-sugar mixture of **4.59** and **4.60** (500 mg, 38%) and some **4.59** (400 mg, 30%) with minor impurities. **4.59** was further purified by crystallization in ether to obtain pure **4.59** as a white solid (360 mg, 27%)

HRMS (ESI+ve): found 315.0851 $[M + Na]^+$; $C_{12}H_{19}FNaO_5^+$ requires 315.0851; m.p.: 124 - 126 °C; $[\alpha]_D^{20}$ -84 (*c* 0.40, MeOH); ν_{max} (thin film): 1760, 1742 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.24 (3H, d, H6, $J_{6,5}$ 6.4), 2.07 (3H, s, CH_3), 2.12 (3H, s, CH_3), 2.16 (3H, s, CH_3), 3.96 (1H, dq, H5, $J_{5,6}$ 6.4, $J_{5,4}$ 8.8), 4.75 (1H, dt, H2, $J_{2,1} = J_{2,3}$ 2.2, $J_{2,F}$ 48.9), 5.18 (1H, ddd, H4, $J_{4,F}$ 2.0, $J_{4,5}$ 8.3, $J_{4,3}$ 10.3), 5.22 (1H, ddd, H3, $J_{3,2}$ 2.6, $J_{3,4}$ 10.3, $J_{3,F}$ 24.2), 6.21 (1H, dd, H1, $J_{1,2}$ 2.2, $J_{1,F}$ 7.0); δ_C ($CDCl_3$, 100 MHz): 17.3 (C6), 20.6 (CH_3), 20.7 (CH_3), 20.9 (CH_3), 68.8 (C5), 69.4 (C3, d, $J_{3,F}$ 17), 70.2 (C4), 86.2 (C2, d, $J_{2,F}$ 180), 90.1 (C1, d, $J_{1,F}$ 31), 168.3 (<u>C</u>=O), 169.6 (<u>C</u>=O), 170.3 (<u>C</u>=O); δ_F ($CDCl_3$, 376 MHz): -203.6 (dddd, $J_{F,4}$ 2.3, $J_{F,1}$ 7.0, $J_{3,F}$ 26.0, $J_{3,F}$ 48.0); *m/z* (ESI+ve): 315 ($[M + H]^+$, 100%).

2,6-Dideoxy-2-fluoro-L-mannose 4.32

Trifluoroacetic acid (1 mL) was added to a solution of **4.59** (170 mg) in water (2 mL). The mixture was stirred at 60 °C for 12 h until TLC (ethyl acetate/methanol 9:1) showed the disappearance of starting material (R_f 0.96) and the formation of one product (R_f 0.68). The

solvent was removed *in vacuo* to obtain 2,6-dideoxy-2-fluoro-mannose **4.32** as a colorless syrup (α:β pyranoside ratio 5:3) (96 mg, 100%).

HRMS (ESI+ve): found 189.0532 $[M + Na]^+$; $C_6H_{11}FNaO_4^+$ requires 189.0534; $[\alpha]_D^{20}$ -3.5 (c 0.91, water, eq); δ_H (D_2O, 400 MHz): 1.29 (3H, d, H6α, $J_{6,5}$ 6.3), 1.31 (3H, d, H6β, $J_{6,5}$ 6.1), 3.40 (1H, dt, H4β, $J_{4,F}$ 1.0, $J_{4,3} = J_{4,5}$ 9.5), 3.45 (1H, t, H4α, $J_{4,3} = J_{4,5}$ 9.7), 3.43 - 3.50 (1H, m, H5β), 4.72 (1H, ddd, H3β, $J_{3,2}$ 2.6, $J_{3,4}$ 9.5, $J_{3,F}$ 30.8), 3.86 (1H, ddd, H3α, $J_{3,2}$ 2.5, $J_{3,4}$ 9.7, $J_{3,F}$ 31.4), 3.90 - 3.95 (1H, m, H5α), 4.75 (1H, dt, H2α, $J_{2,3} = J_{2,1}$ 2.2, $J_{2,F}$ 49.3), 4.79 (1H, dd, H2β, $J_{2,3}$ 2.5, $J_{2,F}$ 51.2), 4.98 (1H, d, H1β, $J_{1,F}$ 20.5), 5.30 (1H, dd, H1α, $J_{1,2}$ 1.8, $J_{1,F}$ 7.5); δ_C (D_2O, 100 MHz): 17.2 (C6α, C6β), 68.9 (C5α), 69.7 (C3α, d, $J_{3,F}$ 18), 72.1 (C3β, d, $J_{3,F}$ 18), 72.4 (H4β), 72.6 (H5β), 72.8 (H4α), 91.1 (C2α, d, $J_{2,F}$ 171), 91.8 (C1α, d, $J_{1,F}$ 45), 92.1 (C2β, d, $J_{2,F}$ 179), 92.7 (C1β, d, $J_{1,F}$ 16); δ_F (D_2O, 376 MHz): -204.5 (ddd, $J_{F,1}$ 7.0, $J_{F,3}$ 32.0, $J_{F,2}$ 49.2), -223.0 (ddd, $J_{F,1}$ 20.6, $J_{F,3}$ 30.9, $J_{F,2}$ 51.5); *m/z* (ESI+ve): 189 ($[M + Na]^+$, 100%).

2,6-Dideoxy-2-fluoro-L-glucose 4.31

Trifluoroacetic acid (2.5 mL) was added to a solution of the mother liquor obtained from the recrystallization of **4.59** (200 mg, containing **4.59** and **4.60**) in water (2.5 mL). The mixture was stirred at 60 °C for 12 h until mass spectrum showed the completion of the reaction. After removing solvent *in vacuo*, **4.31** (5.2 mg) was purified from the mixture (containing R4 and R5) by preparative HPLC.

$[\alpha]_D^{20}$ -38 (*c* 0.26, water, eq) [lit.[80] $[\alpha]_D^{20}$ -35 (*c* 0.27, water)]; δ_H (D_2O, 400 MHz): 1.25 (3H, d, H6α, $J_{6,5}$ 6.3), 1.28 (3H, d, H6β, $J_{6,5}$ 6.3), 3.19 (1H, dd, H4α, $J_{4,3}$ 9.3, $J_{4,5}$ 9.5), 3.21 (1H, dd, H4β, $J_{4,3}$ 9.2, $J_{4,5}$ 9.6), 3.52 (1H, dq, H5β, $J_{5,6}$ 6.3, $J_{5,4}$ 9.6), 3.73 (1H, td, H3β, $J_{3,4} = J_{3,2}$ 9.2, $J_{3,F}$ 15.1), 3.90 (1H, ddd, H3α, $J_{3,4}$ 9.3, $J_{3,2}$ 9.5, $J_{3,F}$ 13.3), 3.91 (1H, dq, $J_{5,6}$ 6.3, $J_{5,4}$ 9.5), 4.09 (1H, ddd, H2β, $J_{2,1}$ 7.9, $J_{2,3}$ 9.2, $J_{2,F}$ 51.5), 4.42 (1H, ddd, H2α, $J_{2,1}$ 4.0, $J_{2,3}$ 9.5, $J_{2,F}$ 49.3), 4.87 (1H, dd, H1β, $J_{1,F}$ 2.5, $J_{1,2}$ 7.9), 5.38 (1H, d, H1α, $J_{1,2}$ 4.0); δ_C (D_2O, 100 MHz): 17.0 (C6α), 17.1 (C6β), 67.8 (C5α, d, $J_{5,F}$ 1.2), 71.4 (C3α, d, $J_{3,F}$ 17), 72.6 (C5β, d, $J_{5,F}$ 1.3), 74.3 (H3β, $J_{3,F}$ 17), 75.0 (H4β, d, $J_{4,F}$ 7.9), 75.1 (H4α, d, $J_{4,F}$ 7.9), 90.0 (C1α, d, $J_{1,F}$ 21.3), 91.0 (C2α, d, $J_{2,F}$ 185.6), 93.7 (C2β, d, $J_{2,F}$ 182.8), 93.8 (C1β, d, $J_{1,F}$ 23.5); δ_F (D_2O,

376 MHz): -199.4 - -199.0 (m, Fα and Fβ); *m/z* (ESI+ve): 189 ([M + Na]$^+$, 100%).

4.5.2 Synthesis of trideoxy-1,5-iminohexitols and trideoxy-1,4-iminohexitols

L-rhamnono-1,4-lactone 4.63

and

L-rhamnono-1,5-lactone 4.64

Bromine (6.3 mL, 120 mmol) was added dropwise to a stirred suspension of barium carbonate (32 g, 164 mmol) in a solution of L-rhamnose **4.1** (20 g, 109 mmol) in water (130 mL) at 0 °C. After stirring at room temperature for 8 h, TLC (methanol/ethyl acetate, 1:9) indicated the formation of a major product (R_f 0.4) and a minor product (R_f 0.45). The reaction mixture was filtered and nitrogen was bubbled through overnight until decolorized. The solvent was removed and the residue was extracted with boiling acetone (250 mL x 3). The resulting mixture was combined, filtered and solvent was removed to obtain a residue that was used for next step without further purification. Small amount of γ-lactone **4.63** was recrystallized from acetone as a white solid for characterization.

m.p. 150 - 152 °C; $[\alpha]_D^{25}$ -41 (*c* 1, water, eq) [lit.[81] m.p. 148 - 150 °C; $[\alpha]_D^{24}$ -40 (*c* 2.0, water)]; ν_{max} (thin film): 3400 (br, OH), 1776 (s, C=O); ^{1}H NMR data of **4.63**: δ_H (D_2O, 400 MHz): 1.25 (3H, d, H6, $J_{6,5}$ 6.3), 4.03 (1H, m, H5), 4.17 (1H, dd, H4, $J_{4,3}$ 2.4, $J_{4,5}$ 8.3), 4.53 (1H, dd, H3, $J_{3,4}$ 2.4, $J_{3,2}$ 4.5), 4.65 (1H, d, H2, $J_{2,3}$ 4.6); *m/z* (ESI+ve): 185 ([M + Na]$^+$, 100%).

2,3-*O*-Isopropylidene-L-rhamnono-1,4-lactone 4.33

and

2,3-*O*-Isopropylidene-L-rhamnono-1,5-lactone 4.65

Sulfuric acid (0.5 mL) and anhydrous $CuSO_4$ (1.0 g) was added to a solution of the crude mixture of **4.63** and **4.64** (10.0 g, 61.7 mmol) in acetone (100 mL). The mixture was stirred at room temperature for 20 h until TLC (cyclohexane/ethyl acetate 1:1) showed the formation of one

major spot (R_f 0.41). Sodium carbonate (~2 g) was added to neutralize the reaction mixture that was then filtered and solvent was removed to give a residue. It was purified by flash chromatography (cyclohexane/ethyl acetate 6:1 to 1:1) to obtain a mixture of γ-lactone **4.33** and δ-lactone **4.65** in a ratio of 5:1 (7.5 g, 60%).

4.33 and **4.65** mixture: δ_H ($CDCl_3$, 400 MHz): 1.38 (3H, d, H6γ, $J_{6,5}$ 6.1), 1.42 (3H, s, CH_3γ), 1.46 (3H, d, H6δ, $J_{6,5}$ 6.3), 1.48 (6H, br-s, CH_3δ, CH_3γ), 1.50 (3H, s, CH_3δ), 3.60 (1H, dd, H4δ, $J_{4,3}$ 6.3, $J_{4,5}$ 9.8), 4.11 (1H, dq, H5γ, $J_{5,6}$ 6.1, $J_{4,5}$ 8.4), 4.19 (1H, dd, H4γ, $J_{4,3}$ 3.8, $J_{4,5}$ 8.4), 4.22-4.27 (1H, m, H5δ), 4.47 (1H, dd, H3δ, $J_{3,4}$ 6.3, $J_{3,2}$ 8.3), 4.72 (1H, d, H2δ, $J_{2,3}$ 8.3), 4.85 (1H, d, H2γ, $J_{2,3}$ 5.7), 4.95 (1H, dd, H3γ, $J_{3,4}$ 3.8, $J_{3,2}$ 5.7); δ_C ($CDCl_3$, 100 MHz): 17.4 (C6δ), 20.3 (C6γ), 24.2 (CH_3δ), 25.4 (CH_3δ), 26.2 (CH_3γ), 27.1 (CH_3γ), 66.1 (C5γ), 72.0 (C2δ), 74.2 (C4δ), 74.9 (C5δ), 76.2, 76.4 (C2γ, C3γ), 79.6 (C3δ), 82.4 (C4γ), 112.5 ($\underline{C}(CH_3)_2$δ), 114.8 ($\underline{C}(CH_3)_2$γ), 169.7 (C1δ), 173.9 (C1γ); *m/z* (ESI+ve): 225 ($[M + Na]^+$, 100%).

5-Azido-5,6-dideoxy-2,3-*O*-isopropylidene-D-gulono-1,4-lactone 4.66

and

4-Azido-4,6-dideoxy-2,3-*O*-isopropylidene-L-talono-1,5-lactone 4.67

Trifluoromethanesulphonic anhydride (0.83 mL, 4.93 mmol) and anhydrous pyridine (0.80 mL, 9.84 mmol) was added dropwise to a solution of the mixture of **4.33** and **4.65** (500 mg, 2.46 mmol) in dichloromethane (15 mL) at -20 °C. After stirring at -20 °C for 3 h, TLC (cyclohexane/ethyl acetate, 1:1) showed the formation of a major product (R_f 0.70) and the disappearance of starting material (R_f 0.41). The reaction mixture was then diluted with dichloromethane (5 mL) and washed with HCl (2 M, aq. 20 mL) and aqueous $NaHCO_3$ (sat., 20 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed *in vacuo* to give a mixture of the crude triflates as a yellow solid (1.0 g).

Sodium azide (191 mg, 2.95 mmol) was added to a solution of the crude triflates (1.0 g) in anhydrous DMF (20 mL). The mixture was stirred at room temperature for 15 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the formation of one major product (R_f 0.59) and one

minor product (R_f 0.20). After dilution with ethyl acetate (10 mL), the mixture was washed with half saturated brine (30 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a residue that was purified by flash chromatography (cyclohexane/ethyl acetate 7:1 to 1:1) to yield the γ-lactone **4.66** as a white solid (337 mg, 60%) and δ-lactone **4.67** as a white solid (100 mg, 18%).

4.66 HRMS (ESI+ve): found 250.0797 $[M + Na]^+$; $C_9H_{13}N_3NaO_4^+$ requires 250.0798; m.p. 66 - 68 °C; $[\alpha]_D^{25}$ -78.3 (*c* 1, $CHCl_3$); ν_{max} (thin film): 2089 (s, N_3), 1769 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.33 (3H, d, H6, $J_{6,5}$ 6.7), 1.39 (3H, s, CH_3), 1.48 (3H, s, CH_3), 3.90 (1H, dq, H5, $J_{5,6}$ 6.7, $J_{4,5}$ 9.2), 4.26 (1H, dd, H4, $J_{4,3}$ 3.5, $J_{4,5}$ 9.2), 4.74 (1H, dd, H3, $J_{3,4}$ 3.5, $J_{3,2}$ 5.2), 4.85 (1H, d, H2, $J_{2,3}$ 5.2); δ_C ($CDCl_3$, 100 MHz): 15.8 (C6), 26.3 (CH_3), 27.1 (CH_3), 57.8 (C5), 75.9 (C3), 76.5 (C2), 82.2 (C4), 114.8 ($\underline{C}(CH_3)_2$), 173.2 (C1); *m/z* (ESI+ve): 250 ($[M + Na]^+$, 100%).

4.67: HRMS (ESI+ve): found 250.0799 $[M + Na]^+$; $C_9H_{13}N_3NaO_4^+$ requires 250.0798; m.p. 60 - 62 °C; $[\alpha]_D^{25}$ +156.1 (*c* 1, $CHCl_3$); ν_{max} (thin film): 2112 (s, N_3), 1747 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.41 (3H, s, CH_3), 1.45 (3H, d, H6, $J_{6,5}$ 6.4), 1.60 (3H, s, CH_3), 4.02 (1H, d, H4, $J_{4,3}$ 4.7), 4.34 (1H, q, H5, $J_{5,6}$ 6.4), 4.63 (1H, d, H2, $J_{2,3}$ 9.2), 4.77 (1H, dd, H3, $J_{3,4}$ 4.7, $J_{3,2}$ 9.2); δ_C ($CDCl_3$, 100 MHz): 18.0 (C6), 24.5 (CH_3), 25.4 (CH_3), 59.5 (C4), 71.2 (C2), 72.1 (C5), 75.2 (C3), 112.2 ($\underline{C}(CH_3)_2$), 168.5 (C1); *m/z* (ESI+ve): 250 ($[M + Na]^+$, 100%).

5-Azido-5,6-dideoxy-2,3-*O*-isopropylidene-D-gulofuranose 4.68

Diisobutylaluminium hydride (25% w/v in toluene, 6.0 ml, 10.56 mmol) was added dropwise to a solution of **4.66** (800 mg, 3.52 mmol) in dichloromethane (20 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 6 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated formation of one product (R_f 0.63) and the disappearance of starting material (R_f 0.59). Then the mixture was diluted with ethyl acetate (10 mL) and potassium sodium tartrate (sat, *aq*., ~1.0 mL) was added. After stirring for 15 h, the mixture was diluted with water (20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a crude that was further purified by flash chromatography

(cyclohexane/ethyl acetate 7:1 to 4:1) to yield the lactol **4.68** as a white solid as single α anomer (742 mg, 92%).

HRMS (ESI+ve): found 252.0956 $[M + Na]^+$; $C_9H_{15}N_3NaO_4^+$ requires 252.0954; m.p.: 40 - 42 °C; ν_{max} (thin film): 3400 (br, OH), 2125 (s, N_3); $[\alpha]_D^{25}$ -46 (*c* 0.83, $CHCl_3$); δ_H (CD_3CN, 400 MHz): 1.22 (3H, d, H6, $J_{6,5}$ 6.6 Hz), 1.29 (3H, s, CH_3), 1.41 (3H, s, CH_3), 3.77 (1H, dq, H5, $J_{5,6}$ 6.6, $J_{5,4}$ 9.3), 3.96 (1H, dd, H4, $J_{4,3}$ 3.7, $J_{4,5}$ 9.3), 4.21 (1H, d, OH, $J_{OH,1}$ 3.8), 4.55 (1H, d, H2, $J_{2,3}$ 5.9), 4.74 (1H, dd, H3, $J_{3,4}$ 3.7, $J_{3,2}$ 5.9), 5.26 (1H, d, H1, $J_{1,OH}$ 3.9); δ_C (CD_3CN, 100 MHz): 16.5 (C6), 25.0 (CH_3), 26.4 (CH_3), 58.7 (C5), 80.7 (C3), 84.3 (C4), 86.9 (C2), 101.7 (C1), 113.1 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 252 ($[M + Na]^+$, 100%).

1,5-Imino-2,3-*O*-isopropylidene-1,5,6-trideoxy-D-gulitol 4.69

Palladium black (10 % wt., 10 mg) was added to a solution of **4.68** (100 mg, 0.43 mmol) in ethanol (5 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 15 h at room temperature under a hydrogen atmosphere until TLC (cyclohexane/ethyl acetate, 1:1) indicated the consumption of starting material (R_f 0.63) and the formation of one product (R_f 0.10). After filtration, the solvent was removed *in vacuo* to afford **4.69** as a yellow solid (81 mg, 100%).

HRMS (ESI+ve): found 188.1281 $[M + H]^+$; $C_9H_{18}NO_3^+$ requires 188.1281; m.p.: 70 - 74 °C; $[\alpha]_D^{25}$ -63 (*c* 0.85, $CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 1.13 (3H, d, H6, $J_{6,5}$ 6.7), 1.34 (3H, s, CH_3), 1.49 (3H, s, CH_3), 2.71 (1H, dd, H1, $J_{1,2}$ 8.2, J_{gem} 11.9), 3.03 (1H, dq, H5, $J_{5,4}$ 1.5, $J_{5,6}$ 6.6), 3.12 (1H, dd, H1', $J_{1',2}$ 7.2, J_{gem} 11.6), 3.74 (1H, br-s, H4), 4.20 - 4.25 (2H, m, H2, H3); δ_C ($CDCl_3$, 100 MHz): 16.9 (C6), 26.7 (CH_3), 28.6 (CH_3), 49.2 (C1), 51.5 (C5), 69.7 (C4), 71.0 (C2), 77.2 (C3), 109.4 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 188 ($[M + H]^+$, 100%).

***N*-Benzyl-1,5-imino-2,3-*O*-isopropylidene-1,5,6-trideoxy-D-gulitol 4.70**

Benzylaldehyde (0.05 mL, 0.48 mmol) and sodium cyanoborohydride (30 mg, 0.48 mmol) were added to a solution of **4.69** (60 mg, 0.32 mmol) in methanol (5 mL). The mixture was stirred at

room temperature for 18 h until TLC (cyclohexane/ethyl acetate 1:1) showed the formation of the only product (R_f 0.40). After removing solvent *in vacuo*, the residue was dissolved in ethyl acetate (5 mL) and washed with water (2 x 10 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a crude residue that was purified by flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1) to yield **4.70** as a white solid (70 mg, 97%).

HRMS (ESI+ve): found 278.1749 $[M + H]^+$; $C_{16}H_{24}NO_3^+$ requires 278.1750; m.p. 26 - 28 °C; $[\alpha]_D^{25}$ +10.4 (*c* 1.46, MeOH), δ_H (CD_3OD, 400 MHz): 1.04 (3H, d, H6, $J_{6,5}$ 6.9), 1.35 (3H, s, CH_3), 1.53 (3H, s, CH_3), 2.82 (1H, dd, H1, $J_{1,2}$ 3.2, J_{gem} 13.7), 2.88 (1H, dd, H1', $J_{1',2}$ 4.1, J_{gem} 13.7), 2.94 (1H, dq, H5, $J_{5,4}$ 4.6, $J_{5,6}$ 6.9), 3.84 (1H, d, CH_2, J_{gem} 13.3), 3.78 (1H, d, CH_2, J_{gem} 13.3), 3.86 (1H, H4, dd, $J_{4,5}$ 4.6, $J_{4,3}$ 7.1), 4.06 (1H, dd, H3, $J_{3,2}$ 6.0, $J_{3,4}$ 7.0), 4.28 (1H, ddd, H2, $J_{2,1}$ 3.2, $J_{2,1'}$ 4.1, $J_{2,3}$ 6.0), 7.26 - 7.41 (5H, m, Ar); δ_C (CD_3OD, 100 MHz): 5.72 (C6), 25.0 (CH_3), 27.2 (CH_3), 56.2 (C1), 58.0 (C5), 47.6 (CH_2), 71.7 (C4), 73.2 (C2), 76.8 (C3), 108.2 ($\underline{C}(CH_3)_2$), 126.7, 127.9, 128.6, 138.7 (Ar); *m/z* (ESI+ve): 278 ($[M + H]^+$, 100%).

1,5-Imino-1,5,6-trideoxy-D-gulitol (5-*epi*-Rhamnojirimycin, 5-*epi*-RHJ) 4.6

4.69 (81 mg, 0.43 mmol) was dissolved in a mixture of trifluoroacetic acid/water/1,4-dioxane (2:1:1, 4 mL). The mixture was stirred at room temperature for 12 h until mass spectrum showed the formation of the only product ($[M+H]^+$ 148). Solvent was removed *in vacuo* to obtain a residue that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After loading, the column was washed with water and 1,4-dioxane and then water again, pure product was released with aqueous ammonia (2 M)/1,4-dioxane (1:1). Then solvent was removed *in vacuo* to yield the title compound **4.6** as a brown oil (60 mg, 95%).

HRMS (ESI+ve): found 148.0968 $[M + H]^+$; $C_6H_{14}NO_3^+$ requires 148.0968; $[\alpha]_D^{25}$ -7.7 (*c* 0.93, water) [lit.[82] $[\alpha]_D^{25}$ -3.0 (*c* 1.0, water)]; δ_H (D_2O, 400 MHz): 1.32 (3H, d, H6, $J_{6,5}$ 6.9), 3.11 (1H, t, H1, $J_{1,2} = J_{gem}$ 11.7), 3.23 (1H, dd, H1', $J_{1',2}$ 4.8, J_{gem} 12.0), 3.59 (1H, br-q, H5, $J_{5,6}$ 6.9), 3.95 (1H, dd, H4,

$J_{4,5}$ 1.7, $J_{4,3}$ 4.7), 4.05 (1H, dd, H3, $J_{3,2}$ 3.2, $J_{3,4}$ 4.2), 4.19 (1H, ddd, H2, $J_{2,3}$ 3.1, $J_{2,1'}$ 4.8, $J_{2,1}$ 11.1); δ_C (D_2O, 100 MHz): 13.6 (C6), 42.8 (C1), 50.7 (C5), 62.8 (C2), 69.1 (C3), 70.0 (C4); *m/z* (ESI+ve): 148 ($[M + H]^+$, 100%).

_N_-Benzyl-1,5-imino-1,5,6-trideoxy-D-gulitol 4.71

4.70 (170 mg, 0.61 mmol) was dissolved in a mixture of trifluoroacetic acid/water/1,4-dioxane (2:1:1, 4 mL). The mixture was stirred at room temperature for 20 h until TLC (ethyl acetate/methanol, 9:1) indicated the formation of product (R_f 0.16) and the consumption of starting material (R_f 0.92). Solvent was removed *in vacuo* to obtain a residue that was then loaded with water (~2 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After loading, the column was washed with water and 1,4-dioxane and then water again and the pure product was released with aqueous ammonia (2 M)/1,4-dioxane (1:1). Then solvent was removed *in vacuo* to give the title compound **4.71** as a light yellow syrup (120 mg, 83%).

HRMS (ESI+ve): found 238.1439 $[M + H]^+$; $C_{13}H_{20}NO_3^+$ requires 238.1438; $[\alpha]_D^{25}$ -5.3 (*c* 0.30, MeOH); δ_H (CD_3OD, 400 MHz): 1.64 (3H, d, H6, $J_{6,5}$ 6.6), 2.96 (2H, d, H1, $J_{1,2}$ 8.1), 3.52 (1H, q, H5, $J_{5,6}$ 6.6), 3.85 (1H, br-s, H4), 3.92 (1H, br-s, H3), 4.16 (1H, br-dd, H2), 4.32 (1H, d, CH_2, 13.3), 4.54 (1H, d, CH_2, 13.3), 7.52 - 7.60 (5H, m, Ar); δ_C (CD_3OD, 100 MHz): 14.8 (C6), 50.8 (C1), 58.1, 58.4 (C5, CH_2), 64.1 (C2), 70.4 (C3), 73.5 (C4), 129.6, 130.5, 131.5, 133.3 (Ar); *m/z* (ESI+ve): 238 ($[M + H]^+$, 100%).

4-Azido-4,6-dideoxy-2,3-*O*-isopropylidene-L-talopyranose 4.72

Diisobutylaluminium hydride (25% w/v in toluene, 0.19 ml, 0.34 mmol) was added slowly to a solution of δ-lactone **4.67** (68.9 mg, 0.31 mmol) in dry THF at -78 °C. After stirring at -78 °C for 3 h, mass spectrometry indicated incomplete conversion. More diisobutylaluminium hydride (25% w/v in toluene, 0.38 ml, 0.64 mmol) was added. After a total of 5 hours, when TLC (acetone/toluene 3:10) showed the formation of two anomers of product (R_f 0.50, 0.63) and

mass spectrometry indicated full conversion ([M + Na]$^+$ 252), the reaction was quenched with saturated aqueous sodium potassium tartrate (3 mL) and left stirring at room temperature overnight. The mixture was diluted with ethyl acetate (50 mL) and washed with water (50 mL), which was further extracted with ethyl acetate (50 mL). The combined organic phases were dried (MgSO4) and reduced to a crude oil, which was purified using flash column chromatography (acetone/toluene 1:20 to 3:10) to yield the product lactol **4.72** as a syrupy mixture of anomers (53 mg, 76 %, α/β 10:1).

HRMS (ESI+ve): found 252.0955 [M + Na]$^+$; $C_9H_{15}N_3NaO_4^+$ requires 252.0955; $[\alpha]_D^{25}$ +109.7 (*c* 1, $CHCl_3$); ν_{max} (thin film): 3419 (br, OH), 2110 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.32 (3H, d, H6α, $J_{6,5}$ 6.5), 1.38 (3H, d, H6β, $J_{6,5}$ 6.1), 1.39 (3H, s, CH_3α), 1.40 (3H, s, CH_3β), 1.62 (3H, s, CH_3α), 1.66 (3H, s, CH_3β), 3.36 (1H, d, OHα, $J_{1,OH}$ 3.5), 3.51 - 3.55 (2H, m, H4β, H5β), 3.57 (1H, dd, H4α, $J_{4,5}$ 1.6, $J_{3,4}$ 5.7), 4.07 (1H, dd, H2α, $J_{1,2}$ 1.0, $J_{2,3}$ 6.4), 4.08 (1H, dq, H5α, $J_{5,4}$ 1.6, $J_{5,6}$ 6.5), 4.21 (1H, dd, H2β, $J_{2,1}$ 3.0, $J_{2,3}$ 6.0), 4.46 (1H, dd, H3α, $J_{3,4}$ 6.0, $J_{3,2}$ 6.4), 4.91 (1H, dd, H1β, $J_{1,2}$ 3.0, $J_{1,OH}$ 11.9), 5.40 (1H, d, H1α, $J_{1,OH}$ 3.5); δ_C ($CDCl_3$, 100 MHz): 18.0 (C6α), 18.1 (C6β), 25.3 (CH_3β), 25.4 (CH_3α), 25.6 (CH_3α), 25.8 (CH_3β), 61.1 (C4β, C5β), 61.2 (C4α), 63.7 (C5α), 68.8 (C4β, C5β), 72.1 (C2β), 72.8 (C3α), 73.3 (C2α), 74.8 (C3β), 92.7 (C1α), 92.9 (C1β), 110.3 (<u>C</u>$(CH_3)_2$α), 111.0 (<u>C</u>$(CH_3)_2$β); *m/z* (ESI+ve): 252 ([M + Na]$^+$, 100%).

_N_-Benzyl-1,4-imino-1,4,6-trideoxy-2,3-_O_-isopropylidene-L-tallitol 4.73

Method 1:

Palladium black (10 % wt., 3 mg) was added to a solution of **4.72** (60.3 mg, 0.26 mmol) in ethanol (2 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. Then the reaction mixture was stirred vigorously overnight at room temperature under a hydrogen atmosphere until mass spectrometry indicated the completion of reaction (*m/z* 188). After filtration with Celite, the solvent was removed *in vacuo* to afford a residue that was dissolved in methanol (2 mL). Benzaldehyde (0.05 mL, 0.53 mmol), $NaBH_3CN$ (41 mg, 0.66 mmol) and acetic acid (0.015 mL, 0.26 mmol) was added and the mixture was stirred overnight

at room temperature. TLC analysis (cyclohexane/ethyl acetate 5:2) indicated incomplete conversion, so benzaldehyde (0.1 mL, 1.06 mmol), $NaBH_3CN$ (82 mg, 1.32 mmol) and acetic acid (0.03 mL, 0.52 mmol) were added and the mixture was again stirred overnight at room temperature until TLC (cyclohexane/ethyl acetate 5:2) indicated the full conversion of the product (R_f 0.28). The mixture was purified by flash column chromatography (cyclohexane/ethyl acetate 20:1 to 5:2, with 1% triethylamine) to afford the **4.73** as a clear oil (62.8 mg, 86 %).

Method 2: **4.70** to **4.73** *via* ring contraction

Trifluoroacetic anhydride (0.06 mL, 0.44 mmol) and triethylamine (0.06 mL, 0.44 mmol) was added into a solution of **4.70** (60.7 mg, 0.22 mmol) in toluene (2.1 mL). The mixture was stirred with refluxing for 3 hour. Then after stirring at room temperature for 30 min, aqueous KOH solution (2M, 4 mL) was added and the mixture was stirred at room temperature overnight and TLC (cyclohexane/ethyl acetate 5:2) showed the formation of one major product (R_f 0.28). The toluene was removed *in vacuo* and the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 x 25 mL). The combined organics was dried ($MgSO_4$) and filtered and the solvent was removed *in vacuo* to obtain a mixture of **4.70** and **4.73** (57.2 mg, 94%) as a ratio of 17:83.

4.73: HRMS (ESI+ve): found 278.1752 $[M + H]^+$; $C_{16}H_{24}NO_3^+$ requires 278.1751; $[\alpha]_D^{25}$ +5.2 (*c* 1, $CHCl_3$); ν_{max} (thin film): 3430 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.17 (3H, d, H6, $J_{6,5}$ 6.1), 1.27 (3H, s, CH_3), 1.56 (3H, s, CH_3), 2.85 (1H, dd, H1, $J_{1,2}$ 4.5, J_{gem} 13.9), 2.95 (1H, d, H4, $J_{4,5}$ 9.4), 3.00 (1H, d, H1', J_{gem} 13.9), 3.22 (1H, dq, H5, $J_{5,6}$ 6.1, $J_{5,4}$ 9.4), 4.01 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.9), 4.13 (1H, d, C$\underline{H}_2$Ph, J_{gem} 12.9), 4.41 (1H, d, H3, $J_{3,2}$ 5.9), 4.67 (1H, dd, H2, $J_{2,1}$ 4.5, $J_{2,3}$ 5.9), 7.16 - 7.35 (5H, m, Ar); δ_C ($CDCl_3$, 100 MHz): 19.5 (C6), 23.6 ($\underline{C}H_3$), 26.8 ($\underline{C}H_3$), 56.1 (C1), 61.9 ($\underline{C}H_2$Ph), 63.9 (C5), 76.9 (C4), 82.8 (C2), 84.1 (C3), 111.9 ($\underline{C}(CH_3)_2$), 127.4, 128.6, 129.1, 139.8 (Ar); *m/z* (ESI+ve): 278 ($[M + H]^+$, 100%).

_N_-Benzyl-1,4-imino-1,4,6-trideoxy-D-tallitol 4.74

4.73 (33 mg, 0.12 mmol) was dissolved in a mixture of trifluoroacetic acid/water (1:1, 2 mL). The mixture was stirred at room temperature for 48 h until TLC (ethyl acetate/methanol, 9:1) indicated the formation of product (R_f 0.36). The solvent was removed *in vacuo* to obtain a residue that was then loaded with water (~2 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After loading, the column was washed with water/1,4-dioxane and then water again and the pure product was released with aqueous ammonia (2 M)/1,4-dioxane (1:1). After being filtered through cotton wool, the solvent was removed *in vacuo* to give the title compound **4.74** as a colorless oil (25.2 mg, 89 %).

HRMS (ESI+ve): found 238.1438 $[M + H]^+$; $C_{13}H_{20}NO_3^+$ requires 238.1438; $[\alpha]_D^{25}$ –62.2 (*c* 1, MeOH); ν_{max} (thin film): 3355 (br, OH); δ_H (CD_3OD, 400 MHz): 1.22 (3H, d, H6, $J_{6,5}$ 6.5), 2.47 (1H, t, H1, $J_{gem} = J_{1,2}$ 8.8), 2.73 (1H, dd, H4, $J_{4,3}$ 2.7, $J_{4,5}$ 5.1), 2.93 (1H, dd, H1', $J_{1',2}$ 5.9, J_{gem} 8.8), 3.58 (1H, d, C$\underline{H}_2$Ph, J_{gem} 13.0), 3.72 (1H, dq, H5, $J_{5,4}$ 5.1, $J_{5,6}$ 6.5), 3.91 (1H, dd, H3, $J_{3,4}$ 2.7, $J_{3,2}$ 4.9), 4.00 (1H, ddd, H2, $J_{2,3}$ 4.9, $J_{2,1'}$ 5.9, $J_{2,1}$ 8.8), 4.06 (1H, d, C$\underline{H}_2$Ph, J_{gem} 13.0), 7.19 - 7.39 (5H, m, Ar); δ_C (CD_3OD, 100 MHz): 19.9 (C6), 58.3 (C1), 62.9 (C$\underline{H}_2$Ph), 69.3 (C5), 72.2 (C2), 74.5 (C3), 77.3 (C4), 128.0, 129.3, 129.9, 140.8 (Ar); *m/z* (ESI+ve): 238 ($[M + H]^+$, 100%).

1,4-imino-1,4,6-trideoxy-D-tallitol 4.75

Palladium on charcoal (10 % wt., 17 mg) was added to a solution of **4.74** (38 mg, 0.16 mmol,) in 1,4-dioxane/water (1:1, 3 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. Then the reaction mixture was stirred vigorously overnight at room temperature under hydrogen atmosphere until mass spectrometry indicates the completion of the reaction (m/z 148). After filtration, the solvent was removed *in vacuo* to afford a residue that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After loading, the column was washed with water and 1,4-dioxane and then water again, pure product was released with aqueous ammonia (2 M). The eluent was filtered through cotton wool and

evaporated to dryness *in vacuo* to afford **4.75** as a colourless solid (23 mg, 98 %).

HRMS (ESI+ve): found 148.0969 $[M + H]^+$; $C_6H_{14}NO_3^+$ requires 148.0968; m.p. 118 - 120 °C; $[\alpha]_D^{25}$ + 40.3 (*c* 1, MeOH); ν_{max} (thin film): 3318 cm^{-1} (br, OH, NH); δ_H (400 MHz, D_2O): 1.25 (3H, d, H6, $J_{6,5}$ 6.5), 2.78 (1H, dd, H1, $J_{1,2}$ 4.0, J_{gem} 12.3), 2.83 (1H, dd, H4, *J* 5.2, *J* 7.2), 3.17 (1H, dd, H1', $J_{1',2}$ 5.2, J_{gem} 12.3), 3.84 - 3.94 (2H, m, H3, H5), 4.11 (1H, ddd, H2, $J_{2,1}$ 4.0, $J_{2,1'}$ 5.2, $J_{2,3}$ 9.4); δ_C (100 MHz, D_2O): 20.2 (C6), 50.4 (C1), 67.3 (C4), 67.8 (C5), 72.0 (C2), 73.7 (C3); *m/z* (ESI+ve): 148 ($[M + H]^+$, 100%)

6-Deoxy-2,3-*O*-isopropylidene-6-deoxy-D-gulono-1,5-lactone 4.79

and

6-Deoxy-2,3-*O*-isopropylidene-6-deoxy-D-gulono-1,4-lactone 4.80

and

6-Deoxy-3,5-*O*-isopropylidene-6-deoxy-D-gulono-1,4-lactone 4.81

Sulfuric acid (0.2 mL) and anhydrous $CuSO_4$ (500 mg) was added to a solution of 6-deoxy-D-gulono-1,4-lactone **4.2** (150 mg, 0.91 mmol) in acetone (20 mL). The mixture was stirred at room temperature for 16 h until TLC (cyclohexane/ethyl acetate, 1:1) showed the formation of three new spots (R_f 0.21, 0.25, 0.32). Sodium carbonate (~500 mg) was added to neutralize the reaction mixture that was then filtered and the solvent was removed to give a residue. Purification by flash chromatography (cyclohexane/ethyl acetate 4:1 to 1:1) afforded products **4.79** (oil, 20 mg, 7%), **4.80** (solid, 70 mg, 35%) and **4.81** (oil, 100 mg, 24%).

NMR data for **4.79**: δ_H (CD_3OD, 400 MHz): 1.37 (3H, s, CH_3), 1.38 (3H, d, H6, $J_{6,5}$ 6.5), 1.47 (3H, s, CH_3), 3.89 (1H, br-d, H4, *J* 1.8), 4.47 (1H, dd, H3, $J_{3,4}$ 2.8, $J_{3,2}$ 7.0), 4.82 (1H, d, H2, $J_{2,3}$ 7.0), 4.74 (1H, dq, H5, $J_{5,4}$ 0.9, $J_{5,6}$ 6.4); δ_C (CD_3OD, 100 MHz): 15.6 (C6), 22.6 ($\underline{C}H_3$), 24.9 ($\underline{C}H_3$), 67.9 (C5), 72.1 (C3), 73.5 (C2), 76.5 (C4), 110.8 ($\underline{C}(CH_3)_2$), 168.8 (C1);

NMR data for **4.80**: δ_H (CD_3OD, 400 MHz): 1.27 (3H, d, H6, $J_{6,5}$ 6.4), 1.37 (3H, s, CH_3), 1.41 (3H, s, CH_3), 4.02 (1H, dq, H5, $J_{5,6}$ 6.4, $J_{5,4}$ 8.5), 4.27 (1H, dd, H4, $J_{4,3}$ 3.4, $J_{4,5}$ 8.5), 4.85 (1H, a-dd, H3, $J_{3,4}$ 3.4, $J_{3,2}$ 5.5), 4.94 (1H, d, H2, $J_{2,3}$ 5.5); δ_C (CD_3OD, 100 MHz): 17.1 (C6), 24.6 ($\underline{C}H_3$), 25.7 ($\underline{C}H_3$), 68.1

(C5), 76.3 (C3), 78.7 (C2), 84.1 (C4), 113.4 ($\underline{C}(CH_3)_2$), 175.2 (C1);

NMR data for **4.81**: δ_H (CD_3OD, 400 MHz): 1.27 (3H, d, H6, $J_{6,5}$ 6.4), 1.37 (3H, s, CH_3), 1.51 (3H, s, CH_3), 4.11 (1H, dd, H4, $J_{4,5}$ 1.8, $J_{4,3}$ 2.1), 4.27 (1H, dq, H5, $J_{5,4}$ 1.8, $J_{5,6}$ 6.4), 4.58 (1H, d, H2, $J_{2,3}$ 4.0), 4.82 (1H, dd, H3, $J_{3,2}$ 4.0, $J_{3,4}$ 2.1); δ_C (CD_3OD, 100 MHz): 17.1 (C6), 19.8 ($\underline{C}H_3$), 29.7 ($\underline{C}H_3$), 65.5 (C5), 70.5 (C3), 72.8 (C2), 83.5 (C4), 99.8 ($\underline{C}(CH_3)_2$), 178.0 (C1);

6-Deoxy-2,3-*O*-(3-pentylidene)-D-gulono-1,4-lactone 4.83

Method 1: from **4.2**

Sulfuric acid (0.4 mL) and anhydrous $CuSO_4$ (1.0 g) was added to a solution of 6-deoxy-D-gulono-1,4-lactone **4.2** (1.0 g, 6.1 mmol) in 3-pentanone (40 mL). The mixture was stirred at 50 °C for 3 h until TLC (cyclohexane/ethyl acetate 1:1) showed the formation of one major product (R_f 0.31). Sodium carbonate (~1 g) was added to neutralize the reaction mixture that was then filtered and solvent was removed to give a residue. Purification by flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1) afforded the diethylketal **4.83** as a colourless oil (911 mg, 65%).

Method 2: from **4.82**

Sulfuric acid (0.1 mL) and anhydrous $CuSO_4$ (500 mg) was added to a solution of diacetondie **4.82** (600 mg, 2.2 mmol) in 3-pentanone (15 mL). The mixture was stirred at 50 °C for 2 h until TLC (cyclohexane/ethyl acetate 1:1) showed the formation of one major product (R_f 0.31). Sodium carbonate (~400 mg) was added to neutralize the reaction mixture that was then filtered and solvent was removed to give a residue. Purification by flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1) afforded the diethylketal **4.83** as a colourless oil (320 mg, 62%).

HRMS (ESI+ve): found 253.1046 $[M + Na]^+$; $C_{11}H_{18}NaO_5^+$ requires 253.1046; ν_{max} (thin film): 1785 (s, C=O); $[\alpha]_D^{25}$ -51 (*c* 0.89, $CHCl_3$); δ_H (CD_3OD, 400 MHz): 0.90 (3H, t, CH_3, *J* 7.5), 0.93 (3H, t, CH_3, *J* 7.3), 1.32 (3H, d, H6, $J_{6,5}$ 6.4), 1.63 (2H, q, $C\underline{H}_2CH_3$, *J* 7.6), 1.65 (2H, q, $C\underline{H}_2CH_3$, *J* 7.5), 4.12 (1H, dq, H5, $J_{5,6}$ 6.6, $J_{4,5}$ 8.5), 4.29 (1H, dd, H4, $J_{4,3}$ 3.5, $J_{4,5}$ 8.5), 4.88 (1H, dd, H3, $J_{3,4}$ 3.5, $J_{3,2}$ 5.5),

4.98 (1H, d, H2, $J_{2,3}$ 5.5); δ_C (CD_3OD, 100 MHz): 6.40 (CH_3), 7.13 (CH_3), 17.1 (C6), 28.9 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 66.2 (C5), 76.5 (C3), 77.0 (C2), 84.3 (C4), 117.3 ($\underline{C}(CH_3)_2$), 174.9 (C1); *m/z* (ESI+ve): 253 ($[M + Na]^+$, 100%).

5-Azido-2,3-*O*-(3-pentylidene)-1,5,6-trideoxy-L-mannono-1,4-lactone 4.84

Triflic anhydride (1.80 mL, 11.0 mmol) and anhydrous pyridine (3.71 mL, 21.9 mmol) was added dropwise to a solution of the pentylidene lactone **4.83** (1.7 g, 7.3 mmol) in dichloromethane (20 mL) at -20 °C. After string at -20 °C for 1.5 h, TLC (cyclohexane/ethyl acetate, 1:1) showed the formation of major product (R_f 0.71) and the disappearance of starting material (R_f 0.35). The reaction mixture was diluted with dichloromethane (10 mL) and washed with HCl (2 M, aq. 30 mL) and aqueous $NaHCO_3$ (sat, 30 mL). The organic layer was dried ($MgSO_4$) and the solvent removed in *vacuo* to give the corresponding triflate as a brown oil (2.0 g).

Sodium azide (520 mg, 8.0 mmol) was added to a solution of the crude triflate (2.0 g) in anhydrous DMF (20 mL). The mixture was stirred at room temperature for 4 h until TLC (cyclohexane/ethyl acetate, 2:1) indicated the formation of one major product (R_f 0.67) and consumption of the crude triflate (R_f 0.57). After being diluted with ethyl acetate (20 mL), the mixture was washed with half saturated brine (40 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain a crude that was purified *via* flash chromatography (cyclohexane/ethyl acetate, 7:1 to 5:1) to yield the azido lactone **4.84** as a white solid (1.1 g, 59%, 2 steps).

HRMS (ESI+ve): found 278.1113 $[M + Na]^+$; $C_{11}H_{17}N_3NaO_4^+$ requires 278.1111; m.p. 40 °C - 42 °C; ν_{max} (thin film): 1794 (s, C=O), 2121 (s, N_3); $[\alpha]_D^{25}$ -3.3 (c 0.90, $CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 0.90 (3H, t, CH_3, *J* 7.5), 0.92 (3H, t, CH_3, *J* 7.5), 1.44 (3H, d, H6, $J_{6,5}$ 6.4), 1.65 - 1.76 (4H, m, 2 x CH_2), 3.95 (1H, dq, H5, $J_{5,6}$ 6.6, $J_{5,4}$ 9.8), 4.07 (1H, dd, H4, $J_{4,3}$ 3.3, $J_{4,5}$ 9.8), 4.85 (1H, d, H2, $J_{2,3}$ 5.5), 4.88 (1H, dd, H3, $J_{3,4}$ 3.3, $J_{3,2}$ 5.5); δ_C ($CDCl_3$, 100 MHz): 8.0 (CH_3), 8.5 (CH_3), 17.8 (C6), 29.8 (CH_2), 30.1 (CH_2), 56.0 (C5), 76.3, 76.7 (C2, C3), 81.0 (C4), 119.0 ($\underline{C}(CH_3)_2$), 173.4 (C1); *m/z* (ESI+ve): 278 ($[M + Na]^+$, 100%).

5-Azido-2,3-*O*-(3-pentylidene)-1,5,6-trideoxy-L-mannofuranose 4.85

Diisobutylaluminium hydride (25% w/v in toluene, 5.5 ml, 9.6 mmol) was added dropwise to a solution of **4.84** (1.1 g, 4.8 mmol) in anhydrous THF (20 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 2 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated formation of one product (R_f 0.80) and mass spectrometry showed the consumption of starting material ([M + Na]$^+$ 278). Then the mixture was diluted with ethyl acetate (10 mL) and potassium sodium tartrate (sat, *aq*., ~2.0 mL) was added. After stirring for 12 h, the mixture was diluted with water (20 mL) and extracted with ethyl acetate (2 x 20 mL). Organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a crude that was further purified *via* flash chromatography (cyclohexane/ethyl acetate 7:1 to 5:1) to yield the lactol **4.85** as a colourless oil (1.1 g, 100%, α/β 20:1).

HRMS (ESI+ve): found 280.1268 [M + Na]$^+$; $C_{11}H_{19}N_3NaO_4^+$ requires 280.1268; ν_{max} (thin film): 2092 (s, N_3); $[\alpha]_D^{25}$ +18 (*c* 0.61, $CHCl_3$); NMR data of α anomer of **4.85**: δ_H ($CDCl_3$, 400 MHz): 0.88 (3H, t, CH_3, *J* 7.5), 0.91 (3H, t, CH_3, *J* 7.5), 1.36 (3H, d, H6, $J_{6,5}$ 6.1), 1.57 - 1.74 (4H, m, 2 x CH_2), 2.51 (1H, d, OH, $J_{OH,1}$ 2.3), 3.84 - 3.89 (2H, m, H4, H5), 4.61 (1H, d, H2, $J_{2,3}$ 5.8), 4.79 (1H, dd, H3, $J_{3,4}$ 2.8, $J_{3,2}$ 5.9), 5.37 (1H, d, H1, $J_{1,OH}$ 2.0); δ_C ($CDCl_3$, 100 MHz): 8.0 (CH_3), 8.8 (CH_3), 17.7 (C6), 29.1 (CH_2), 29.4 (CH_2), 55.9 (C5), 80.2 (C3), 83.4 (C4), 86.1 (C2), 101.9 (C1), 117.3 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 280 ([M + Na]$^+$, 100%).

1,5-Imino-2,3-*O*-(3-pentylidene)-1,5,6-trideoxy-L-mannitol 4.86

Palladium black (10 % wt., 110 mg) was added to a solution of **4.85** (1.1 g, 4.30 mmol) in ethanol (20 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 12 h at room temperature under hydrogen atmosphere until TLC (cyclohexane/ethyl acetate, 1:1) indicated the consumption of starting material (R_f 0.80) and the formation of one product (R_f 0.1). After filtration, the solvent was removed *in vacuo* to afford **4.86** as a yellow oil (900 mg, 98%).

HRMS (ESI+ve): found 216.1594 $[M + H]^+$; $C_{11}H_{22}NO_3^+$ requires 216.1594; $[\alpha]_D^{25}$ +69 (*c* 0.56, MeOH); δ_H (CD_3OD, 400 MHz): 0.92 (3H, t, CH_3, $J_{CH3,CH2}$ 7.5), 1.01 (3H, t, CH_3, $J_{CH3,CH2}$ 7.5), 1.19 (3H, d, H6, $J_{6,5}$ 6.4), 1.63 - 1.71 (2H, m, CH_2), 1.75 - 1.85 (2H, m, CH_2), 2.36 (1H, dq, H5, $J_{5,6}$ 6.4, $J_{5,4}$ 10.1), 2.97 (1H, dd, H1, $J_{1,2}$ 3.2, J_{gem} 15.0), 3.23 (1H, dd, H4, $J_{4,3}$ 7.5, $J_{4,5}$ 10.0), 3.29 (1H, br-d, H1', J_{gem} 15.0), 3.88 (1H, dd, H3, $J_{3,2}$ 5.8, $J_{3,4}$ 7.3), 4.24 (1H, ddd, H2, $J_{2,1'}$ 1.0, $J_{2,1}$ 3.1, $J_{2,3}$ 5.7); δ_C (CD_3OD, 100 MHz): 7.5 (CH_3), 7.6 (CH_3), 16.7 (C6), 28.2 (CH_2), 30.0 (CH_2), 45.2 (C1), 54.2 (C5), 73.9 (C2), 76.4 (C4), 79.8 (C3), 112.8 ($\underline{C}(CH_3)_2$); *m/z* (ESI+ve): 238 ($[M + Na]^+$, 100%).

_N_-Benzyl-1,5-imino-2,3-_O_-(3-pentylidene)-1,5,6-trideoxy-L-mannitol 4.87

Benzylaldehyde (0.55 mL, 5.44 mmol) and sodium cyanoborohydride (342 mg, 5.44 mmol) were added to a solution of **4.86** (900 mg, 4.19 mmol) in methanol (15 mL). The mixture was stirred at room temperature for 20 h until TLC showed the formation of the only product (R_f 0.72). After removing the solvent *in vacuo*, the residue was dissolved in ethyl acetate (20 mL) and washed with water (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain a crude that was further purified *via* flash chromatography (cyclohexane/ethyl acetate 7:1) to yield **4.87** as a yellow oil (1.0 g, 79%).

HRMS (ESI+ve): found 306.2604 $[M + H]^+$; $C_{18}H_{28}NO_3^+$ requires 306.2604; $[\alpha]_D^{25}$ +3.3 (*c* 0.48, $CHCl_3$); δ_H (CD_3OD, 400 MHz): 0.90 (3H, t, $C\underline{H}_3$, $J_{CH3,CH2}$ 7.5), 1.01 (3H, t, $C\underline{H}_3$, $J_{CH3,CH2}$ 7.5), 1.30 (3H, d, H6, $J_{6,5}$ 6.4), 1.60 – 1.70 (2H, m, $C\underline{H}_2$), 1.75 - 1.85 (2H, m, $C\underline{H}_2$), 2.35 (1H, dq, H5, $J_{5,6}$ 6.4, $J_{5,4}$ 8.5), 2.48 (1H, dd, H1, $J_{1,2}$ 4.0, J_{gem} 13.9), 3.03 (1H, dd, H1', $J_{1',2}$ 3.4, J_{gem} 13.9), 4.43 (1H, d, $C\underline{H}_2Ph$, J_{gem} 13.3), 3.51 (1H, dd, H4, $J_{4,3}$ 7.0, $J_{4,5}$ 8.5), 3.93 (1H, dd, H3, $J_{3,2}$ 6.4, $J_{3,4}$ 6.7), 4.02 (1H, d, $C\underline{H}_2$, J_{gem} 13.3), 4.25 (1H, br-ddd, H2, $J_{2,1'}$ 3.2, $J_{2,1}$ 3.8, $J_{2,3}$ 6.7), 7.25 - 7.37 (5H, m, -Ar); δ_C (CD_3OD, 100 MHz): 7.4 ($\underline{C}H_3$), 7.5 ($\underline{C}H_3$), 14.0 (C6), 28.2 ($\underline{C}H_2$), 29.8 ($\underline{C}H_2$), 50.3 (C1), 56.2 (CH_2), 59.2 (C5), 72.9 (C2), 74.3 (C4), 79.1 (C3), 112.8 ($\underline{C}(CH_3)_2$), 126.6, 126.8, 127.9, 128.0, 128.9 (Ar); *m/z* (ESI+ve): 306 ($[M + H]^+$, 100%).

1,5-Imino-1,5,6-trideoxy-L-mannitol (1-Deoxy-rhamnojirimycin, RHJ) 4.16

4.86 (100 mg, 0.47 mmol) was dissolved in a mixture of trifluoroacetic acid/water/1,4-dioxane (2:1:1, 4 mL). The mixture was stirred at room temperature for 12 h until mass spectrometry showed the formation of the only product ($[M+H]^+$ 148). The solvent was removed *in vacuo* to obtain a residue that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After loading, the column washing again with water, 1,4-dioxane and then water, pure product was released with aqueous ammonia (2 M). Then solvent was removed *in vacuo* to yield the title compound **4.16** as an oil (65 mg, 96%).

HRMS (ESI+ve): found 148.0967 $[M + H]^+$; $C_6H_{14}NO_3^+$ requires 148.0968; $[\alpha]_D^{25}$ +28.5 (*c* 1.0, water) [lit.[74] $[\alpha]_D^{22}$ +32 (*c* 1.0, water)]; δ_H (D_2O, 400 MHz): 1.25 (3H, d, H6, $J_{6,5}$ 6.3), 2.66 (1H, dq, H5, $J_{5,6}$ 6.3, $J_{5,4}$ 9.6), 2.87 (1H, d, H1, J_{gem} 14.0), 3.07 (1H, dd, H1', $J_{1',2}$ 2.1, J_{gem} 14.0), 3.43 (1H, t, H4, $J_{4,3} = J_{4,5}$ 9.6), 3.56 (1H, dd, H3, $J_{3,2}$ 2.9, $J_{3,4}$ 9.6), 4.06 (1H, br-s, H2); δ_C (D_2O, 100 MHz): 16.8 (C6), 48.6 (C1), 55.9 (C5), 68.9 (C2), 73.3 (C4), 74.1 (C3); *m/z* (ESI+ve): 148 ($[M + H]^+$, 100%).

N-Benzyl-1,5-imino-1,5,6-trideoxy-L-mannitol 4.88

4.87 (40 mg, 0.13 mmol) was dissolved in a mixture of trifluoroacetic acid/water/1,4-dioxane (2:1:1, 2 mL). The mixture was stirred at room temperature for 15 h until mass spectrometry showed the formation of the only product ($[M+H]^+$ 238). Solvent was removed *in vacuo* to obtain a residue that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After loading, the column washing again with water and 1,4-dioxane and then water, and the pure product was released with aqueous ammonia (2 M)/1,4-dioxane (1:1). Then solvent was removed *in vacuo* to yield the title compound **4.88** as a white solid (30 mg, 97%).

HRMS (ESI+ve): found 238.1437 $[M + H]^+$; $C_{13}H_{20}NO_3^+$ requires 238.1438; m.p. 110 °C - 114 °C; $[\alpha]_D^{25}$ +77 (*c* 0.47, MeOH); δ_H (CD_3OD, 400 MHz): 1.42 (3H, d, H6, $J_{6,5}$ 6.1), 2.13 - 2.19 (1H, m, H1, H5), 2.90 (1H, dd, H1', $J_{1',2}$ 3.7, J_{gem} 12.5), 3.29 (1H, dd, H3, $J_{3,2}$ 3.5, $J_{3,4}$ 9.3), 3.32 (1H, d, CH_2, J_{gem} 13.6), 3.42 (1H, t, H4, $J_{4,3} = J_{4,5}$ 9.2), 3.80 (1H, br-dt, H2, $J_{2,1}$ 1.8, $J_{2,1'} = J_{2,3}$ 3.2), 4.12 (1H, d, CH_2, J_{gem}

13.6), 7.25 - 7.38 (5H, m, -Ar); δ_C (CD_3OD, 100 MHz): 16.3 (C6), 56.8 (C1), 58.4 (CH_2Ph), 63.1 (C5), 60.5 (C2), 74.8 (C4), 76.4 (C3), 128.3, 129.4, 130.6, 139.3 (Ar); *m/z* (ESI+ve): 238 ($[M + H]^+$, 100%).

_N_-Benzyl-1,4-imino-1,4,6-trideoxy-2,3-_O_-(3-pentylidene)-D-allitol 4.89

Trifluoroacetic anhydride (0.24 mL, 1.70 mmol) and triethylamine (0.24 mL, 1.70 mmol) was added into a solution of **4.87** (260 mg, 0.85 mmol) in toluene (5 mL). The mixture was stirred with refluxing for 4 hour. Then after stirring at room temperature for 30 min, aqueous NaOH solution (2M, 5 mL) was added and the mixture was stirred at room temperature overnight and TLC (hexane/ethyl acetate 4:1) showed the formation of one major product (R_f 0.38) and a small amount of starting material (R_f 0.44). Then toluene was removed *in vacuo* and the mixture was extracted with ethyl acetate (3 x 10 mL). The organics were dried ($MgSO_4$) and filtered and solvent was remove *in vacuo* to obtain a residue (**4.87**:**4.89** = 12:82) that was purified *via* flash column chromatography (ethyl acetate/hexane 1:9) to yield **4.89** (180 mg, 70%) as a colorless oil.

HRMS (ESI+ve): found 306.2604 $[M + H]^+$; $C_{18}H_{28}NO_3^+$ requires 306.2604; $[\alpha]_D^{25}$ -33 (*c* 0.47, $CHCl_3$); δ_H (CD_3OD, 400 MHz): 0.89 (3H, t, CH_3, *J* 7.5), 0.94 (3H, t, CH_3, *J* 7.5), 1.26 (3H, d, H6, $J_{6,5}$ 6.6), 1.63 (2H, q, CH_2, *J* 7.5), 1.63 (2H, q, CH_2, *J* 7.5), 2.49 (1H, dd, H1, $J_{1,2}$ 5.0, J_{gem} 9.8), 2.63 (1H, dd, H4, $J_{4,5}$ 2.6, $J_{4.3}$ 3.4), 3.19 (1H, dd, H1', $J_{1',2}$ 6.3, J_{gem} 9.8), 4.50 (1H, d, CH_2, J_{gem} 12.9), 4.05 (1H, d, CH_2, J_{gem} 13.3), 4.13 (1H, dq, H5, $J_{5,4}$ 2.3, $J_{5,6}$ 6.4), 4.58 (1H, br-dt, H2, $J_{2,1}$ = $J_{2,1'}$ 6.2, $J_{2,3}$ 7.2), 4.70 (1H, dd, H3, $J_{3,4}$ 3.8, $J_{3,2}$ 7.2), 7.26-7.37 (5H, m, Ar); δ_C (CD_3OD, 100 MHz): 6.9 (CH_3), 7.5 (CH_3), 18.5 (C6), 28.7 (CH_2), 28.6 (CH_2), 56.7 ($\underline{C}H_2Ph$), 58.5 (C1), 63.5 (C5), 73.5 (C4), 77.8 (C2), 79.8 (C3), 116.9 ($\underline{C}(CH_3)_2$), 126.6, 126.8, 126.9, 127.9, 128.7 (Ar); *m/z* (ESI+ve): 306 ($[M + H]^+$, 100%).

_N_-Benzyl-1,4-imino-1,4,6-trideoxy-2,3-D-allitol 4.90

4.89 (110 mg, 0.36 mmol) was dissolved in a mixture of trifluoroacetic acid/water/1,4-dioxane (2:1:1, 4 mL). The mixture was stirred at room temperature for 5 h until mass spectrum showed

the formation of the only product ([M+H]$^+$ 238). Solvent was removed *in vacuo* to obtain a residue that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After loading, the column was washed with water and 1,4-dioxane and then water again, pure product was released with aqueous ammonia (2 M)/1,4-dioxane (1:1). Then solvent was removed *in vacuo* to yield the title compound **4.90** as a yellow oil (70 mg, 82%).

HRMS (ESI+ve): found 238.1437 [M + H]$^+$; $C_{13}H_{20}NO_3^+$ requires 238.1438; $[\alpha]_D^{25}$ -27.5 (*c* 0.24, MeOH); δ_H (D_2O, 400 MHz): 1.18 (3H, d, H6, $J_{6,5}$ 6.4), 3.43 (1H, dd, H1, $J_{1,2}$ 4.5, J_{gem} 12.7), 3.53 - 3.58 (2H, m, H4, H5), 3.64 (1H, dd, H1', $J_{1',2}$ 4.5, J_{gem} 12.5), 4.35 (1H, q, H2, $J_{2,1} = J_{2,1'} = J_{2,3}$ 4.5), 4.43 (1H, br-dd, H3, $J_{3,2}$ 4.9, $J_{3,4}$ 5.8), 4.56 (1H, s, C$\underline{H}_2$Ph), 7.37 - 7.55 (5H, m, Ar); δ_C (D_2O, 100 MHz): 19.0 (C6), 57.7 (C1), 61.7 (CH_2), 64.3 (C5), 70.0, 70.3 (C2, C3), 74.3 (C4), 128.1, 129.4, 130.1, 140.8 (Ar); *m/z* (ESI+ve): 238 ([M + H]$^+$, 100%).

1,4-Imino-1,4,6-trideoxy-D-allitol 4.91

Palladium on charcoal (10 % wt., 6 mg) was added to a solution of **4.90** (60 mg, 4.20 mmol) in ethanol (5 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 15 h at room temperature under hydrogen atmosphere until mass spectrometry indicate the completion of the reaction ([M+H]$^+$ 148). After filtration, the solvent was removed *in vacuo* to afford a residue that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). After loading, the column was washed with water and 1,4-dioxane and then water again and the pure product was released with aqueous ammonia (2 M). The solvent was removed *in vacuo* to yield pure **4.91** as a brown glass (35 mg, 95%).

HRMS (ESI+ve): found 148.0968 [M + H]$^+$; $C_6H_{14}NO_3^+$ requires 148.0968; $[\alpha]_D^{25}$ +10.9 (*c* 0.32, water); δ_H (D_2O, 400 MHz): 1.28 (3H, d, H6, $J_{6,5}$ 6.4), 3.06 (1H, dd, H1, $J_{1,2}$ 2.1, J_{gem} 12.5), 3.21 (1H, br-t, H4, $J_{4,3} = J_{4,5}$ 4.7), 3.26 (1H, br-dd, H1', $J_{1',2}$ 3.2, J_{gem} 12.2), 4.04 (1H, q, H5, $J_{5,4}$ 4.8, $J_{5,6}$ 6.4),

4.20 - 4.23 (2H, m, H2, H3); δ_C (D_2O, 100 MHz): 19.4 (C6), 50.3 (C1), 66.6 (C5), 67.0 (C4), 71.5, 72.1 (C2, C3); *m/z* (ESI+ve): 148 ($[M + H]^+$, 100%).

4.5.3 Synthesis of 2,5,6-trideoxy-2,5-iminohexitols

2-Azido-*(R)*-3,5-*O*-benzylidene-2,6-dideoxy-L-glucitol 4.120

Diisobutylaluminum hydride (25% w/v in toluene, 0.31 ml, 0.54 mmol) was added dropwise to a solution of the lactone **4.46** (100 mg, 0.36 mmol) in anhydrous dichloromethane (3.6 mL) at -78 °C. The reaction mixture was stirred at -78°C for 1 h until mass spectrometry showed the formation of desired product peak ($[M + Na]^+$ 300) and disappearance of the starting material peak ($[M + Na]^+$ 298). The reaction mixture was diluted with ethyl acetate (15 mL) and potassium sodium tartrate (sat. aq., ~1 mL) was added. The reaction mixture was stirred for 8 h, then diluted with water (15 mL) and extracted with ethyl acetate (3 x 15 mL). The organic phase was dried ($MgSO_4$) and the solvent was removed *in vacuo*; the residue was dissolved in methanol (3.6 mL) and then sodium borohydride (14 mg, 0.36 mmol) was added. After stirring at room temperature for one hour, TLC (ethyl acetate/cyclohexane 2:3) showed the formation of a single spot (R_f 0.18). Acetic acid was added to neutralize the reaction mixture. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (ethyl acetate/cyclohexane 3:7 to 1:1) to afford the diol **4.120** (93.6 mg, 92 %) as a white solid.

HRMS (ESI+ve): found 302.1111 $[M + Na]^+$; $C_{13}H_{17}N_3O_4Na^+$ requires 302.1111; m.p. 105 - 106 °C; $[\alpha]_D^{25}$ –5.2 (*c* 1.0, CH_3CN); ν_{max} (thin film): 3395 (br, OH), 2102 cm^{-1} (s, N_3); δ_H (CD_3CN, 400 MHz): 1.43 (3H, d, H6, $J_{5,6}$ 7.2), 3.11 (1H, br-t, OH, $J_{OH,1} = J_{OH,1'}$ 5.5), 3.39 (1H, dt, H4, $J_{4,3} = J_{4,5}$ 1.2, $J_{4,OH}$ 9.5), 3.53 (1H, d, OH, $J_{OH,4}$ 9.5), 3.57 - 3.66 (1H, m, H1), 3.68 - 3.78 (2H, m, H1', H3), 4.17 - 4.27 (2H, m, H2, H5), 5.94 (1H, s, H7), 7.32 - 7.57 (5H, m, Ar); δ_C (100 MHz, CD_3CN): 15.4 (C6), 61.2 (C1), 65.5 (C3), 67.2 (C4), 76.2 (C2), 76.8 (C5), 94.9 (C7), 127.3, 129.1, 129.7, 139.8 (-Ar); *m/z* (ESI+ve): 302 ($[M + Na]^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,6-dideoxy-L-glucitol 4.121

tert-Butyldimethylsilyl triflate (0.51 mL, 2.2 mmol) and anhydrous pyridine (0.22 mL, 2.7 mmol) were added to a solution of diol **4.120** (250 mg, 0.90 mmol) in anhydrous dichloromethane (6 mL) at 0 °C. After stirring at 0 °C for 1 hour, the mixture was stirred at rt for another 3 hours. TLC (ethyl acetate/cyclohexane 1:19) showed the formation of one spot (R_f 0.27). The solvent was removed *in vacuo*; the residue was dissolved in cyclohexane (25 mL) and washed with water (2 x 25 mL). Organic phase was dried ($MgSO_4$), filtered and the solvent removed to obtain a residue that was purified by flash column chromatography (ethyl acetate/cyclohexane 0:10 to 1:24) to afford the silyl ether **4.121** (444 mg, 98 %) as a white solid.

HRMS (ESI+ve): found 530.2843 $[M + Na]^+$; $C_{25}H_{45}N_3O_4Si_2Na^+$ requires 530.2841; m.p. 61-62 °C; $[\alpha]_D^{25}$ -33.2 (*c* 1.0, MeCN); ν_{max} (thin film): 2098 (s, N_3); δ_H (CD_3CN, 400 MHz): 0.11 (3H, s, CH_3), 0.12 (3H, s, CH_3), 0.13 (3H, s, CH_3), 0.16 (3H, s, CH_3), 0.93 (9H, s, 3 x CH_3), 0.94 (9H, s, 3 x CH_3), 1.42 (3H, d, H6, $J_{6,5}$ 7.2), 3.58 (1H, s, H4), 3.64 (1H, a-dt, H2, $J_{2,1'} = J_{2,1}$ 4.5, $J_{2,3}$ 8.8), 3.77 (1H, dd, H1, $J_{1,2}$ 4.5, J_{gem} 11.1), 3.85 (1H, dd, H1', $J_{1',2}$ 4.5, J_{gem} 11.1), 4.24 - 4.32 (2H, m, H3, H5), 5.92 (1H, s, H7), 7.33 - 7.54 (5H, m, Ar); δ_C (CD_3CN, 100 MHz): -5.4 ($\underline{C}H_3$), -5.3 ($\underline{C}H_3$), -4.4 ($\underline{C}H_3$), -3.7 ($\underline{C}H_3$), 15.6 (C6), 18.7 ($\underline{C}(CH_3)_3$), 18.8 ($\underline{C}(CH_3)_3$), 26.1 ($C(\underline{C}H_3)_3$), 26.2 ($C(\underline{C}H_3)_3$), 62.9 (C1), 64.6 (C2), 68.6 (C4), 75.1 (C3), 75.7 (C5), 94.8 (C7), 127.3, 129.0, 129.7, 140.1 (-Ar); *m/z* (ESI+ve): 530 ($[M + Na]^+$, 100%).

2-Azido-3-*O*-benzoyl-5-bromo-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,5,6-trideoxy-D-iditol 4.123

N-Bromosuccinimide (185 mg, 1.0 mmol) and barium carbonate (256 mg, 1.3 mmol) were added to a solution of **4.121** (440 mg, 0.87 mmol) in carbon tetrachloride (5.8 mL). The stirred reaction mixture was refluxed for 1 hour until TLC (ethyl acetate/cyclohexane (1:19) indicated the disappearance of starting material (R_f 0.27) and the formation of one major product (R_f 0.52). After removal of the solvent *in vacuo*, the residue was purified by flash column chromatography

(ethyl acetate/hexane 0:10 to 1:24) to give the bromide **4.123** (437 mg, 86%) as a colorless oil. HRMS (ESI+ve): found 586.2127, 588.2107 $[M + H]^+$; $C_{25}H_{45}BrN_3O_4Si_2^+$ requires 586.2127, 588.2106; $[\alpha]_D^{25}$ - 17.5 (*c* 1.0, MeCN); ν_{max} (thin film): 1728 (s, C=O), 2102 cm^{-1} (s, N_3); δ_H (400 MHz, CD_3CN): -0.10 (3H, s, CH_3), 0.00 (3H, s, CH_3), 0.02 (3H, s, CH_3), 0.11 (3H, s, CH_3), 0.83 (9H, s, CH_3), 0.86 (9H, s, CH_3), 1.74 (3H, d, H6, $J_{6,5}$ 6.9), 3.75 - 3.84 (2H, m, H1, H2), 3.86 - 3.94 (1H, m, H1'), 4.06 (1H, dd, H4, $J_{4,5}$ 3.0, $J_{4,3}$ 6.6), 4.40 (1H, dq, H5, $J_{5,4}$ 3.0, $J_{5,6}$ 6.9), 5.52 (1H, dd, H3, $J_{3,2}$ 2.9, $J_{3,4}$ 6.6), 7.51 (2H, t, Ar, *J* 7.7), 7.65 (1H, t, Ar, *J* 7.4), 8.01 - 8.08 (2H, m, Ar); δ_C (100 MHz, CD_3CN): -5.5 (CH_3Si), -5.4 (CH_3Si), -3.7 (CH_3Si), -3.6 (CH_3Si), 18.7 ($\underline{C}(CH_3)_3$), 18.8 ($\underline{C}(CH_3)_3$), 23.5 (C6), 26.1 ($C(\underline{C}H_3)_3$), 26.2 ($C(\underline{C}H_3)_3$), 52.9 (C5), 63.0 (C2), 64.4 (C1), 74.9 (C4), 75.4 (C3), 129.6, 130.8, 130.9, 134.5 (Ar), 166.3 (C=O); *m/z* (ESI+ve): 586, 588 ($[M + H]^+$, 100%, 93%).

3-*O*-Benzoyl-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,5-imino-2,5,6-trideoxy-L-glucitol 4.124

Palladium on charcoal (10 % wt., 72 mg) and sodium acetate (84 mg, 1.0 mmol) were added to a solution of **4.123** (399 mg, 0.68 mmol) in ethanol (20 mL). The reaction mixture was flushed sequentially with nitrogen, argon and hydrogen gas. Then the reaction mixture was stirred vigorously overnight at room temperature under a hydrogen atmosphere until TLC (ethyl acetate/cyclohexane 3:7) showed the formation of one major product (R_f 0.32). After filtration, the solvent was removed *in vacuo* and the residue purified by flash column chromatography (triethylamine/ethyl acetate/cyclohexane 0.01:3:7) to afford **4.124** (236 mg, 72%) as a yellow oil.

HRMS (ESI+ve): found 480.2957 $[M + H]^+$; $C_{25}H_{45}NO_4Si_2^+$ requires 480.2960; $[\alpha]_D^{25}$ +17.4 (*c* 1.0, MeCN); ν_{max} (thin film): 1722 (s, C=O); δ_H (CD_3CN, 400 MHz): -0.10 (3H, s, CH_3), -0.07 (3H, s, CH_3), 0.03 (3H, s, CH_3), 0.07 (3H, s, CH_3), 0.80 (9H, s, CH_3), 0.86 (9H, s, 3 x CH_3), 1.16 (3H, d, H6, $J_{6,5}$ 6.4), 2.96 (1H, quintet, H5, $J_{5,4}$ 6.3), 3.43-3.49 (1H, m, H2), 3.58 (1H, dd, H1, $J_{1,2}$ 4.9, J_{gem} 10.1), 3.66 (1H, dd, H1', $J_{1',2}$ 6.6, J_{gem} 10.1), 3.86 (1H, dd, H4, $J_{4,3}$ 4.2, $J_{4,5}$ 6.3), 5.25 (1H, dd, H3, $J_{3,4}$ 4.2, $J_{3,2}$ 6.2), 7.46-7.54 (2H, m, Ar), 7.59 - 7.66 (1H, m, Ar), 8.01 - 8.01 (2H, m, Ar); δ_C (CD_3CN, 100 MHz): -5.4 ($\underline{C}H_3$), -5.3 ($\underline{C}H_3$), -4.5 ($\underline{C}H_3$), -4.3 ($\underline{C}H_3$), 18.5 $\underline{C}(CH_3)_3$, 18.7 ($\underline{C}(CH_3)_3$), 19.6 (C6), 26.1 ($C(\underline{C}H_3)_3$),

26.2 (C($\underline{C}H_3)_3$), 60.2 (C5), 60.9 (C2), 63.1 (C1), 81.9 (C3), 83.6 (C4), 129.5, 130.4, 131.2, 134.2 (Ar), 166.6 (C=O); *m/z* (ESI+ve): 480 ([M + H]$^+$, 100%).

2,5-Imino-2,5,6-trideoxy-L-glucitol (6-Deoxy-L-DGDP) 4.112

4.124 (236 mg, 0.49 mmol) was dissolved in a mixture of trifluoroacetic acid/water/1,4-dioxane (1:1:1, 6 mL). The solution was stirred at 50 °C for 24 hours until mass spectrometry showed the deprotection of the silyl groups ([M + H]$^+$ 252). The solvent was removed *in vacuo*; the residue was dissolved in aqueous sodium hydroxide (aq., 2M, 10 mL) and stirred at 50 °C for 24 hours until mass spectrometry showed the formation of desired product ([M + H]$^+$ 148). After removal of the solvent *in vacuo*, the residue was dissolved in ethanol (5 mL), filtered and then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin (pre-washed with water, 1,4-dioxane and water sequentially until the eluent was neutral). The ion exchange column was then washed with water, 1,4-dioxane and then water and the pure product was then eluted with aqueous ammonia (2 M). Removal of the solvent *in vacuo* gave the unprotected pyrrolidine **4.112** as a yellow oil (58 mg, 81%).

HRMS (ESI+ve): found 148.0967 [M + H]$^+$; $C_6H_{14}NO_3^+$ requires 148.0968; $[\alpha]_D^{25}$ –12.1 (*c* 0.45, water) [lit[83] for the enantiomer of **4.112**: $[\alpha]_D^{29}$ +5.2 (*c* 1.0, MeOH)]; ν_{max} (thin film): 3300 (br, NH, OH); δ_H (D_2O, 400 MHz): 1.25 (3H, d, H6, $J_{6,5}$ 6.6), 2.92 (1H, quintet, H5, $J_{5,4}$ = $J_{5,6}$ 6.6), 3.05 (1H, q, H2, $J_{2,1}$ = $J_{2,1'}$ = $J_{2,3}$ 6.0), 3.64 (1H, dd, H4, $J_{4,3}$ 3.5, $J_{4,5}$ 6.6), 3.67 (1H, dd, H1, $J_{1,2}$ 6.0, J_{gem} 11.4), 3.79 (1H, dd, H1', $J_{1',2}$ 6.0, J_{gem} 11.4), 4.12 (1H, dd, H3, $J_{3,4}$ 3.5, $J_{3,2}$ 6.0); δ_C (D_2O, 100 MHz): 17.9 (C6), 59.2 (C5), 60.6 (C1), 61.2 (C2), 78.3 (C3), 84.4 (C4); *m/z* (ESI+ve): 148 ([M + H]$^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-1,4-di-*O*-benzoyl-2,6-dideoxy-L-glucitol 4.125

Benzoyl chloride (0.24 mL, 2.2 mmol) and pyridine (0.24 mL, 2.8 mmol) were added to a solution of the diol **4.120** (200 mg, 0.72 mmol) in anhydrous dichloromethane (7 mL). The reaction mixture was stirred at rt for 16 hours until TLC (ethyl acetate/cyclohexane 1:4) showed the formation of one major product (R_f 0.41). The solvent was removed *in vacuo* and the residue

purified by flash column chromatography (ethyl acetate/cyclohexane 1:19 to 1:4) to form the dibenzoate **4.125** (300 mg, 86%) as a clear oil; trituration of the oil with acetonitrile gave a white crystalline solid.

HRMS (ESI+ve): found 510.1634 $[M + Na]^+$; $C_{27}H_{25}N_3O_6Na^+$ requires 510.1636; m.p. 111 - 114 °C; $[\alpha]_D^{25}$ –29.6 (*c* 1.0, MeCN); ν_{max} (thin film): 1716 (s, C=O), 2104 (s, N_3); δ_H (CD_3CN, 400 MHz): 1.58 (3H, d, H6, $J_{6,5}$ 7.2), 4.14 - 4.21 (1H, m, H2), 4.35 (1H, dd, H1, $J_{1,2}$ 5.8, J_{gem} 12.1), 4.46 (1H, br-q, H5, $J_{5,6}$ 7.2), 4.50 (1H, dd, H1', $J_{1',2}$ 3.3, J_{gem} 12.1), 4.68 (1H, dd, H3, $J_{3,4}$ 1.4, $J_{3,2}$ 7.9), 5.02 (1H, br-s, H4), 6.11 (1H, s, H7), 7.37 - 7.68 (10H, m, Ar x 2), 7.95 - 8.01 (2H, m, Ar), 8.09 - 8.15 (2H, m, Ar); δ_C (CD_3CN, 100 MHz): 15.4 (C6), 60.8 (C2), 64.0 (C1), 69.8 (C4), 73.8 (C5), 74.5 (C3), 95.1 (C7), 127.3, 129.2, 129.6, 129.7, 129.9, 130.4, 130.6, 130.9, 134.4, 134.5, 139.5 (-Ar), 166.7 (C=O), 166.8 (C=O); *m/z* (ESI+ve): 510 ($[M + Na]^+$, 100%).

2-Azido-5-bromo-1,3,4-tri-*O*-benzoyl-2,5,6-trideoxy-D-iditol 4.128a

and

2-Azido-5-bromo-1,3,4-tri-*O*-benzoyl-2,5,6-trideoxy-L-glucitol 4.128b

N-Bromosuccinimide (116 mg, 0.65 mmol) and barium carbonate (160 mg, 0.81 mmol) were added to a solution of **4.125** (265 mg, 0.54 mmol) in carbon tetrachloride (3.5 mL). The stirred reaction mixture was refluxed for 1 h until TLC (ethyl acetate/cyclohexane (1:4) indicated the disappearance of starting material (R_f 0.39) and the formation of two products (R_f 0.45, 0.5). After removal of the solvent, the residue was purified by flash column chromatography (ethyl acetate/hexane 0:10 to 1:4) to give a mixture of the inseparable epimeric bromides **4.128a** and **4.128b** (5:1, 280 mg, 91%) as a light yellow syrup.

4.128a: δ_H (CD_3CN, 400 MHz): 1.76 (3H, d, H6, $J_{6,5}$ 6.8), 4.29 (1H, ddd, H2, $J_{2,3}$ 3.0, $J_{2,1'}$ 5.2, $J_{2,1}$ 6.6), 4.35 (1H, dd, H1, $J_{1,2}$ 6.6, J_{gem} 11.4), 4.63 – 4.71 (2H, m, H1', H5,), 5.72 (1H, dd, H4, $J_{4,5}$ 3.1, $J_{4,3}$ 7.3), 5.95 (1H, dd, H3, $J_{3,2}$ 3.1, $J_{3,4}$ 7.3), 7.39 - 7.72 (15H, m, -Ar x 3), δ_C (CD_3CN, 100 MHz): 24.8 (C6), 50.4 (C5), 60.9 (C2), 66.0 (C1), 75.4 (C3), 76.5 (C4), 130.9 – 136.1 (-Ar x 3), 167.7 (C=O), 167.8 (C=O), 167.9 (C=O);

4.128b: δ_H (CD_3CN, 400 MHz): 1.79 (3H, d, H6, $J_{6,5}$ 6.7), 4.20 (1H, dt, H2, $J_{2,3}$ = $J_{2,1'}$ 4.2, $J_{2,1}$ 6.2), 4.54 - 4.71 (3H, m, H1, H1', H5,), 5.89 (1H, t, H4, $J_{4,5}$ = $J_{4,3}$ 5.4), 5.98 (1H, dd, H3, $J_{3,2}$ 4.5, $J_{3,4}$ 5.4), 7.39 - 7.72 (15H, m, -Ar x 3), δ_C (CD_3CN, 100 MHz): 22.9 (C6), 48.1 (C5), 62.3 (C2), 66.3 (C1), 73.4 (C3), 77.4 (C4), 131.1 – 136.4 (-Ar x 3);

1,3,4-Tri-*O*-benzoyl-2,5-imino-2,5,6-trideoxy-L-glucitol 4.129a

and

1,3,4-Tri-*O*-benzoyl-2,5-imino-2,5,6-trideoxy-D-iditol 4.129b

Palladium on charcoal (10 % wt., 30 mg) and sodium acetate (49 mg, 0.6 mmol) were added to a solution of the mixture of **4.128a** and **4.128b** (260 mg, 0.46 mmol) in ethanol (15 mL). The reaction mixture was flushed sequentially with nitrogen, argon and hydrogen and then stirred overnight at room temperature under a hydrogen atmosphere for 12 h when TLC (ethyl acetate/cyclohexane 1:1) showed the formation of two products (R_f 0.14, 0.17). The solvent was removed and the residue purified by flash column chromatography (triethylamine/ethyl acetate/cyclohexane 0.01:1:5 to 0.01:2:1) to afford a mixture of 5-epimers **4.129a** and **4.129b** (4:1, 173 mg, 82%) as a white solid. The mixture was not clean enough for assignment of the tribenzoates by NMR; this crude product was directly used in the deprotection step.

2,5-Imino-2,5,6-trideoxy-L-glucitol 4.112

and

2,5-Imino-2,5,6-trideoxy-D-iditol 4.113

The mixture of **4.129a** and **4.129b** (100 mg, 0.22 mmol) was dissolved in sodium hydroxide (aq, 2M, 10 mL) and stirred at 50 °C for 48 hours until mass spectrometry showed the formation of desired product ($[M+H]^+$ 148). The solvent was removed *in vacuo*; the residue was dissolved in ethanol (5 mL) and passed through glass fiber to afford a crude salt that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin. The ion exchange column was then washed with water, 1,4-dioxane and then water; the pure product was then eluted with

aqueous ammonia (2 M). Removal of solvent *in vacuo* gave a mixture of the unprotected pyrrolidines **4.112** and **4.113** as a yellow oil (5 :1 according to ^{1}H spectrum, 23 mg, 73%).

2-Azido-*(R)*-3,5-*O*-benzylidene-2,6-dideoxy-L-mannitol 4.130

Diisobutylaluminium hydride (25% w/v in toluene, 2.33 ml, 4.05 mmol) was added dropwise to a solution of **4.47** (500 mg, 1.80 mmol) in anhydrous dichloromethane (18 mL) at -78 °C. The reaction mixture was stirred at -78°C for 1.5 h until mass spectrometry showed the formation of desired product peak ($[M + Na]^+$ 300) and disappearance of starting material peak ($[M+Na]^+$ 298). Then the mixture was diluted with ethyl acetate (25 mL) and potassium sodium tartrate (sat, aq., ~5 mL) was added and allowed to stand at rt for 8h. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). Organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a residue that was dissolved in methanol (18 mL) and sodium borohydride (69 mg, 1.80 mmol) was added. After stirring at room temperature for one hour, TLC (ethyl acetate/cyclohexane 2:3) showed the formation of a single spot (R_f 0.32). Acetic acid was added to neutralize the reaction mixture. After removing the solvent *in vacuo*, the crude product was purified by flash column chromatography (ethyl acetate/cyclohexane 3:7 to 1:1) to afford the diol **4.130** (452 mg, 89 %) as a white solid.

HRMS (ESI+ve): found 302.1111 $[M + Na]^+$; $C_{13}H_{17}N_3O_4Na^+$ requires 302.1111; m.p. 108 - 109 °C; $[\alpha]_D^{25}$ +19.8 (*c* 1.0, MeCN); ν_{max} (thin film): 3406 (br, OH), 2102 (s, N_3); δ_H (CD_3CN, 400 MHz): 1.44 (3H, d, H6, $J_{6,5}$ 7.2), 3.11 (1H, br-t, OH, $J_{OH,1} = J_{OH,1'}$ 5.7), 3.43 - 3.51 (2H, m, H4, OH), 3.65 (1H, dt, H1, $J_{1,2}$ 5.9, J_{gem} 11.8), 3.73 (1H, ddd, H2, $J_{2,1'}$ 2.6, $J_{2,1}$ 5.9, $J_{2,3}$ 9.1), 3.88 (1H, ddd, H1', $J_{1',2}$ 2.6, $J_{1',OH}$ 5.7, J_{gem} 11.8), 4.01 (1H, d, H3, $J_{3,2}$ 9.1), 4.26 (1H, q, H5, $J_{5,6}$ 7.2), 5.85 (1H, s, H7), 7.33 - 7.53 (5H, m, Ar); δ_C (CD_3CN, 100 MHz): 15.2 (C6), 62.4 (C1), 63.4 (C2), 66.8 (C4), 73.9 (C3), 76.9 (C5), 94.7 (H7), 127.2, 129.1, 129.7, 139.8 (Ar); *m/z* (ESI+ve): 302 ($[M + Na]^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,6-dideoxy-L-mannitol 4.131

tert-Butyldimethylsilyl triflate (1.0 mL, 4.50 mmol) and anhydrous pyridine (0.45 mL, 5.4 mmol) were added to a solution of **4.130** (500 mg, 1.80 mmol) in anhydrous dichloromethane (12 mL) at 0 °C. After stirring at 10 °C for 3 hour, TLC (ethyl acetate/cyclohexane 1:4) showed the formation of a new spot (R_f 0.73) but reaction was not complete. Further *tert*-butyldimethylsilyl triflate (0.33 mL, 1.49 mmol) and anhydrous pyridine (0.15 mL, 1.80 mmol) were added to the reaction mixture which was stirred at -18 °C for 16 hours until TLC indicated the completion of reaction. After removing the solvent *in vacuo*, the residue was dissolved in ethyl acetate (50 mL) and washed with half saturated brine (50 mL) and then brine (50 mL). The organic phase was dried ($MgSO_4$), filtered and solvent was removed to obtain a residue that was purified by flash column chromatography (ethyl acetate/cyclohexane 0:10 to 1:4) to afford **4.131** (852 mg, 94 %) as a colorless oil.

HRMS (ESI+ve): found 530.2838 $[M + Na]^+$; $C_{25}H_{45}N_3O_4Si_2Na^+$ requires 530.2841; $[\alpha]_D^{25}$ +7.6 (*c* 1.0, MeCN); ν_{max} (thin film): 2099 (s, N_3); δ_H (CD_3CN, 400 MHz): 0.08 (6H, s, CH_3), 0.150 (3H, s, CH_3), 0.153 (3H, s, CH_3), 0.92 (9H, s, 3 x CH_3), 0.95 (9H, s, 3 x CH_3), 1.41 (3H, d, H6, $J_{6,5}$ 7.2), 3.63 (1H, br-s, H4), 3.66 (1H, ddd, H2, $J_{2,1'}$ 1.9, $J_{2,1}$ 5.0, $J_{2,3}$ 7.5), 3.87 (1H, dd, H1, $J_{1,2}$ 5.0, J_{gem} 10.9), 4.01 (1H, dd, H3, $J_{3,4}$ 1.1, $J_{3,2}$ 7.5), 4.06 (1H, dd, H1', $J_{1',2}$ 1.9, J_{gem} 10.9), 4.25 (1H, q, H5, $J_{5,6}$ 7.2), 5.80 (1H, s, H7), 7.32 - 7.47 (5H, m, Ar); δ_C (CD_3CN, 100 MHz): -5.3 (CH_3Si), -5.3 (CH_3Si), -4.7 (CH_3Si), -4.2 (CH_3Si), 15.4 (C6), 18.79 ($\underline{C}(CH_3)_3$), 18.84 ($\underline{C}(CH_3)_3$), 26.1 ($C(\underline{C}H_3)_3$), 26.2 ($C(\underline{C}H_3)_3$), 62.1 (C2), 64.1 (C1), 67.8 (C4), 73.3 (C3), 76.2 (C5), 94.5 (C7), 127.2, 129.0, 129.7, 140.1 (Ar); *m/z* (ESI+ve): 530 ($[M + Na]^+$, 100%).

2-Azido-3-*O*-benzoyl-5-bromo-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,5,6-trideoxy-D-gulitol 4.132

N-Bromosuccinimide (358 mg, 2.0 mmol) and barium carbonate (496 mg, 2.5 mmol) were added to a solution of **4.131** (851 mg, 1.70 mmol) in carbon tetrachloride (11.2 mL). The mixture was refluxed with stirring for 50 min until TLC (ethyl acetate/cyclohexane (1:19) indicated the disappearance of starting material (R_f 0.50) and the formation of one major product (R_f 0.62).

After removal of the solvent *in vacuo*, the residue was purified by flash column chromatography (ethyl acetate/hexane 0:10 to 1:24) to afford **4.132** (909 mg, 92%) as a colorless oil.

HRMS (ESI+ve): found 608.1944, 610.1924 $[M + Na]^+$; $C_{25}H_{44}BrN_3O_4Si_2Na^+$ requires 608.1946, 608.1926; $[\alpha]_D^{25}$ -22.2 (*c* 1.0, MeCN); ν_{max} (thin film): 2098 (s, N_3), 1728 (s, C=O); δ_H (CD_3CN, 400 MHz): 0.03 (3H, s, CH_3), 0.04 (3H, s, CH_3), 0.06 (3H, s, CH_3), 0.14 (3H, s, CH_3), 0.89 (9H, s, 3 x CH_3), 0.91 (9H, s, 3 x CH_3), 1.64 (3H, d, H6, $J_{6,5}$ 6.9), 3.77 - 3.89 (2H, m, H1, H2), 3.94 (1H, dd, H1', $J_{1',2}$ 3.7, J_{gem} 10.0), 4.19 (1H, t, H4, $J_{4,3} = J_{4,5}$ 4.0), 4.32 (1H, dq, H5, $J_{5,4}$ 4.0, $J_{5,6}$ 6.9), 5.58 (1H, dd, H3, $J_{3,4}$ 4.0, $J_{3,2}$ 6.3), 7.47 - 7.55 (2H, m, -Ar), 7.61 - 7.68 (1H, m, -Ar), 7.99 - 8.07 (2H, m, -Ar); δ_C (CD_3CN, 100 MHz): -5.5 (CH_3Si), -5.4 (CH_3Si), -3.9 (CH_3Si), -3.7 (CH_3Si), 18.8 ($\underline{C}(CH_3)_3$), 22.3 (C6), 26.1 ($C(\underline{C}H_3)_3$), 26.3 ($C(\underline{C}H_3)_3$), 52.6 (C5), 63.2 (C2), 63.7 (C1), 73.5 (C3), 74.3 (C4), 129.7, 130.6, 130.9, 134.5 (-Ar), 166.2 (C=O); *m/z* (ESI+ve): 586, 588 ($[M + H]^+$, 100%, 97%).

3-*O*-Benzoyl-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,5-imino-2,5,6-trideoxy-L-mannitol 4.133

Palladium on charcoal (10 % wt., 165 mg) and sodium acetate (191 mg, 2.3 mmol) were added to a solution of **4.132** (909 mg, 1.5 mmol) in ethanol (45 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. Then the reaction mixture was stirred vigorously overnight at room temperature under a hydrogen atmosphere for 12 h until TLC (ethyl acetate/cyclohexane 1:4) showed the formation of one major product (R_f 0.17). Filtration and evaporation *in vacuo* gave a crude bromide salt (~1.2 g). Part of the salty residue (120 mg) was purified by flash column chromatography (triethylamine/ethyl acetate/cyclohexane 0.01:3:7) to give a pure sample of **4.133** (64 mg, 71%) as a yellow oil for full characterization. The crude salt was used in the next step.

HRMS (ESI+ve): found 480.2959 $[M + H]^+$; $C_{25}H_{45}NO_4Si_2^+$ requires 480.2960; $[\alpha]_D^{25}$ +10.6 (*c* 1.0, MeCN); ν_{max} (thin film): 3350 (br, NH), 1722 (s, C=O); δ_H (CD_3OD, 400 MHz): 0.03 (3H, s, CH_3), 0.12 (3H, s, CH_3), 0.13 (3H, s, CH_3), 0.16 (3H, s, CH_3), 0.87 (9H, s, 3 x CH_3), 0.94 (9H, s, 3 x CH_3), 1.32 (3H, d, H6, $J_{6,5}$ 6.6), 3.35 - 3.49 (2H, m, H2, H5), 3.92 (1H, dd, H1, $J_{1,2}$ 3.9, J_{gem} 10.6), 4.03 (1H,

dd, H1', $J_{1',2}$ 4.4, J_{gem} 10.6), 4.16 (1H, dd, H4, $J_{4,3}$ 5.2, $J_{4,5}$ 7.3), 5.32 (1H, t, H3, $J_{3,2}$ = $J_{3,4}$ 4.6), 7.51 (2H, t, -Ar, *J* 7.7), 7.64 (1H, t, -Ar, *J* 7.4), 8.07 (2H, d, -Ar, *J* 7.4); δ_C (CD_3OD, 100 MHz): -5.3 (CH_3Si), -5.2 (CH_3Si), -4.6 (CH_3Si), -4.2 (CH_3Si), 17.1 (C6), 18.7 ($\underline{C}(CH_3)_3$), 19.1 ($\underline{C}(CH_3)_3$), 26.2 ($C(\underline{C}H_3)_3$), 26.4 ($C(\underline{C}H_3)_3$), 60.2 (C5), 64.0 (C2), 64.7 (C1), 82.8 (C3), 83.3 (C4), 129.7, 130.7, 130.8, 134.6 (Ar), 167.4 (C=O); *m/z* (ESI+ve): 480 ($[M + H]^+$, 100%).

2,5-Imino-2,5,6-trideoxy-L-mannitol (6-Deoxy-L-DMDP) 4.114

The crude product **4.133** (617 mg) was dissolved in a solution of trifluoroacetic acid/water/1,4-dioxane (2:1:1, 20 mL). The solution was stirred at 50 °C for 18 hours until mass spectrometry showed the deprotection of the silyl groups ($[M + H]^+$ 252). After the solvent was removed *in vacuo*, the residue was dissolved into sodium hydroxide solution (aq, 2M, 5 mL) and stirred at 50 °C for 24 hours until mass spectrometry showed the formation of desired product ($[M + H]^+$ 148). The mixture was neutralized with HCl (2M, aq) and the solvent removed *in vacuo*. The residue was dissolved in ethanol (5 mL) and filtered (glass microfiber) to afford a crude salt that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin. The ion exchange column was then washed with water, 1,4-dioxane and then water; the pure product was then eluted with aqueous ammonia (2 M). Removal of solvent *in vacuo* gave a residue which was subjected to a further purification cycle by resin column to yield 6-deoxy-L-DMDP **4.114** as a yellow oil (121 mg, 64% from crude salt of **4.133**).

HRMS (ESI+ve): found 148.0968 $[M + H]^+$; $C_6H_{13}NO_3^+$ requires 148.0968; $[\alpha]_D^{25}$ -42 (*c* 1.0, MeOH) [lit.[84] $[\alpha]_D^{25}$ -33 (*c* 1.2, MeOH)]; ν_{max} (thin film): 3277 (br, NH, OH); δ_H (D_2O, 400 MHz): 1.19 (3H, d, H6, $J_{6,5}$ 6.4), 2.96 (1H, dq, H5, $J_{5,6}$ 6.4, $J_{5,4}$ 7.3), 3.05 (1H, br-q, H2, *J* 6.5), 3.58 – 3.64 (2H, m, H4, H1), 3.68 (1H, dd, H1', $J_{1',2}$ 4.6, J_{gem} 11.4), 3.81 (1H, t, H3, $J_{3,4}$ = $J_{3,2}$ 6.8); δ_C (D_2O, 100 MHz): 17.8 (C6), 58.2 (C5), 62.0, 63.0 (C1, C2), 78.7 (C3), 83.3 (C4); *m/z* (ESI+ve): 148 ($[M + H]^+$, 100%); *m/z* (ESI+ve): 148 ($[M + H]^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-1,4-di-*O*-benzoyl-2,6-dideoxy-L-mannitol 4.134

Benzoyl chloride (0.12 mL, 1.1 mmol) and pyridine (0.12 mL, 1.4 mmol) was added into a solution of diol **4.130** (100 mg, 0.36 mmol) in anhydrous dichloromethane (7 mL). The mixture was stirred at rt for 18 hours until TLC (ethyl acetate/cyclohexane, 1:4) showed the formation of one major product (R_f 0.42). The solvent was *in vacuo* and the residue was purified by flash column chromatography (ethyl acetate/cyclohexane 1:8 to 1:4) to afford the dibenzoate **4.134** (196 mg, 97%) as a white solid.

HRMS (ESI+ve): found 510.1633 $[M + Na]^+$; $C_{27}H_{25}N_3O_6Na^+$ requires 510.1636; m.p. 79 - 85 °C; $[\alpha]_D^{25}$ +37.9 (*c* 1.0, MeCN); ν_{max} (thin film): 2103 (s, N_3), 1719 (s, C=O); δ_H (CD_3CN, 400 MHz): 1.59 (3H, d, H6, $J_{6,5}$ 7.2), 4.23 (1H, ddd, H2, $J_{2,1'}$ 2.7, $J_{2,1}$ 7.4, $J_{2,3}$ 9.7), 4.43 (1H, dd, H3, $J_{3,4}$ 1.5, $J_{3,2}$ 9.7), 4.50 (1H, dd, H1, $J_{1,2}$ 7.4, J_{gem} 11.7), 4.51 (1H, dq, H5, $J_{5,4}$ 1.5, $J_{5,6}$ 7.2), 4.80 (1H, dd, H1', $J_{1',2}$ 2.7, J_{gem} 11.7), 5.05 (1H, t, H4, $J_{4,3} = J_{4,5}$ 1.5), 6.01 (1H, s, H7), 7.34 - 7.69 (10H, m, -Ar x 2), 8.02 - 8.18 (4H, m, -Ar); δ_C (CD_3CN, 100 MHz): 15.4 (C6), 60.7 (C2), 65.2 (C1), 69.3 (C4), 72.8 (C3), 73.7 (C5), 94.7 (C7), 127.2, 129.2, 129.6, 129.7, 129.9, 130.47, 130.52, 130.7, 130.9, 134.3, 134.5, 139.4 (-Ar x 2), 166.6, 166.8 (C=O); *m/z* (ESI+ve): 510 ($[M + Na]^+$, 100%).

2-Azido-1,3,4-tri-*O*-benzoyl-5-bromo-2,5,6-trideoxy-D-gulitol 4.135a

and

2-Azido-1,3,4-tri-*O*-benzoyl-5-bromo-2,5,6-trideoxy-L-mannitol 4.135b

N-Bromosuccinimide (156 mg, 0.88 mmol) and barium carbonate (217 mg, 1.1 mmol) were added into a solution of **4.134** (357 mg, 0.73 mmol) in carbon tetrachloride (5.0 mL). The mixture was refluxed with stirring for 1 hour until mass spectrometry indicated the completion of reaction ($[M + Na]^+$ 588, 590) The solvent was removed *in vacuo* and the residue purified by flash column chromatography (ethyl acetate/hexane 1:9 to 3:7) to give a mixture of the bromide epimers **4.135a** and **4.135b** (4:1, 388 mg, 94%) as a colorless oil. Separation of the epimers **4.135a** and **4.135b** by flash column chromatography was not efficient.

HRMS (ESI+ve): found 588.0740, 590.0719; $C_{27}H_{24}BrN_3O_6Na^+$ requires 588.0741, 590.0720; ν_{max} (thin film): 2108 (s, N_3), 1722 (s, C=O); *m/z* (ESI+ve): 588, 590 ($[M + Na]^+$, 100%, 95%)

4.135a: δ_H (CD_3CN, 400 MHz): 1.76 (3H, d, H6, $J_{6,5}$ 6.8), 4.31 (1H, dt, H2, $J_{2,1'}$ 3.7, $J_{2,1}$ = $J_{2,3}$ 6.7), 4.46 (1H, dd, H1, $J_{2,1}$ 6.7, J_{gem} 12.0), 4.55 - 4.63 (1H, m, H5), 4.70 (1H, dd, H1', $J_{1',2}$ 3.7, J_{gem} 12.0), 5.72 (1H, t, H4, $J_{4,3}$ = $J_{4,5}$ 5.0), 5.78 (1H, dd, H3, $J_{4,3}$ 5.0, $J_{3,2}$ 6.7), 7.41 - 7.71 (9H, m, -Ar), 7.95 - 7.17 (6H, m, -Ar); δ_C (CD_3CN, 100 MHz): 23.1 (C6), 49.4 (C5), 61.0 (C2), 64.3 (C1), 72.4 (C3), 75.1 (C4), 129.6-130.8, 134.5, 134.7, 134.8 (-Ar), 166.1 (C=O), 166.4 (C=O), 166.7 (C=O).

4.135b: δ_H (CD_3CN, 400 MHz): 1.72 (3H, d, H6, $J_{6,5}$ 6.7), 4.25 (1H, ddd, H2, $J_{2,1'}$ 3.7, $J_{2,1}$ 6.7, $J_{2,3}$ 7.8), 4.42 (1H, dd, H1, $J_{1,2}$ 6.7, J_{gem} 11.9), 4.41 - 4.49 (1H, m, H5), 4.63 (1H, dd, H1', $J_{2,1'}$ 3.7, J_{gem} 11.9), 5.82 (1H, dd, H4, $J_{3,4}$ 2.7, $J_{4,5}$ 7.7), 5.87 (1H, dd, H3), 7.41 - 7.71 (9H, m, -Ar), 7.95 - 7.17 (6H, m, -Ar); δ_C (CD_3CN, 100 MHz): 22.1 (C6), 46.8 (C5), 61.1 (C2), 64.4 (C1), 71.2 (C3), 75.5 (C4), 129.6 - 130.8, 134.4, 134.87, 134.92 (-Ar), 166.1 (C=O), 166.4 (C=O), 166.6 (C=O).

1,3,4-Tri-*O*-benzoyl-2,5-imino-2,5,6-trideoxy-L-mannitol 4.136a

and

1,3,4-Tri-*O*-benzoyl-2,5-imino-2,5,6-trideoxy-D-gulitol 4.136b

Palladium on charcoal (10 % wt., 73 mg) and sodium acetate (84 mg, 0.6 mmol) were added to a solution of **4.135a/b** (388 mg, 0.69 mmol) in ethanol (15 mL). The reaction mixture was flushed sequentially with nitrogen, argon and hydrogen. The reaction mixture was stirred at room temperature under hydrogen atmosphere for 16 h until TLC (ethyl acetate/cyclohexane 3:7) showed the formation of two products (R_f 0.12, 0.14). The reaction mixture was filtered and evaporated to dryness and the residue was purified by flash column chromatography (triethylamine/ethyl acetate/ petroleum ether 0.01:1:8 to 0.01:2:1) to obtain a mixture of 5-epimers **4.136a** and **4.136b** (10:7, 280 mg, 89%) as a yellow oil. Attempted separation of **4.136a** and **4.136b** by flash column chromatography was not successful.

4.136a: δ_H (CD_3CN, 400 MHz): 1.34 (3H, d, H6, $J_{6,5}$ 6.6), 3.57 (1H, br-dq, H5, $J_{5,4}$ 4.2, $J_{5,6}$ 6.6), 3.82 (1H, dt, H2, $J_{2,3}$ 4.2, $J_{2,1}$ = $J_{2,1'}$ 5.6), 4.50 (1H, dd, H1, $J_{1,2}$ 5.6, J_{gem} 10.9), 4.53 - 4.60 (1H, m, H1'), 5.72 (1H, dd, H4, $J_{4,3}$ 3.7, $J_{4,5}$ 4.2), 5.58 (1H, dd, H3, $J_{3,4}$ 3.7, $J_{3,2}$ 4.1), 7.34 – 7.78 (15H, -Ar x 3); δ_C (CD_3CN, 100 MHz): 20.3 (C6), 60.2 (C5), 63.4 (C2), 67.7 (C1), 83.4 (C3), 86.9 (C4), 120.7 – 135.8

(-Ar x 3), 168.0 (C=O), 168.1 (C=O), 168.4 (C=O).

4.136b: δ_H (CD_3CN, 400 MHz): 1.21 (3H, d, H6, $J_{6,5}$ 6.3), 3.61 – 3.68 (2H, m, H2, H5), 4.45 – 4.60 (2H, m, H1, H1'), 5.42 (1H, dd, H4, *J* 1.9, *J* 4.9), 4.48 (1H, dd, H3, *J* 1.9, *J* 4.5), 7.34 – 8.08 (15H, m, -Ar x 3); δ_C (CD_3CN, 100 MHz): 16.0 (C6), 58.6 (C5), 64.8 (C2), 67.6 (C1), 82.8 (C3), 83.3 (C4), 120.7 – 135.8 (-Ar x 3), 167.8 (C=O), 167.9 (C=O), 168.3 (C=O).

2,5-Imino-2,5,6-trideoxy-L-mannitol 4.114

and

2,5-Imino-2,5,6-trideoxy-D-gulitol 4.115

The epimeric mixture of the tribenzoates **4.136a** and **4.136b** (200 mg, 0.44 mmol) was dissolved into sodium hydroxide solution (aq., 2M, 15 mL) and stirred at 50 °C for 48 hours until mass spectrometry showed the formation of desired product ($[M + H]^+$ 148). The solvent was removed *in vacuo*, the residue was dissolved in ethanol (5 mL) and filtered (glass fiber) to afford a crude salt that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin. The ion exchange column was then washed with water, 1,4-dioxane and then water; the pure product was then eluted with aqueous ammonia (2 M). Removal of solvent *in vacuo* yielded a mixture of **4.114** and **4.115** as a yellow oil (5:3 according to ^1H spectrum, 46 mg, 70%).

***(R)*-3,5-*O*-Benzylidene-6-deoxy-D-gulono-1,4-lactone 4.137**

Concentrated hydrochloride acid (37%, 4 mL) was added to a suspension of 6-deoxy-D-gulono-1,4-lactone **4.2** (887 mg, 5.5 mmol) in benzaldehyde (15 mL). The mixture was stirring at room temperature for 15 h during which time a solid product separated. Then the reaction mixture was filtered and the product washed with diethyl ether (20 mL) to yield the benzylidene acetal **4.137** (1.2 g, 92%) as a white crystals.

HRMS (ESI+ve): found 273.0733 $[M + Na]^+$; $C_{13}H_{14}O_5Na^+$ requires 273.0733; m.p. 226 - 230 °C; $[\alpha]_D^{25}$ -54 (*c* 0.8, MeCN); ν_{max} (thin film) 1785 (s, C=O); δ_H (CD_3CN, 500 MHz): 1.38 (3H, d, H6, $J_{6,5}$

6.7), 3.82 (1H, br-d, OH, $J_{OH,2}$ 8.9), 4.21 (1H, t, H4, $J_{4,3}$ = $J_{4,5}$ 2.0), 4.27 (1H, dq, H5, $J_{5,4}$ 1.9, $J_{5,6}$ 6.6), 4.60 (1H, dd, H2, $J_{2,3}$ 4.1, $J_{2,OH}$ 8.2), 4.70 (1H, dd, H3, $J_{3,4}$ 2.0, $J_{3,2}$ 4.1), 5.70 (1H, s, H7), 7.40 - 7.50 (5H, m, -Ar); δ_C (CD_3CN, 100 MHz): 18.1 (C6), 73.7 (C2), 74.1 (C5), 74.4 (C4), 76.8 (C3), 101.2 (C7), 128.6, 130.5, 131.4, 140.2 (-Ar), 177.7 (C=O); *m/z* (ESI+ve): 273 ([M + Na]$^+$, 100%).

_(R)_-3,5-_O_-Benzylidene-6-deoxy-2-_O_-trifluoromethanesulfonyl-D-gulono-1,4-lactone 4.138

Anhydrous pyridine (0.03 mL, 0.36 mmol) and triflic anhydride (0.05 mL, 0.31 mmol) were sequentially added dropwise to a solution of the benzylidene lactone **4.137** (600 mg, 2.40 mmol) in anhydrous THF (10 mL) at -20 °C. After 3 h, TLC (cyclohexane/ethyl acetate, 1:1) indicated the formation of one product (R_f 0.57). The reaction mixture was diluted with dichloromethane (10 mL) and washed with HCl (2 M, aq, 2 x 10 mL). The organic layer was dried ($MgSO_4$); the solvent was removed *in vacuo* to give a residue that was purified by flash chromatography (ethyl acetate/cyclohexane, 1:5 to 1:2) to obtain pure triflate **4.138** (898 mg, 98%) as a white solid.

HRMS (ESI+ve): Found 405.2226 [M + Na]$^+$; $C_{13}H_{14}O_5Na^+$ requires 405.2226; m.p. 198 – 200 °C (decomp); $[\alpha]_D^{25}$ -64 (*c* 0.79, MeCN); ν_{max} (thin film): 1795 (s, C=O); δ_H (CD_3CN, 400 MHz): 1.42 (3H, d, H6, $J_{6,5}$ 6.6), 4.34 (1H, dq, H5, $J_{5,4}$ 1.4, $J_{5,6}$ 6.6), 4.42 (1H, br-t, H4, $J_{5,4}$ = $J_{3,4}$ 1.5), 5.10 (1H, dd, H3, $J_{3,4}$ 1.9, $J_{3,2}$ 4.0), 5.75 (1H, s, H7), 5.89 (1H, d, H2, $J_{2,3}$ 4.0), 7.42 - 7.44 (5H, m, -Ar); δ_C (CD_3CN, 100 MHz): 18.0 (C6), 73.7 (C5), 75.1, 75.2 (C3, C4), 82.8 (C2), 101.2 (C7), 128.5, 130.7, 131.7, 139.5 (-Ar), 170.6 (C1); *m/z* (ESI+ve): 405 ([M + Na]$^+$, 100%).

2-Azido-_(R)_-3,5-_O_-benzylidene-2,6-dideoxy-D-idono-1,4-lactone 4.139

Sodium azide (168 mg, 2.58 mmol) was added to a solution of triflate **4.138** (898 mg, 2.35 mmol) in anhydrous DMF (10 mL). The reaction mixture was stirred at -10 °C for 20 h until TLC (cyclohexane/ethyl acetate, 1:1) indicated the formation of a major product (R_f 0.70). After being diluted with ethyl acetate (10 mL), the mixture was washed with half saturated brine (20 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain a crude that was purified by flash chromatography (ethyl acetate/cyclohexane, 1:7 to 1:5)

to yield the azido lactone **4.139** (500 mg, 77%) as a white solid.

HRMS (ESI+ve): found 276.0980 $[M + H]^+$; $C_{13}H_{14}O_4N_3^+$ requires 276.0979; m.p.: 68 - 69 °C; $[\alpha]_D^{25}$ -89 (*c* 0.88, $CHCl_3$); ν_{max} (thin film) 2112 (s, N_3), 1780 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.51 (3H, d, H6, $J_{6,5}$ 6.6), 4.20 (1H, dq, H5, $J_{5,4}$ 1.8, $J_{5,6}$ 6.6), 4.21 (1H, br-s, H4), 4.35 (1H, dd, H3, $J_{3,4}$ 1.9, $J_{3,2}$ 2.2), 4.44 (1H, d, H2, $J_{2,3}$ 2.2), 5.65 (1H, s, H7), 7.37 - 7.45 (5H, m, -Ar); δ_C ($CDCl_3$, 100 MHz): 17.5 (C6), 63.3 (C5), 72.4 (C4), 76.1, 76.2 (C2, C3), 100.1 (C7), 126.5, 128.8, 129.9, 136.9 (-Ar), 171.4 (C1); *m/z* (ESI+ve): 298 ($[M + Na]^+$, 100%).

(R)-3,5-*O*-Benzylidene-6-deoxy-D-idono-1,4-lactone 4.141

Caesium trifluoroacetate (48 mg, 0.19 mmol) was added to a solution of **4.138** (50 mg, 0.13 mmol) in anhydrous DMF (1.5 mL). The reaction mixture was then stirred at 60 °C for 50 h until TLC (ethyl acetate/cyclohexane, 2:1) indicated the disappearance of the starting material (R_f 0.68) and the formation of one product (R_f 0.62). After dilution with ethyl acetate (5 mL), the reaction mixture was washed with half saturated brine (10 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to give a residue that was purified by flash chromatography (ethyl acetate/cyclohexane, 1:5 to 1:3) to obtain **4.141** (32.5 mg, 100%) as a white solid.

HRMS (ESI+ve): found 273.0734 $[M + Na]^+$; $C_{13}H_{14}O_5Na^+$ requires 273.0733; m.p. 82 - 84 °C; $[\alpha]_D^{25}$ -39 (*c* 0.78, CH_3CN); ν_{max} (thin film) 3220 (br, OH), 1776 (s, C=O); δ_H (CD_3CN, 400 MHz): 1.41 (3H, d, H6, $J_{6,5}$ 6.6), 4.12 (1H, br-s, H3), 4.31 (1H, dq, H5, $J_{5,4}$ 1.7, $J_{5,6}$ 6.6), 4.53 – 4.55 (1H, m, H4), 5.70 (1H, s, H7), 5.89 (1H, d, H2, $J_{2,3}$ 4.0), 7.39 - 7.46 (5H, m, -Ar); δ_C (CD_3CN, 100 MHz): 17.3 (C6), 72.7 (C5), 73.7 (C3), 76.9 (C4), 78.1 (C2), 99.8 (C7), 127.0, 129.1, 130.0, 138.8 (-Ar), 175.7 (C1); *m/z* (ESI+ve): 273 ($[M + Na]^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-2,6-dideoxy-D-gulono-1,4-lactone 4.140

Method 1: from **4.138**

Sodium azide (228 mg, 3.36 mmol) was added to a solution of the *gulono*-triflate **4.138** (838 mg,

2.19 mmol) in anhydrous DMF (10 mL). The mixture was stirred at room temperature for 42 h until TLC (ethyl acetate/cyclohexane, 1:1) indicated the formation of major product (R_f 0.20). After being diluted with ethyl acetate (10 mL), the mixture was washed with half saturated brine (20 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain a crude that was purified by flash chromatography (ethyl acetate/cyclohexane 1:5 to 1:1) to yield the azido lactone **4.140** (470 mg, 78%) as a white solid, identical to material obtained by Method 2 below.

Method 2: from **4.141**

Anhydrous pyridine (0.03 mL, 0.36 mmol) and triflic anhydride (0.05 mL, 0.31 mmol) were sequentially added dropwise to a solution of the *idono*-lactone **4.141** (60 mg, 0.24 mmol) in anhydrous THF (3 mL) at -20 °C. After 4 h, TLC (ethyl acetate/cyclohexane, 1:1) indicated the formation of the only product (R_f 0.83). The reaction mixture was diluted with dichloromethane (5 mL) and washed with HCl (2 M, aq, 2 x 5 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed *in vacuo* to give the residue (~100 mg) that was used without further purification; in contrast to *gulono*-triflate **4.39**, this triflate was not stable.

Then sodium azide (23 mg, 0.36 mmol) was added to a solution of the crude triflate (~100 mg) in anhydrous DMF (2 mL). The mixture was stirred at room temperature for 30 h until TLC (ethyl acetate/cyclohexane, 1:1) indicated the formation of the product (R_f 0.20). After being diluted with ethyl acetate (5 mL), the mixture was washed with half saturated brine (10 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to obtain a crude that was purified by flash chromatography (ethyl acetate/cyclohexane 1:5 to 1:1) to yield the azido lactone **4.140** as a white solid (30 mg, 45%).

HRMS (ESI+ve): Found 298.0799 $[M + Na]^+$; $C_{13}H_{13}O_4N_3Na^+$ requires 298.0798; m.p. 144 - 148 °C; $[\alpha]_D^{25}$ -76 (*c* 0.70, $CHCl_3$); ν_{max} (thin film) 2108 (s, N_3), 1775 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.49 (3H, d, H6, $J_{6,5}$ 6.6), 4.04 (1H, d, H2, $J_{2,3}$ 3.8), 4.14 (1H, br-s, H4), 4.22 (1H, dq, H5, $J_{5,4}$ 1.2, $J_{5,6}$ 6.6), 4.78 (1H, dd, H3, $J_{3,4}$ 1.8, $J_{3,2}$ 3.8), 5.61 (1H, s, H7), 7.37 - 7.49 (5H, m, -Ar); δ_C ($CDCl_3$, 100 MHz): 17.0 (C6), 62.1 (C2), 72.5 (C5), 73.7 (C4), 75.2 (C3), 100.1 (C7), 126.5, 128.8, 129.8, 136.8 (-Ar),

170.9 (C1); *m/z* (ESI+ve): 298 ([M + Na]$^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-2,6-dideoxy-D-iditol 4.142

Diisobutylaluminium hydride (25% w/v in toluene, 4.83 ml, 8.50 mmol) was added dropwise to a solution of **4.139** (467 mg, 1.70 mmol) in anhydrous dichloromethane (15 mL) at -78 °C. The reaction mixture was stirred at -78°C for 3 h until mass spectrometry showed the formation of desired product peak ([M+Na]$^+$ 300) and disappearance of starting material peak ([M + Na]$^+$ 298). Then the mixture was diluted with ethyl acetate (20 mL) and potassium sodium tartrate (sat, aq., ~2 mL) was added. After stirring for 12 h, the mixture was diluted with water (30 mL) and extracted with ethyl acetate (3 x 20 mL). Organic phase was dried ($MgSO_4$), filtered and solvent was removed *in vacuo* to obtain a residue that was dissolved in methanol (15 mL) and sodium borohydride (84 mg, 2.20 mmol) was added. After stirring at room temperature for one hour, TLC (ethyl acetate/cyclohexane 1:1) showed the formation of a new spot (R_f 0.32). Acetic acid was added to neutralize the reaction mixture. After removing solvent *in vacuo*, the resulting crude was purified by flash column chromatography (ethyl acetate/cyclohexane 1:4 to 1:1) to afford the *ido*-diol **4.142** (312 mg, 66 %) as a pale syrup.

HRMS (ESI+ve): Found 302.1111 [M + Na]$^+$; $C_{13}H_{17}O_4N_3Na^+$ requires 302.1111; $[\alpha]_D^{25}$ +5.2 (*c* 0.78, $CHCl_3$); ν_{max} (thin film) 3400 (br, OH), 2108 (s, N_3); δ_H ($CDCl_3$, 400MHz): 1.37 (3H, d, H6, $J_{6,5}$ 6.4), 2.24 (1H, br-s, OH), 3.48 (1H, br-s, H4), 3.76 (1H, dd, H1, $J_{1,2}$ 4.2, J_{gem} 12.0), 3.82 (1H, dd, H1', $J_{1',2}$ 3.1, J_{gem} 12.0), 3.90 (1H, ddd, H2, $J_{2,1'}$ 3.1, $J_{2,1}$ 4.2, $J_{2,3}$ 8.5), 4.06 (1H, dq, H5, $J_{5,4}$ 0.6, $J_{5,6}$ 6.5), 4.35 (1H, dd, H3, $J_{3,4}$ 0.6, $J_{3,2}$ 8.5), 5.69 (1H, s, H7), 7.36 - 7.56 (5H, m, -Ar); δ_C ($CDCl_3$, 100MHz): 17.2 (C6), 61.2 (C1), 64.3 (C2), 66.6 (C4), 76.6 (C5), 81.5 (C3), 101.4 (C7), 126.1, 128.6, 129.3, 137.6 (-Ar); *m/z* (ESI+ve): 302 ([M + Na]$^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,6-dideoxy-D-iditol 4.143

tert-Butyldimethylsilyl triflate (1.03 mL, 4.5 mmol) and anhydrous pyridine (0.59 mL, 7.2 mmol)

were added to a solution of **4.142** (250 mg, 0.90 mmol) in anhydrous dichloromethane (6 mL) at 0 °C. Then the mixture was stirred at room temperature for 18 h until TLC (ethyl acetate/cyclohexane, 1:4) showed the formation of a major spot (R_f 0.78). Removal of the solvent *in vacuo* gave a residue that was dissolved in cyclohexane (25 mL) and washed with water (2 x 25 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent removed to obtain a residue that was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:9) to afford the fully protected **4.143** (334 mg, 74 %) as a colorless oil.

HRMS (ESI+ve): found 530.2840 $[M + Na]^+$; $C_{25}H_{45}N_3O_4Si_2Na^+$ requires 530.2841; $[\alpha]_D^{25}$ -35 (*c* 0.81, $CHCl_3$); ν_{max} (thin film): 2100 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 0.10 (3H, s, CH_3), 0.11 (6H, s, 2 x CH_3), 0.15 (3H, s, CH_3), 0.92 (9H, s, 3 x CH_3), 0.97 (9H, s, 3 x CH_3), 1.31 (3H, d, H6, $J_{6,5}$ 6.4), 3.56 (1H, br-s, H4), 3.60 (1H, ddd, H2, $J_{2,1'}$ 3.2, $J_{2,1}$ 4.6, $J_{2,3}$ 9.0), 3.75 (1H, dd, H1, $J_{1,2}$ 4.6, J_{gem} 10.7), 3.82 (1H, dd, H1', $J_{1',2}$ 3.1, J_{gem} 10.7), 3.92 (1H, q, H5, $J_{5,6}$ 6.5), 4.00 (1H, d, H3, $J_{3,2}$ 9.0), 5.62 (1H, s, H7), 7.33 - 7.55 (5H, m, -Ar); δ_C ($CDCl_3$, 100 MHz): -5.2 (CH_3Si), -5.1 (CH_3Si), -2.8 (2 x CH_3Si), 18.6 (2 x ($\underline{C}(CH_3)_3$)), 19.1 (C6), 26.1 ($C(\underline{C}H_3)_3$), 26.5 ($C(\underline{C}H_3)_3$), 62.4, 62.5 (C1, C2), 68.4 (C4), 76.9 (C5), 79.8 (C3), 102.2 (C7), 126.8, 128.6, 129.2, 138.6 (-Ar); *m/z* (ESI+ve): 530 ($[M + Na]^+$, 100%).

2-Azido-3-*O*-benzoyl-5-bromo-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,5,6-trideoxy-L-glucitol 4.144

N-Bromosuccinimide (146 mg, 0.83 mmol) and barium carbonate (205 mg, 1.04 mmol) were added into a solution of **4.143** (350 mg, 0.69 mmol) in carbon tetrachloride (5 mL). The reaction mixture was refluxed with stirring for 1 hour until TLC (ethyl acetate/cyclohexane, 1:19) indicated the disappearance of the starting material (R_f 0.52) and the formation of one major product (R_f 0.71). After removing the solvent *in vacuo*, the residue was purified by flash column chromatography (ethyl acetate/hexane 1:9) to afford the bromide **4.144** (400 mg, 99%) as a yellow oil.

HRMS (ESI+ve): found 586.2127, 588.2105 $[M + H]^+$; $C_{25}H_{45}BrN_3O_4Si_2^+$ requires 586.2127, 588.2106; $[\alpha]_D^{25}$ -11 (*c* 0.66, $CHCl_3$); ν_{max} (thin film): 2098 (s, N_3), 1728 (s, C=O); δ_H (400 MHz,

$CDCl_3$): 0.03 (3H, s, CH_3), 0.04 (3H, s, CH_3), 0.08 (3H, s, CH_3), 0.18 (3H, s, CH_3), 0.86 (9H, s, 3 x CH_3), 0.87 (9H, s, 3 x CH_3), 1.74 (3H, d, H6, $J_{6,5}$ 6.9), 3.69 – 3.73 (1H, m, H2), 3.79 (1H, dd, H1, $J_{1,2}$ 7.0, J_{gem} 10.3), 3.87 (1H, dd, H1', $J_{1',2}$ 4.9, J_{gem} 10.2), 4.26 (1H, t, H4, $J_{4,3}$ = $J_{4,5}$ 5.0), 4.41 (1H, dq, H5, $J_{5,4}$ 4.8, $J_{5,6}$ 6.7), 5.28 (1H, dd, H3, $J_{3,2}$ 3.7, $J_{3,4}$ 5.2), 7.46 (2H, t, -Ar, *J* 7.7), 7.60 (1H, t, -Ar, *J* 7.3), 8.08 – 8.10 (2H, m, -Ar); δ_C (100 MHz, $CDCl_3$): -5.3 (2 x CH_3Si), -3.9 (CH_3Si), -3.8 (CH_3Si), 18.5 ($\underline{C}(CH_3)_3$), 18.6 ($\underline{C}(CH_3)_3$), 22.1 (C6), 26.1 ($C(\underline{C}H_3)_3$), 26.2 ($C(\underline{C}H_3)_3$), 49.5 (C5), 62.2 (C2), 63.8 (C1), 73.8 (C4), 75.9 (C3), 128.6, 129.9, 130.4, 133.7 (-Ar), 165.9 (C=O); *m/z* (ESI+ve): 586, 588 ($[M + H]^+$, 100%, 98%).

3-*O*-Benzoyl-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,5-imino-2,5,6-trideoxy-L-glucitol 4.145

Palladium on charcoal (10 % wt., 60 mg) and sodium acetate (82 mg, 0.99 mmol) were added to a solution of the bromide **4.144** (385 mg, 0.66 mmol) in ethanol (20 mL). The reaction mixture was flushed with nitrogen, argon and hydrogen gas sequentially. Then the reaction mixture was stirred vigorously at room temperature under hydrogen atmosphere for 2 h until TLC (ethyl acetate/cyclohexane 1:4) showed the formation of one major product (R_f 0.30). The reaction mixture was filtered, the solvent removed and the resultant crude product **4.144** (400 mg) was used for the next step without further purification. A small portion of the crude material was purified for characterization by flash column chromatography (triethylamine/ethyl acetate/cyclohexane 0.01:3:7) to afford **4.145** as a yellow oil.

HRMS (ESI+ve): found 480.2955 $[M + H]^+$; $C_{25}H_{45}BrNO_4Si_2^+$ requires 480.2960; $[\alpha]_D^{25}$ -13 (*c* 1.45, MeOH); ν_{max} (thin film): 1723 (s, C=O); δ_H (CD_3OD, 400 MHz): -0.09 (3H, s, CH_3), 0.01 (3H, s, CH_3), 0.17 (3H, s, CH_3), 0.26 (3H, s, CH_3), 0.82 (9H, s, 3 x CH_3), 0.99 (9H, s, 3 x CH_3), 1.20 (3H, d, H6, $J_{6,5}$ 6.6), 3.44 (1H, dq, H5, $J_{5,4}$ 3.5, $J_{5,6}$ 6.6), 3.74 – 3.85 (3H, m, H1, H1', H2), 4.10 (1H, br-d, H4, *J* 2.0), 5.39 (1H, br-d, H3, *J* 2.1), 7.52 (2H, t, -Ar, *J* 7.7), 7.65 (1H, t, -Ar, *J* 7.4), 8.08 (2H, d, -Ar, *J* 7.3); δ_C (CD_3OD, 100 MHz): -4.6 (CH_3Si), -4.5 (CH_3Si), -4.1 (CH_3Si), -3.6 (CH_3Si), 15.5 (C6), 19.8 ($\underline{C}(CH_3)_3$), 19.7 ($\underline{C}(CH_3)_3$), 27.1 ($C(\underline{C}H_3)_3$), 27.2 ($C(\underline{C}H_3)_3$), 58.3 (C5), 62.1 (C2), 63.3 (C1), 79.6 (C4), 81.5 (C3), 130.5, 131.5, 132.2, 135.3 (-Ar), 167.7 (C=O); *m/z* (ESI+ve): 480 ($[M + H]^+$, 100%).

2,5-Imino-2,5,6-trideoxy-D-iditol 4.113

The crude protected pyrrolidine **4.145** (400 mg) was dissolved in a solution of trifluoroacetic acid/water/1,4-dioxane (2:1:1, 10 mL). The solution was stirred at 50 °C for 24 hours until mass spectrometry showed the deprotection of the silyl groups ($[M + H]^+$ 252). The solvent was removed and the residue dissolved in sodium hydroxide solution (aq., 2M, 8 mL); the reaction mixture was then stirred at 50 °C for 24 hours until mass spectrometry showed the formation of the desired product ($[M + H]^+$ 148). The reaction mixture was neutralized with HCl (2M, aq) and the solvent removed *in vacuo*. The residue was dissolved in ethanol (5 mL) and filtered (glass microfiber) to afford a crude salt that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin. The ion exchange column was then washed with water, 1,4-dioxane and then water; the pure product was then eluted with aqueous ammonia (2 M). Removal of solvent *in vacuo* gave a residue which was subjected to a further purification cycle by resin column to give the title compound **4.113** (72 mg, 74% 3 steps from **4.144**) as a yellow oil.

HRMS (ESI+ve): found 148.0968 $[M + H]^+$; $C_6H_{14}N_3O_3^+$ requires 148.0968; $[\alpha]_D^{25}$ +2.4 (*c* 0.17, water); ν_{max} (thin film): 3300 (br, NH, OH); δ_H (D_2O, 400 MHz): 1.15 (3H, d, H6, $J_{6,5}$ 6.7), 3.43 (1H, dq, H5, $J_{5,4}$ 3.7, $J_{5,6}$ 6.7), 3.05 (1H, ddd, H2, $J_{2,3}$ 4.9, $J_{2,1'}$ 6.6, $J_{2,1}$ 7.2), 3.65 (1H, dd, H1, $J_{1,2}$ 7.2, J_{gem} 11.4), 3.77 (1H, dd, H1', $J_{1',2}$ 6.6, J_{gem} 11.4), 3.98 (1H, dd, H4, $J_{4,3}$ 1.5, $J_{4,5}$ 3.6), 4.24 (1H, dd, H3, $J_{3,4}$ 1.4, $J_{3,2}$ 4.9); δ_C (D_2O, 100 MHz): 13.2 (C6), 55.5 (C5), 60.7, 60.8 (C1, C2), 77.2 (C3), 78.9 (C4); *m/z* (ESI+ve): 148 ($[M + H]^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-2,6-dideoxy-D-gulitol 4.146

Lithium borohydride (2M in THF, 0.74 mL, 1.47 mmol) was added to a solution of lactone **4.140** (270 mg, 0.98 mmol) in anhydrous THF at -20 °C. The reaction mixture was stirred at -20 °C for 2 hours when TLC (ethyl acetate/cyclohexane 2:1) showed the consumption of starting material (R_f 0.51) and the formation of a new spot (R_f 0.71). After addition of acetic acid (0.4 mL) to the mixture at -20 °C with stirring, the solvent was removed *in vacuo* to obtain a residue that was

redissovled in ethyl acetate (20 mL) and washed with water (20 mL). The aqueous layer was back extracted with ethyl acetate (2 x 15 mL). The organic phase was combined, dried ($MgSO_4$) and the solvent was removed *in vacuo* a yield a residue that was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:2 to 2:1) to afford the diol **4.146** (246 mg, 90%) as a white solid.

HRMS (ESI+ve): Found 302.1112 $[M + Na]^+$; $C_{13}H_{17}N_3O_4Na^+$ requires 302.1113; m.p.: 100 – 102 °C; $[\alpha]_D^{25}$ +19 (*c* 0.69, $CHCl_3$); ν_{max} (thin film): 2102 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.40 (3H, d, H6, $J_{6,5}$ 6.4), 3.58 (1H, s, H4), 3.82 (1H, dd, H1, $J_{1,2}$ 4.9, J_{gem} 11.4), 3.85 (1H, d, H3, $J_{3,2}$ 9.1), 3.88 – 3.92 (1H, m, H4), 3.98 (1H, dd, H1', $J_{1',2}$ 3.2, J_{gem} 12.0), 3.90 (1H, ddd, H2, $J_{2,1'}$ 3.1, $J_{2,1}$ 4.2, $J_{2,3}$ 8.5), 4.07 (1H, q, H5, $J_{5,6}$ 6.4), 5.60 (1H, s, H7), 7.37 - 7.48 (5H, m, -Ar); δ_C ($CDCl_3$, 100 MHz): 17.5 (C6), 62.4, 62.5 (C1, C2), 66.2 (C4), 76.9 (C5), 79.1 (C3), 101.5 (C7), 126.2, 128.7, 129.5, 137.6 (-Ar); *m/z* (ESI+ve): 302 ($[M + Na]^+$, 100%).

2-Azido-*(R)*-3,5-*O*-benzylidene-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,6-dideoxy-D-gulitol 4.147

tert-Butyldimethylsilyl triflate (0.27 mL, 1.18 mmol) and anhydrous pyridine (0.11 mL, 1.41 mmol) were added to a solution of **4.146** (130 mg, 0.47 mmol) in anhydrous dichloromethane (6 mL) at 0 °C. The reaction mixture was stirred at room temperature for 15 h until TLC (ethyl acetate/cyclohexane, 1:9) showed the formation of a major spot (R_f 0.73). Removal of solvent *in vacuo* gave a residue that was dissolved in cyclohexane (20 mL) and washed with water (2 x 15 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed to obtain a residue that was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:9) to afford the silyl ether **4.147** (200 mg, 84%) as a colorless oil.

HRMS (ESI+ve): found 530.2840 $[M + Na]^+$; $C_{25}H_{45}N_3O_4Si_2Na^+$ requires 530.2841; $[\alpha]_D^{25}$ +6.6 (*c* 0.80, $CHCl_3$); ν_{max} (thin film): 2103 (s, N_3); δ_H (400 MHz, $CDCl_3$): 0.04 (3H, s, CH_3), 0.05 (3H, s, CH_3), 0.14 (3H, s, CH_3), 0.17 (3H, s, CH_3), 0.90 (9H, s, 3 x CH_3), 0.99 (9H, s, 3 x CH_3), 1.30 (3H, d, H6, $J_{6,5}$ 6.4), 3.53 (1H, ddd, H2, $J_{2,1'}$ 2.3, $J_{2,1}$ 4.9, $J_{2,3}$ 10.0), 3.66 (1H, s, H4), 3.76 (1H, d, H3, $J_{3,2}$ 10.0),

3.89 – 3.95 (2H, m, H1, H5), 4.01 (1H, dd, H1', $J_{1',2}$ 2.4, J_{gem} 10.7), 5.51 (1H, s, H7), 7.32 - 7.47 (5H, m, -Ar); δ_C (100 MHz, $CDCl_3$): -5.3 (CH_3Si), -5.2 (CH_3Si), -3.6 (CH_3Si), -3.4 (CH_3Si), 18.6 ($\underline{C}(CH_3)_3$), 18.8 ($\underline{C}(CH_3)_3$), 22.3 (C6), 19.0 ($\underline{C}(CH_3)_3$), 26.1 ($C(\underline{C}H_3)_3$), 26.6 ($C(\underline{C}H_3)_3$), 59.9 (C2), 63.2 (C1), 66.7 (C4), 76.9 (C5), 78.1 (C3), 102.1 (C7), 126.9, 128.6, 129.3, 138.8 (-Ar); *m/z* (ESI+ve): 530 ($[M + Na]^+$, 100%).

2-Azido-3-*O*-benzoyl-5-bromo-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,5,6-trideoxy-L-mannitol 4.148

N-Bromosuccinimide (55 mg, 0.47 mmol) and barium carbonate (115 mg, 0.59 mmol) were added to a solution of **4.147** (200 mg, 0.39 mmol) in carbon tetrachloride (6 mL). The reaction mixture was refluxed for 1 hour until TLC (ethyl acetate/cyclohexane, 1:19) indicated the disappearance of starting material (R_f 0.70) and the formation of one major product (R_f 0.62). After removal of the solvent *in vacuo*, the residue was purified by flash column chromatography (ethyl acetate/hexane, 1:9) to give the *manno*-bromide **4.148** (173 mg, 76%) as a yellow oil.

HRMS (ESI+ve): found 586.2128, 588.2107 $[M + H]^+$; $C_{25}H_{45}BrN_3O_4Si_2^+$ requires 586.2127, 588.2106; $[\alpha]_D^{25}$ –8.1 (*c* 0.93, $CHCl_3$); ν_{max} (thin film): 2103 (s, N_3), 1730 (s, C=O); δ_H ($CDCl_3$, 400 MHz): -0.01 (3H, s, CH_3), 0.01 (3H, s, CH_3), 0.21 (3H, s, CH_3), 0.22 (3H, s, CH_3), 0.87 (9H, s, 3 x CH_3), 0.98 (9H, s, 3 x CH_3), 1.69 (3H, d, H6, $J_{6,5}$ 6.9), 3.70 – 3.81 (2H, m, H1, H2), 3.86 (1H, dd, H1', $J_{1',2}$ 2.7, J_{gem} 10.4), 4.16 (1H, dq, H5, $J_{5,4}$ 5.7, $J_{5,6}$ 6.7), 4.29 (1H, dd, H4, $J_{4,3}$ 1.7, $J_{4,5}$ 5.7), 5.41 (1H, dd, H3, $J_{3,4}$ 1.7, $J_{3,2}$ 9.2), 7.46 (2H, t, -Ar, *J* 7.6), 7.59 (1H, t, -Ar, *J* 7.5), 8.05 (2H, d, -Ar, *J* 7.3); δ_C ($CDCl_3$, 100 MHz): -5.3 (2 x CH_3Si), -3.9 (CH_3Si), -3.8 (2 x CH_3Si), 18.5 ($\underline{C}(CH_3)_3$), 18.8 ($\underline{C}(CH_3)_3$), 22.3 (C6), 26.1 ($C(\underline{C}H_3)_3$), 26.4 ($C(\underline{C}H_3)_3$), 50.3 (C5), 61.9 (C2), 64.0 (C1), 71.9 (C3), 75.4 (C4), 128.9, 130.0, 130.3, 133.7 (-Ar), 165.7 (C=O); *m/z* (ESI+ve): 586, 588 ($[M + H]^+$, 100%, 98%).

3-*O*-Benzoyl-1,4-*bis*-(*O*-*tert*-butyldimethylsilyl)-2,5-imino-2,5,6-trideoxy-D-gulitol 4.149

Palladium on charcoal (10 % wt., 40 mg) and sodium acetate (49 mg, 0.60 mmol) was added to a solution of **4.148** (233 mg, 0.40 mmol) in ethanol (15 mL). The reaction mixture was flushed

with nitrogen, argon and hydrogen gas sequentially. Then the reaction mixture was stirred vigorously at room temperature under hydrogen atmosphere for 5 h until TLC (ethyl acetate/cyclohexane 1:4) showed the formation of one major product (R_f 0.14). After filtered and evaporated to dryness *in vacuo*, the crude protected pyrrolidine **4.149** (170 mg) was used for the next step without further purification. A small portion was purified for characterization by flash column chromatography (triethylamine/ethyl acetate/cyclohexane 0.01:2:1) to afford a pure sample of **4.149** as a yellow oil.

HRMS (ESI+ve): found 480.2958 $[M + H]^+$; $C_{25}H_{45}BrNO_4Si_2^+$ requires 480.2960; $[\alpha]_D^{25}$ -23 (*c* 0.90, MeOH); ν_{max} (thin film): 1721 (s, C=O); δ_H (CD_3OD, 400 MHz): 0.13 (6H, s, 2 x CH_3), 0.15 (3H, s, CH_3), 0.23 (3H, s, CH_3), 0.93 (9H, s, 3 x CH_3), 0.98 (9H, s, 3 x CH_3), 1.23 (3H, d, H6, $J_{6,5}$ 6.6), 3.25 (1H, dt, H2, $J_{2,3}$ 3.2, $J_{2,1}$ = $J_{2,1'}$ 6.0), 3.31 (1H, dq, H5, $J_{5,4}$ 3.5, $J_{5,6}$ 6.6), 3.85 (1H, dd, H1, $J_{1,2}$ 6.4, J_{gem} 10.1), 3.92 3.85 (1H, dd, H1', $J_{1',2}$ 6.0, J_{gem} 10.1), 4.15 (1H, br-d, H4, $J_{4,5}$ 3.2), 5.19 (1H, br-d, H3, $J_{3,2}$ 2.8), 7.52 (2H, t, -Ar, *J* 7.6), 7.65 (1H, t, -Ar, *J* 7.3), 8.06 (2H, d, -Ar, *J* 7.2); δ_C (CD_3OD, 100 MHz): -4.4 (CH_3Si), -4.3 (CH_3Si), -4.1 (CH_3Si), -3.5 (CH_3Si), 15.0 (C6), 19.9 ($\underline{C}(CH_3)_3$), 20.0 ($\underline{C}(CH_3)_3$), 27.2 ($C(\underline{C}H_3)_3$), 27.3 ($C(\underline{C}H_3)_3$), 59.9 (C5), 65.3 (C2), 67.7 (C1), 80.9 (C4), 84.7 (C3), 130.5, 131.5, 132.0, 135.3 (-Ar), 167.9 (C=O); *m/z* (ESI+ve): 480 ($[M + H]^+$, 100%).

2,5-Imino-2,5,6-trideoxy-D-gulitol 4.115

A solution of the protected pyrrolidine **4.149** (170 mg) in trifluoroacetic acid/water/1,4-dioxane (2:1:1, 4 mL) was stirred at 50 °C for 24 hours until mass spectrometry showed the deprotection of the silyl groups ($[M + H]^+$ 252). The solvent was removed and the residue dissolved in sodium hydroxide solution (aq, 2M, 3 mL) and stirred at 50 °C for 24 hours until mass spectrometry showed the formation of desired product ($[M + H]^+$ 148). The mixture was neutralized with HCl (2M, aq) and the solvent removed *in vacuo*. The residue was dissolved in ethanol (5 mL) and filtered (glass microfiber) to afford a crude salt that was then loaded with water (~1 mL) onto a short column of DOWEX® 50WX8-200 resin. The ion exchange column was then washed with water, 1,4-dioxane and then water; the pure product was then eluted with

aqueous ammonia (2 M). Removal of solvent *in vacuo* gave a residue which was subjected to a further purification by resin column to yield the *gulo*-pyrrolidine **4.115** (30 mg, 51% 3 steps from **4.148**) as a light yellow gum.

HRMS (ESI+ve): found 148.0968 $[M + H]^+$; $C_6H_{14}N_3O_3^+$ requires 148.0968; $[\alpha]_D^{25}$ –8.9 (*c* 0.57, water) [lit.[69] for the enantiomer of **4.115**: $[\alpha]_D^{24}$ +17 (*c* 0.50, water)]; ν_{max} (thin film): 3290 (br, NH, OH); δ_H (D_2O, 400 MHz): 1.21 (3H, d, H6, $J_{6,5}$ 6.7), 3.13 (1H, ddd, H2, $J_{2,3}$ 4.4, $J_{2,1'}$ 4.9, $J_{2,1}$ 6.7), 3.45 (1H, dq, H5, $J_{5,4}$ 4.3, $J_{5,6}$ 6.7), 3.71 (1H, dd, H1, $J_{1,2}$ 7.0, J_{gem} 11.8), 3.80 (1H, dd, H1', $J_{1',2}$ 4.9, J_{gem} 11.8), 3.93 (1H, dd, H3, $J_{3,4}$ 1.8, $J_{3,2}$ 4.4), 3.99 (1H, dd, H4, $J_{4,3}$ 1.8, $J_{4,5}$ 4.3); δ_C (D_2O, 100 MHz): 12.6 (C6), 57.1 (C5), 61.9 (C1), 66.7 (C2), 78.9 (C3), 79.4 (C4); *m/z* (ESI+ve): 148 ($[M + H]^+$, 100%).

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Publication and Conference

Related to Chapter 2:

Liu, Z.; Jenkinson, S. F.; Vermaas T.; Adachi I.; Wormald, M. R.; Hata Y.; Kurashima Y.; Kaji A.; Yu, C; Kato A.; and Fleet, G. W. J., 3-Fluoroazetidinecarboxylic acids and trans,trans-3,4-difluoroproline as peptide scaffolds: inhibition of pancreatic cancer cell growth by a fluoroazetidine iminosugar, *The Journal of Organic Chemistry*, 2015, 80 (9), 4244-58.

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Related to Chapter 3:

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Related to Chapter 4:

Liu, Z *et al*. 6-Deoxyhexoses from L-rhamnose in the search for inducers of the rhamnose operon: synergy of chemistry and biotechnology, **in submission**

Kelly, C.; **Liu, Z** *et al.* Synthetic chemical inducers and genetic simplification enable orthogonal and improved control of the rhaBAD promoter system, **submitted**

Conference:

Dec. 2015, Poster presentation, **Pacifichem 2015**, Hawaii, USA
Aug. 2014, Poster presentation, 248th **ACS National Meeting & Exposition**, SF, USA
May. 2014, Poster and oral presentations, **RSC Carbohydrate Group Meeting**, UK
Sep. 2013, Poster presentation, **1st Joint RSC Fluorine-Carbohydrate Subject Meeting**, UK