The Synthesis of Branched Sugars and Iminosugars

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Abstract

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Iminosugars, carbohydrate analogues in which nitrogen replaces the endocyclic oxygen, have attracted much interest due to their biological activity. Iminosugars inhibit carbohydrate-processing enzymes, thereby affecting many biological processes. Several iminosugars are licensed drugs, with many more compounds undergoing clinical trials. The main subject of this thesis is the synthesis and evaluation of novel iminosugars, particularly the effects of structural modifications on the biological activity of these compounds.

Chapter 1 describes the role of carbohydrate-processing enzymes in the body, and explores the therapeutic applications of iminosugars that arise from their activity against these enzymes. Examples of substituted iminosugars are reviewed, and the effects of substituents on enzyme inhibition are described.

Chapter 2 concerns methyl-branched swainsonine derivatives. Swainsonine has shown potential as a cancer treatment through its inhibition of α-mannosidase. The synthesis of (6*R*)- and (6*S*)-*C*-methyl D-swainsonine is described; both compounds were potent and selective α-mannosidase inhibitors (IC_{50} 3.8 μM, 14 μM). Although less active than the parent compound, their selectivity for Golgi mannosidase over lysosomal mannosidase may be more important than the absolute value against the model enzyme.

Chapter 3 describes the synthesis of 2-*C*-methyl L-fucose derivative. A diastereoselective Kiliani reaction allowed the formation of a single lactone bearing a new quaternary centre. The utility of this intermediate in accessing di-branched iminosugars was explored; however, attempts to introduce nitrogen to the lactone lacked the necessary stereoselectivity.

Chapter 4 relates to the synthesis of pyrrolidine iminosugars, specifically methyl amides. Two enantiomeric dihydroxyproline amides were synthesised; the D-proline derivative was a potent β-*N*-acetylhexosaminidase inhibitor (IC_{50} values of up to 3.6 μM), but the L-enantiomer was completely inactive. Inhibition of *N*-acetylhexosaminidases is relevant to the treatment of cancer and lysosomal storage diseases, and this work contributed to a wider project investigating the effects of altered stereochemistry on the biological activity of pyrrolidine amides.

Acknowledgments

First and foremost I would like to thank my supervisor Professor George Fleet for his guidance and support during my time in his research group. As well as being a fountain of chemistry knowledge, he is a really *great guy*.

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…and James.

Abbreviations

The following abbreviations are used in this thesis:

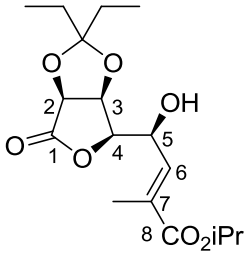
a-	apparent
Å	Ångström
Ac	acetyl
aq	aqueous
Ar	aryl; aromatic
Asn	asparagine
atm	atmosphere(s)
ATP	adenosine triphosphate
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
br	broad
Bu	butyl
Bzh	benzhydryl, diphenylmethyl
c	concentration
°C	degrees Celsius
cat.	catalytic amount
CI	chemical ionisation
cm	centimetre(s)
conc.	concentrated
COSY	correlation spectroscopy
CRC	colorectal carcinoma
CS	castanospermine
d	doublet
DAB	1,4-dideoxy-1,4-imino-D-arabinitol
DCM	dichloromethane
dd	double doublet
ddd	double double doublet
ddq	double double quartet
ddt	double double triplet
DEPT	distortionless enhancement by polarisation transfer
DGJ	1-deoxygalactonojirimycin
dGMII	drosophila Golgi α-mannosidase II
DHP	dihydroxyproline
DIBAL-H	diisobutyl aluminium hydride
dm	decimeter
DMDP	2,5-dideoxy-2,5-imino-D-mannitol
DMF	*N*,*N*-dimethylformamide
DNA	deoxyribonucleic acid
DNJ	1-deoxynojirimycin
dq	double quartet
dt	double triplet
E_1cB	unimolecular elimination (conjugate base)
eq	equivalent(s)
eqm	equilibrium
ER	endoplasmic reticulum
ESI	electrospray ionisation
Et	ethyl
FAD	flavine adenine dinucleotide
FI	field ionisation
Fmoc	9-fluorenylmethyloxycarbonyl
Fuc	fucose
g	gram(s)
Gal	galactose
GalNAc	*N*-acetylgalactosamine
GalNAcase	*N*-acetylgalactosaminidase
GCase	β-glucocerebrosidase
GDP	guanosine diphosphate
Glc	glucose
GlcNAc	*N*-acetylglucosamine
GlcNAcase	*N*-acetylglucosaminidase
GMII	Golgi α-mannosidase II
GSL	glycosphingolipid

h	hour(s)
hGMII	human Golgi α-mannosidase II
hLM	human lysosomal α-mannosidase
HMQC	heteronuclear multiple quantum coherence
HNJ	homonojirimycin
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	Hertz
IC_{50}	half maximal inhibitory concentration
IFG	isofagomine
imid.	imidazole
IPA	*iso*-propyl alcohol
*i*Pr	*iso*-propyl
IR	infrared
IUPAC	International Union for Pure and Applied Chemistry
J	scalar coupling constant
JBM	jack bean α-mannosidase
L	litre(s)
LAB	1,4-dideoxy-1,4-imino-L-arabinitol
L-DMDP	2,5-dideoxy-2,5-imino-L-mannitol
LG	leaving group
Lit.	literature value
LM	lysosomal α-mannosidase
LRMS	low resolution mass spectroscopy
LSD	lysosomal storage disease
m	multiplet
m	meta
M	molar, $mol \cdot dm^{-3}$
MAF	macrophage activating factor
mbar	millibar(s)
Me	methyl
MEM	β-methoxyethoxy methyl ether
mg	milligram(s)
MHz	megahertz
min	minute(s)
mL	millilitre(s)
mmol	millimole(s)
mol	mole(s)
m.p.	melting point
Ms	mesyl, methanesulfonyl
MTBE	methyl *tert*-butyl ether
m/z	mass to charge ratio
μM	micromolar
N	mol·kg
NAD	nicotinamide adenine dinucleotide
*N*B-DNJ	*N*-butyl 1-deoxynojirimycin
*n*Bu	normal butyl
NI	no inhibition
nm	nanometre(s)
nM	nanomolar
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
o	ortho
p	para
[P]	generic protecting group
Ph	phenyl
ppm	parts per million
Pro	proline
*p*TSA	*para*-toluenesulfonic acid
py.	pyridine
q	quartet
quant.	quantitative
R	generic group
RCC	renal cell carcinoma
Red-Al®	sodium bis(2-methoxyethoxy) aluminumhydride
R_f	retention factor
RNA	ribonucleic acid
RT	room temperature
s	singlet (NMR); strong (IR)
sept	septet
S_N1	unimolecular nucleophilic substitution

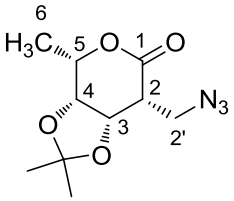
S_N2	bimolecular nucleophilic substitution
SW	swainsonine
t	triplet
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
*t*Bu	*tert*-butyl
Tf	trifluromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
t.l.c.	thin layer chromatography
Trt	trityl, triphenylmethyl
UV	ultraviolet
v	very
v/v	volume to volume
w	weak
% wt	% by weight

Note Concerning Nomenclature

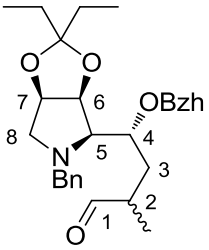
The numbering conventions used in this thesis for assignment of spectroscopic data is derived from the systematic naming of materials according to IUPAC recommendations.[1] Some examples are given below for reference.



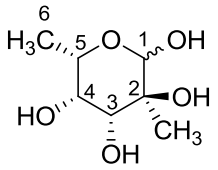
iso-Propyl (*E*)-6,7-dideoxy-2,3-*O*-diethylketal-7-*C*-methyl-L-*galactono*-1,4-lactono-oct-6-enoate



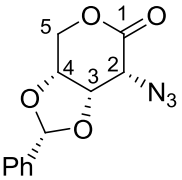
2-Azidomethyl-2,6-dideoxy-L-talono-1,5-lactone



4-*O*-Benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2-*C*-methyl-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octose



6-Deoxy-2-*C*-methyl-L-galactose



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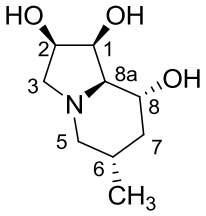
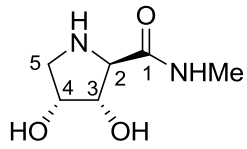


(6*S*)-*C*-Methyl-D-swainsonine



Methyl (3*S*,4*R*)-dihydroxy-D-proline amide

[1] McNaught, A.D., *Carbohydrate Research*, **1997**, 297, 1-92

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Chapter 1

General Introduction

1.1 Carbohydrates: overview

Carbohydrates are the most abundant class of organic compounds in nature,[1] and were originally defined by the chemical formula $C_x(H_2O)_y$.[2] However, many related compounds with different compositions are now classed as carbohydrates, including deoxysugars such as 2-deoxyribose **1.2** (a component of DNA), and compounds that contain heteroatoms, such as *N*-acetyl neuraminic acid **1.3** (the most common sialic acid, found in many animal tissues). Carbohydrates can therefore be more accurately considered as polyhydroxylated aldehydes and ketones.

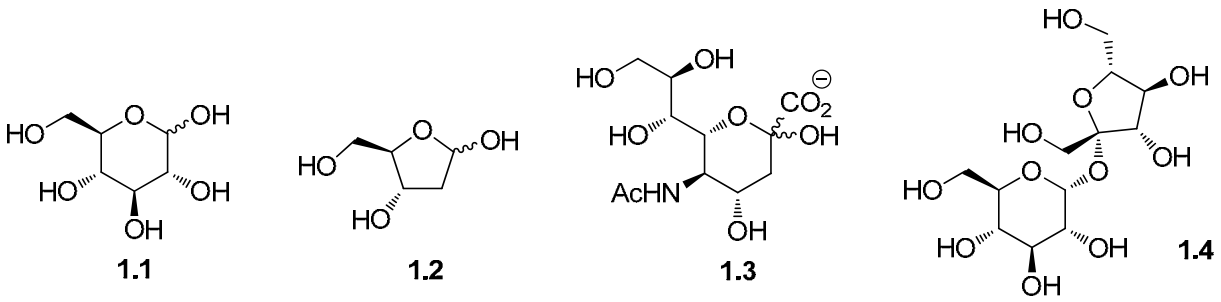
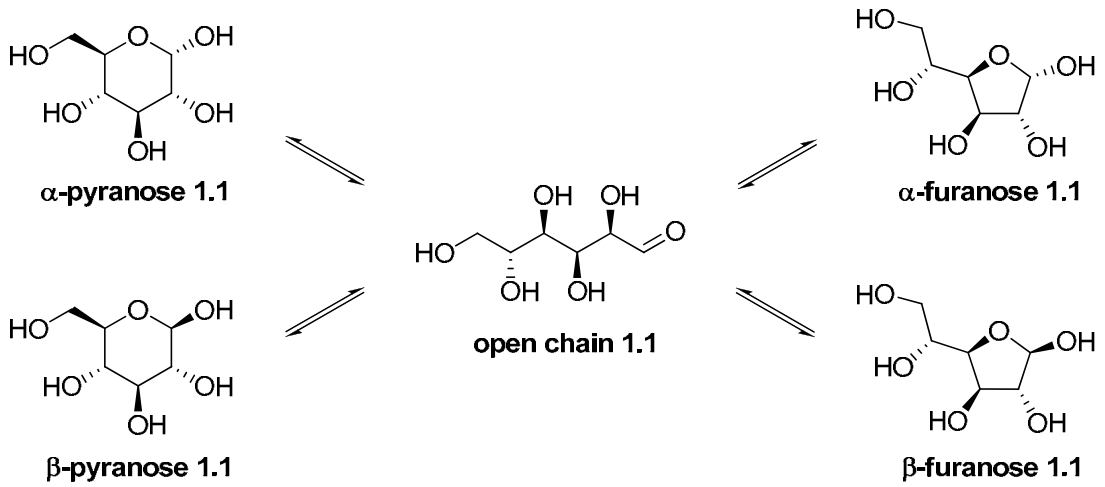


Figure 1.1 The carbohydrates D-glucose, 2-deoxy-D-ribose, *N*-acetyl neuraminic acid (sialic acid), and sucrose.

Carbohydrates vary in the number of saccharide units they contain. Monosaccharides (*e.g.* glucose **1.1**) are single units; disaccharides consist of two monosaccharide units linked *via* a glycosidic bond (*e.g.* sucrose **1.4** is made by the condensation of glucose and fructose). Oligosaccharides contain about 3-10 saccharide units, and polysaccharides contain much larger numbers of subunits.



Scheme 1.1 Equilibria between different forms of glucose.

Monosaccharides often exist as mixtures of open-chain, furanose (five-membered ring) and pyranose (six-membered ring) forms; the anomeric group in furanose or pyranose rings can adopt the α or β orientation (**Scheme 1.1**). These properties, along with the density of stereocentres in each saccharide unit, give rise to great variation in the structure of carbohydrates.

1.2 Biological importance of carbohydrates

Polysaccharides fulfil some important biological roles. Starch and glycogen, which are branched α(1→4) polysaccharides of glucose, act as convenient stores of chemical energy. Cellulose is a β(1→4) polysaccharide of glucose that is the structural element of plant cell walls, and chitin, its *N*-acetylglucosamine (GlcNAc) analogue, is the major component of arthropod shells. However, this thesis derives from interest in monosaccharides (specifically branched monosaccharides) and oligosaccharides, which will be outlined in the following sections.

1.2.1 Monosaccharides

Monosaccharides have a variety of biological roles, aside from their well-known function of providing organisms with chemical energy *via* glycolysis and the Krebs cycle. Deoxyribose forms the backbone of DNA, and the absence of the 2-hydroxyl group means that DNA is more flexible than RNA, allowing it to adopt a double helix conformation. Ribose is a component of the coenzymes FAD and NAD, which are both redox cofactors involved in the electron transport chain of oxidative phosphorylation; it is also a component of ATP, the universal energy currency of cells.[3]

1.2.1.1 Branched monosaccharides

Branched monosaccharides are a class of sugars that possess one or more additional groups attached to the carbon backbone. Common substituents in natural branched sugars are methyl and hydroxymethyl groups. The first branched sugars to be discovered were apiose **1.5**, found in parsley, and hamamelose **1.6**, isolated from witch hazel.[4]

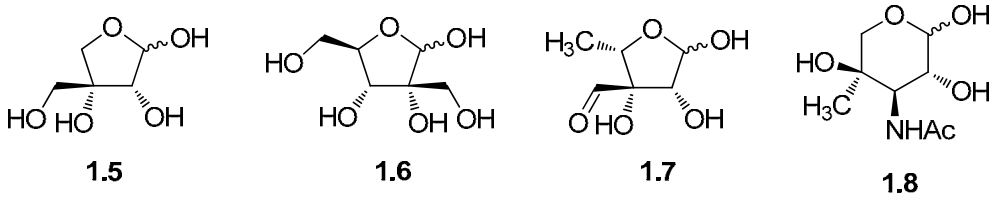


Figure 1.2 Some naturally-occurring branched sugars.

A variety of other branched sugars have since been discovered. Many are found in antibiotics, such as streptose **1.7** from streptomycin, and garosamine **1.8** from gentamycin. Others are components of bacterial cell walls; it is thought that the different cell surface polysaccharides of bacteria may exist as a form of antigenic variation.

The presence of a branch can have a large effect on the biological behaviour of a sugar, increasing activity, reducing activity, or even rendering the compound completely inactive. There is considerable interest in the synthesis of branched sugars, and this topic will be discussed further in **Chapter 3**.

1.2.2 Oligosaccharides

Glycoconjugates consist of a carbohydrate and a non-carbohydrate moiety, and include structures such as glycoproteins and glycolipids. In general the carbohydrate is an oligosaccharide, and this portion of the molecule is called a glycan. In eukaryotic organisms, the non-carbohydrate portion of the molecule is embedded in the cell membrane, and the glycan is displayed on the cell surface. The cell surface displays a dense array of glycans, referred to as the glycocalyx.

These glycans are biologically important, for example serving as markers for cell-cell recognition. Although the exact mechanisms are not well understood, this interaction is vital in biological events such as egg fertilisation,[5] microbial infection,[6] inflammation, and cancer growth and metastasis.[7]

1.2.2.1 Biosynthesis of glycans

In glycoproteins, there are two types of glycan. The more common *N*-linked glycans are found attached to the nitrogen of the side chain of asparagine, and *O*-linked glycans are attached through serine or threonine side chains. *N*-Linked glycans fall into three classes: high mannose type, hybrid type, and complex type.

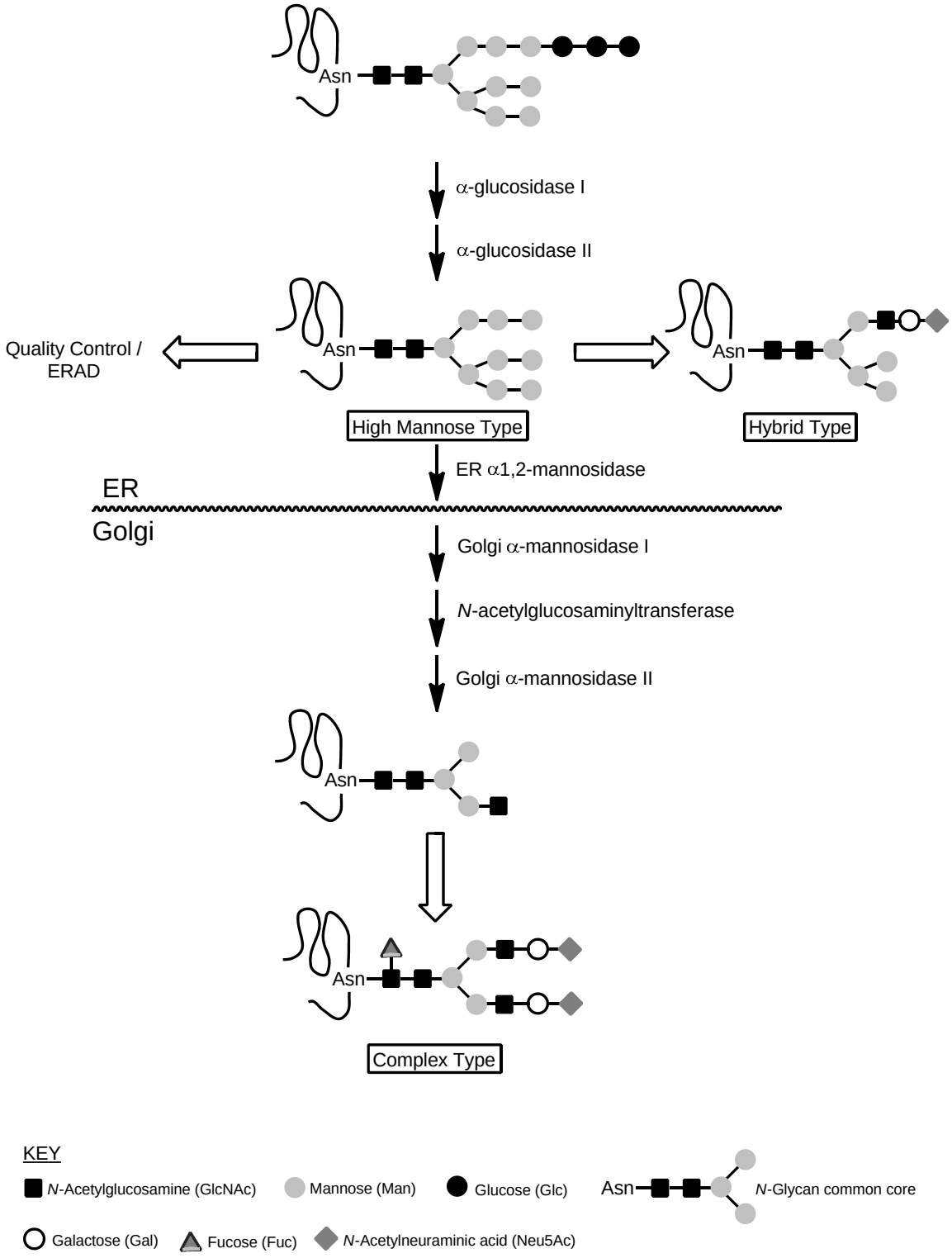


Figure 1.3 Assembly of glycans in the cell. Reprinted from *Progress In Medicinal Chemistry* Vol. 50, G. Horne and F. X. Wilson, Therapeutic Application of Iminosugars: Current Perspectives and Future Opportunities, 2011, with permission from Elsevier.[8]

Glycans must be assembled and then modified before they are functional; this process is shown in **Figure 1.3**. Assembly takes place in the endoplasmic reticulum, and in the case of complex type oligosaccharides, the precursors are moved to the Golgi apparatus prior to their modification. Once modified, the glycans move to the cell surface.

Key steps in this pathway are catalysed by glycosidases and glycosyl transferases. Given the importance of cell surface glycans in many biological processes, it is clear that the inhibition of these carbohydrate-processing enzymes is an important therapeutic target.

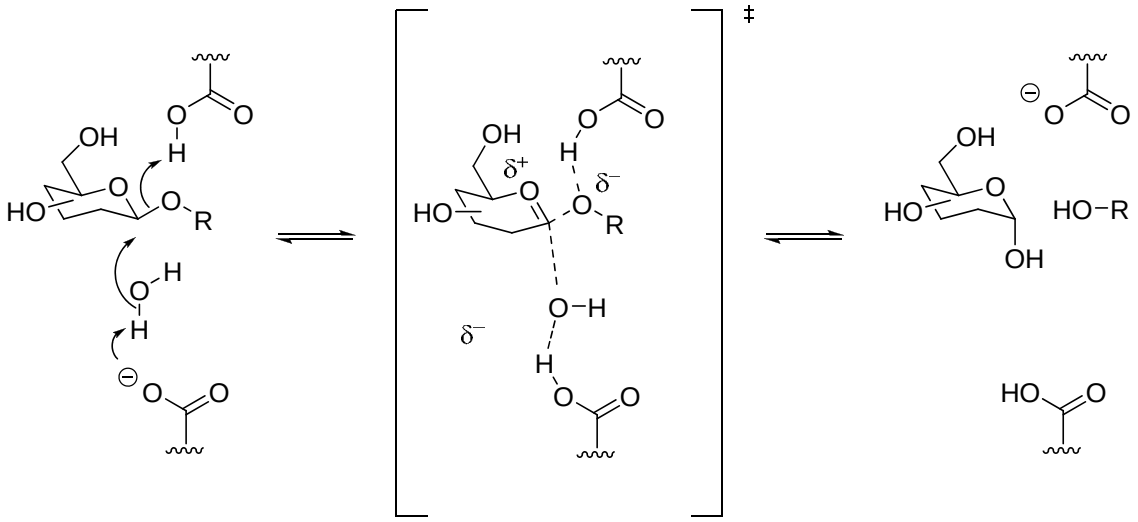
1.3 Glycosidases

Carbohydrate-processing enzymes fall into two broad classes: glycosyl transferases, which form glycosidic bonds, and glycoside hydrolases (or glycosidases), which cleave them. Glycosidic bonds are stable under physiological conditions; however, glycosidases may catalyse their hydrolysis at a rates of up to $1000s^{-1}$.[9] Glycosidases are necessary for key biological processes such as intestinal digestion, post-translational modification of glycoproteins, and lysosomal catabolism.[10]

Glycosidases promote the hydrolysis of glycosidic bonds *via* nucleophilic substitution. Depending on the mechanism, the process may be inverting or retaining. The mechanisms of glycoside hydrolysis have been studied in detail.[11-13]

1.3.1 Hydrolysis with inversion

The general mechanism for the hydrolysis of a glycosidic bond with inversion of configuration is shown in **Scheme 1.2**.



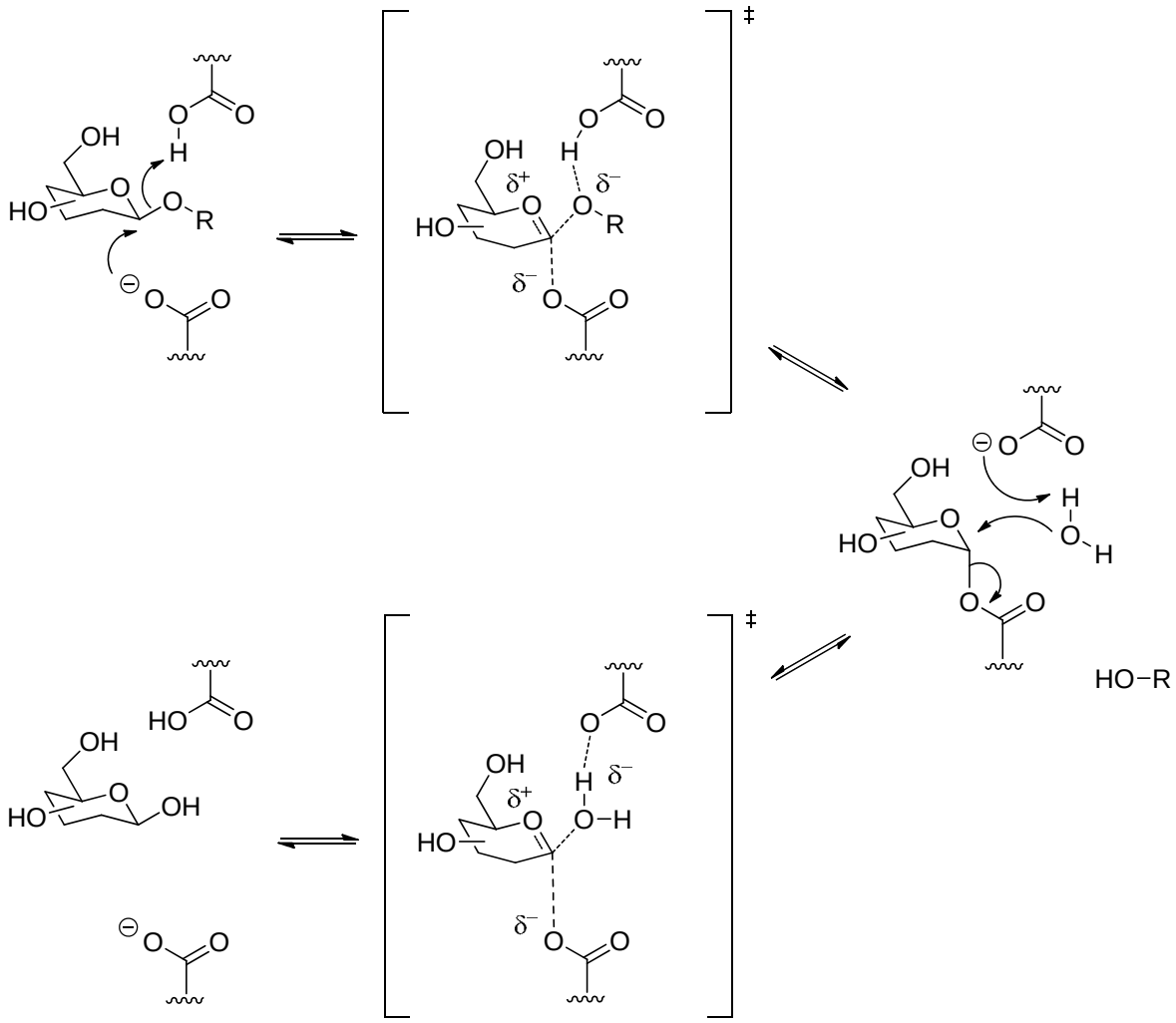
Scheme 1.2 General mechanism for glycoside hydrolysis with inversion.

Within the active site, two carboxylic acid residues act as general acid, general base, or nucleophilic catalysts. In the case of inverting glycosidases, these residues are around 10.5 Å apart, allowing enough space for the substrate and a water molecule to bind between them. This mechanism involves a single direct displacement: the anomeric oxygen is protonated by the carboxylic acid residue, and the carboxylate group acting as a general base directs the water molecule to attack the anomeric carbon.

1.3.2 Hydrolysis with retention

Hydrolysis with retention is a slightly more involved mechanism involving two displacements to give a net retention of configuration. The general mechanism for this is shown in **Scheme 1.3**. Within the active site of retaining glycosidases, the carboxylic acid residues are in close proximity (around 5.5 Å apart) compared to inverting glycosidases. Initially the carboxylate attacks the anomeric carbon, while the anomeric substituent is ejected, to give the glycosyl-ester intermediate. The other carboxylate group then deprotonates a water molecule as water attacks

the anomeric centre, displacing the ester and forming the hemiacetal with net retention of configuration.



Scheme 1.3 General mechanism for hydrolysis with retention.

It is notable that in both inverting and retaining glycosidases, the transition states resemble an oxocarbenium ion, with the anomeric carbon possessing considerable sp^2 character.[14]

1.4 Iminosugars

1.4.1 Overview and natural occurrence

Iminosugars are carbohydrate analogues in which the endocyclic oxygen is replaced by a nitrogen atom.[15] Iminosugars are an abundant class of natural products, having been isolated from plants, fungi, and bacteria; many artificial iminosugars have also been synthesised.[16] The five classes of naturally-occurring iminosugars are the monocyclic five-membered pyrrolidines and six-membered piperidines, and the bicyclic [3.3.0] pyrrolizidines, [4.3.0] indolizidines, and [3.2.1] nortropanes (**Figure 1.4**).

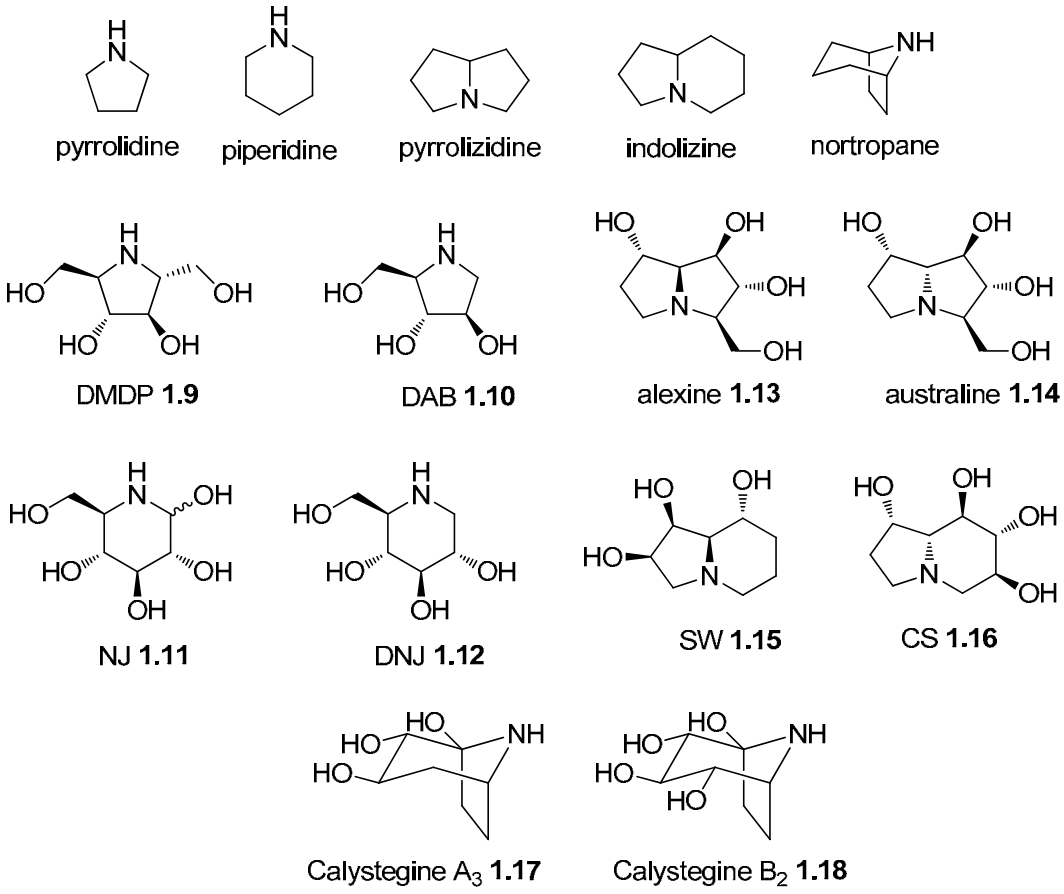


Figure 1.4 Generic iminosugar scaffolds, and naturally-occurring examples of each.

Nojirimycin (NJ) **1.11** was the first iminosugar to be isolated, in the 1960s.[17-20] NJ is the nitrogen analogue of D-glucose, and was found to have antibiotic properties. The related compound 1-deoxynojirimycin (DNJ) **1.12** was subsequently isolated from the mulberry plant[21] and from bacterial culture.[22] Both NJ and DNJ are potent glucosidase inhibitors; however, the absence of the hemiaminal group in DNJ greatly increases the stability of this compound. The first pyrrolidine iminosugar was isolated a few years later from the plant *Derris elliptica*: 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) **1.9** was found to be another glucosidase inhibitor.[23] Following these early examples of biologically active iminosugars, a large number of other compounds have been isolated from natural sources. The first indolizidine iminosugars to be isolated were swainsonine (SW) **1.15** from *Swainsona canescens*,[24] and castanospermine (CS) **1.16** from *Castanospermum australe*;[25] both are Australian plants that were known to be toxic to livestock. The pyrrolizidines alexine **1.13**[26] and australine **1.14,**[27] and the nortropane iminosugars calystegines, were discovered soon afterwards.[28]

1.4.2 Glycosidase inhibition

Many iminosugars are glycosidase inhibitors.[29] In the majority of cases, the inhibition is due to the iminosugar mimicking the transition state of the substrate in the active site.[12, 30] At physiological pH, iminosugars are protonated at the nitrogen atom, thus mimicking the positively charged carbocation/oxocarbenium transition state of the enzyme's natural substrate.[31] Pyrrolidine and piperidine iminosugars usually have a close structural resemblance to the natural substrate; bicyclic inhibitors often have the same stereochemistry as the substrate, and the conformational rigidity of the bicyclic skeleton may lead to stronger inhibitor-enzyme interactions.

However, there are examples of L-iminosugars inhibiting enzymes that process D-sugars; this inhibition often occurs in a non-competitive fashion, indicating the presence of allosteric binding sites in these enzymes.[16] Additionally, modifications to iminosugars (*e.g.* in stereochemistry, ring size, substituents, *N*-alkylation) can alter the properties of the molecule, such as strength of enzyme inhibition, selectivity, and ease of transport into the cell.

The glycosidase inhibition shown by iminosugars makes them attractive targets for medicinal use; these applications are discussed in the following section.

1.5 Therapeutic applications of iminosugars

Iminosugars have a number of advantages as drug candidates. They are highly water soluble and readily absorbed compared to most other small molecules; in some cases they may benefit from biological mechanisms for transporting carbohydrates,[8] which may in turn allow them to cross the blood brain barrier.[32] As highly oxidised species, iminosugars are generally metabolically stable and are excreted unchanged in the urine.

Iminosugars have a large number of potential therapeutic applications,[8, 15, 33] mainly through modulating the activity of carbohydrate-processing enzymes. Conditions that are amenable to this approach include metabolic disorders, cancers, viral infection, neurodegenerative disorders, and inflammation. Some key therapeutic applications of iminosugars will be discussed below.

1.5.1 Lysosomal Storage Diseases

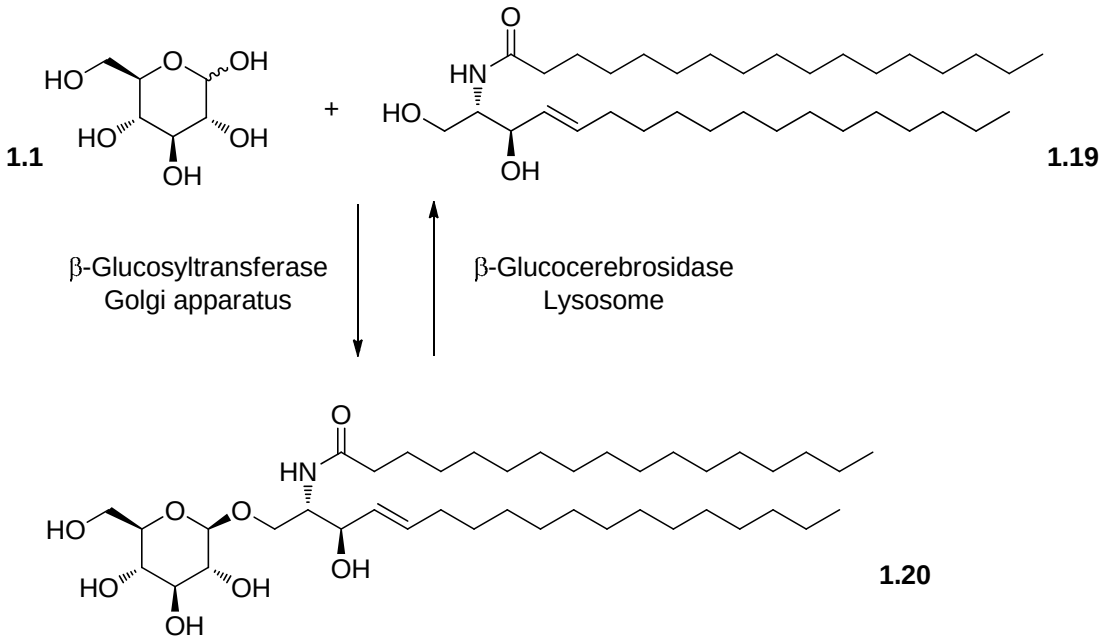
Lysosomal storage diseases (LSDs) are a family of around 50 inherited metabolic disorders that arise from defects in the function of the lysosome.[34] Lysosomes are chiefly responsible for the degradation of macromolecules and for the recycling of the resulting breakdown products. A

deficiency of a particular carbohydrate-processing enzyme will lead to accumulation of the partially degraded substrate (for example glycosphingolipids or glycoproteins) in the lysosomes, causing the cells to swell and symptoms of the disease to follow.[35, 36] Symptoms depend on the type and severity of the disease, but can include enlarged liver and spleen, developmental and neurological problems, bone abnormalities, and cardiac problems. Sufferers often have a very low life expectancy. Two therapeutic strategies that rely on the interaction between iminosugars and carbohydrate-processing enzymes can be used to treat such diseases.

1.5.1.1 Substrate Reduction Therapy

Substrate Reduction Therapy is an approach to diseases arising from a defective enzyme. If a glycosidase exhibits some residual activity, and so can break down intermediates at a slower rate, inhibition of the corresponding glycosyltransferase that makes these intermediates can reduce the workload of the defective enzyme to a manageable level. This ameliorates the symptoms that arise from high concentrations of the intermediate in question. The LSD Gaucher disease can be treated using this approach, as described below.

In all eukaryotic cells, there is a balance between the synthesis and degradation of glycosphingolipids (GSLs), which are a type of glycolipid displayed on the cell surface and involved in cell-cell interactions. One step in this biosynthetic path is the synthesis of glucosylceramide **1.20** from glucose and ceramide (**Scheme 1.4**). Glucosylceramide is made in the Golgi apparatus by β-glucosylceramidetransferase, and broken down in the lysosome by β-glucocerebrosidase (GCase). It is an intermediate in the synthesis and breakdown of all GSLs.



Scheme 1.4 Biosynthesis and catabolism of glucosylceramide.

Patients with Gaucher disease are deficient in GCase, due to a mutation, which leads to accumulation of glucosylceramide in the lysosome.[37, 38] Gaucher disease is the most common LSD, affecting around 1 in 60,000 live births, with the incidence rising to around 1 in 800 among the Ashkenazi Jewish population.

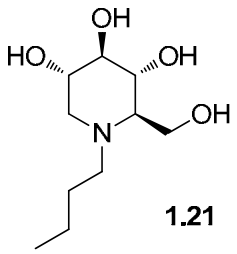


Figure 1.5 *N*-Butyl-DNJ, miglustat, Zavesca®.

N-Butyl-DNJ **1.21**, known generically as miglustat and marketed as Zavesca®, is licensed to treat Type I Gaucher disease.[38-40] *N*B-DNJ inhibits the glucosylceramidetransferase, reducing biosynthesis of glucosylceramide, thereby reducing the accumulation of this product in the lysosomes. There have been indications that *N*B-DNJ can cross the blood-brain barrier, in

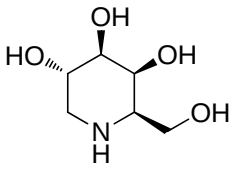
contrast to the first line of treatment, Enzyme Replacement Therapy. It could therefore help Types II and III Gaucher patients, who suffer from neurological symptoms.

*N*B-DNJ is also licensed for the treatment of Niemann-Pick Disease Type C.[41-43] This LSD arises from a deficiency of the enzyme acid sphingomyelinase, which leads to accumulation of cholesterol in the lysosomes, insufficient cholesterol in cell membranes, and associated symptoms. As a glucosylceramide synthase inhibitor, *N*B-DNJ can be used as substrate reduction therapy in this case as well.

1.5.1.2 Chaperone-Mediated Therapy

In some genetic diseases, a mutation causes an enzyme to misfold during its biosynthesis in the endoplasmic reticulum (ER). The protein may be still have some residual function, but quality control mechanisms in the ER cause the nascent enzyme to be degraded rather than trafficked to the lysosome.[33] In such cases, chaperone-mediated therapy (CMT) can be a viable approach.[44] The rationale behind CMT is that a pharmacological chaperone may bind to the protein so as to act as a template for the correct folding of the enzyme, restoring normal function. As long as there is a subinhibitory concentration of chaperone, once the enzyme-inhibitor complex reaches the lysosome, the high local concentration of substrate allows the enzyme to function as normal. Thus the chaperone leads to an overall increase in lysosomal enzyme activity and prevents the accumulation of unwanted substrate.

Fabry disease is an LSD caused by a deficiency of α-galactosidase A, which result in the inability to catabolise an intermediate trisaccharide ceramide. A number of different mutations all cause this enzyme to malfunction.[45]



1.22

Figure 1.6 DGJ, migalastat, Amigal®.

DGJ (1-deoxygalactonojirimycin) **1.22** resembles galactose and is a potent and specific inhibitor of α-galactosidases. Known as migalastat, this compound is currently in Phase III clinical trials to treat Fabry disease.[46-48]

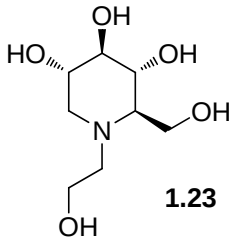
In a second example, DNJ is in clinical trials for the treatment of Pompe disease.[49, 50] This LSD is a glycogen storage disease resulting from a lack of acid α-glucosidase. DNJ is an effective chaperone, significantly increasing enzymes levels in experiments on fibroblasts.

1.5.2 Diabetes

Diabetes mellitus is a disease characterised by a lack of control over blood glucose levels. The two major types are Type 1 diabetes, in which the pancreas cannot produce insulin, the hormone required to remove glucose from the bloodstream into the cells; and Type 2 diabetes, in which cells gradually become insensitive to insulin; this insulin resistance means that the pancreas has to secrete increasing levels of the hormone to have the necessary effect. Eventually the pancreas may become unable to secrete insulin. The more recently discovered Type 3 diabetes is caused by lack of insulin in the brain, and may be linked in Alzheimer's disease.[51, 52]

Diabetes can cause acute problems such as ketoacidosis and hypoglycaemia; in the longer term, persistently high blood sugar leads to microangiopathy (damage to the small blood vessels), which leads to cardiovascular disease, neuropathy, and blindness. The estimated number of Type 2 diabetes worldwide is estimated at 311 million,[53] with the number expected to rise even

further due to factors such as rising obesity, lack of exercise, and an ageing world population.[54] An estimated 3.4 million people die each year from the complications of diabetes.



1.23

Figure 1.7 *N*-Hydroxyethyl-DNJ, miglitol, Glyset®.

Intestinal glucosidases, such as sucrase, maltase, isomaltase, and lactase, are targets for inhibition in the treatment of Type 2 diabetes,[15] since inhibition would reduce the rate of glucose absorption from the gut. *N*-Hydroxyethyl-DNJ **1.23**, known as miglitol and marketed under the name Glyset®,[55, 56] has been licensed to treat Type 2 diabetes since 1996. This compound acts *via* inhibition of intestinal α-1,4-glucosidases. Glucosidase inhibitors have advantages over insulin therapy and other treatments for diabetes: miglitol is free of serious side effects, and any instances of hypoglycaemia can be readily reversed by oral administration of glucose.

1.5.3 Cancer

Cancer is a family of diseases characterised by uncontrolled cell division and invasion of other tissues. Worldwide there were 7.8 million deaths from cancer in 2008, with the number projected to reach 11 million per year by 2030.[57] Cancer is a very complex and variable disease, and there are several different strategies for the treatment of cancer *via* inhibition of carbohydrate-processing enzymes.

The glycosidases and mannosidases involved in the biosynthesis of *N*-linked glycans (**Figure 1.3**) are well-studied targets for the potential treatment of cancer. Compared to normal cells, cancer cells display large numbers of abnormal *N*-linked glycans. Therefore inhibition of the enzymes required for the biosynthesis of these glycans would target cancer cells over normal cells. The α-glucosidases DNJ **1.12** and castanospermine **1.16** have been investigated as anti-cancer agents, and the use of α-mannosidase inhibitors in the treatment of cancer is described in more detail in **Chapter 2**.

Another enzyme involved in the progression of cancer is α-*N*-acetylgalactosaminidase (α-GalNAcase), which acts as an immunosuppressant. Under normal circumstances, cancer cells are destroyed by macrophages, a process mediated by the messenger Macrophage Activating Factor (MAF). Melanoma cells have been shown to secrete α-GalNAcases that destroy the precursor to the active MAF, allowing the cancer cells to survive and the disease to progress.[58, 59] An inhibitor of α-*N*-acetylgalactosaminidase could therefore restore normal function of MAF and destruction of cancer cells by the immune system.

A third approach to cancer treatment targets β-*N*-acetylglucosaminidase (β-GlcNAcase). Secretion of β-GlcNAcase by cancer cells allows degradation of the extracellular matrix,[60] a key step in allowing cells to break away from the primary tumour, enter the bloodstream, and metastasise.[61] β-*N*-Acetylglucosaminidase inhibitors are discussed in **Chapter 4**.

1.6 Carbon-branched iminosugars

In many cases, the presence of a substituent on the iminosugar carbon skeleton will decrease or remove completely the biological activity.[62] However, there are some exceptions, which are

interesting as they can give information on the type of binding and activity between the iminosugar and the enzyme. In some cases these are valuable drug targets.

1.6.1 Intestinal glucosidase inhibition

Work by Compain, Gardin, Asano *et al.* showed that the addition of a 1-*C*-alkyl substituent to DNJ altered the level of inhibition of intestinal maltase, sucrase and isomaltase.[63, 64] Inhibition of these digestive glucosidases is relevant to the treatment of Type 2 diabetes (**Section 1.5.2**).

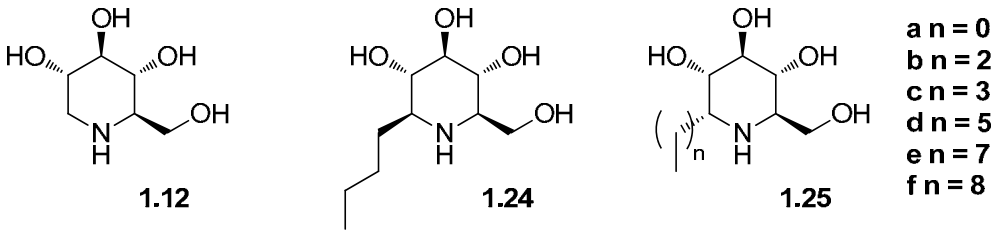


Figure 1.8 DNJ, β-1-*C*-butyl DNJ, and some α-1-*C*-alkyl DNJ derivatives.

While the presence of a β-butyl group decreased inhibition, for α-substituents increasing the chain length correlated roughly with stronger isomaltase inhibition. α-1-*C*-Nonyl-DNJ **1.25f** was 100 times more potent than DNJ **1.12**, and 25 times more potent than even the octyl derivative **1.25e**. With an IC_{50} value of 3.5 nM, the nonyl derivative is the most potent isomaltase derivative reported to date.

A similar pattern was observed with the incorporation of a hydroxymethyl group at the same position (giving α- and β-homonojirimycins, HNJs).[65]

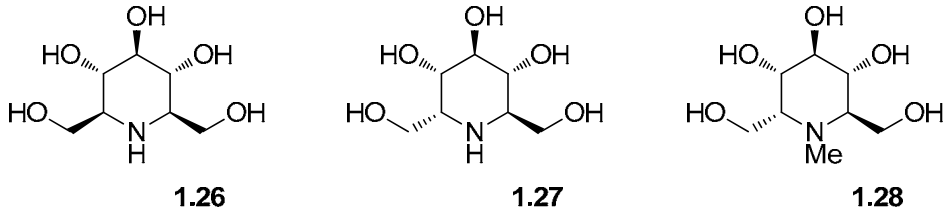
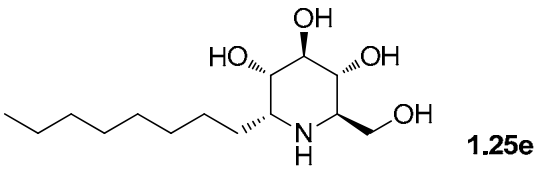


Figure 1.9 β-HNJ, α-HNJ, and *N*-methyl HNJ.

β-HNJ **1.26** was a poorer inhibitor of intestinal maltase and sucrase than DNJ; α-HNJ **1.27** was a slightly better inhibitor; and *N*-methyl α-HNJ **1.28** was a markedly stronger inhibitor of maltase and sucrase (twofold and tenfold, respectively).

1.6.2 β-Glucocerebrosidase inhibition

As well as being an inhibitor of intestinal glucosidases, the octyl derivative of DNJ **1.25e** was found to be a good candidate for chaperone therapy for Gaucher disease.[66, 67]



1.25e

Figure 1.10 Octyl DNJ.

The compound is around 500 times more potent than DNJ as an inhibitor of GCase, the misfolded enzyme in Gaucher patients. It is also selective for this β-glucosidase over lysosomal α-glucosidase, which is important for the avoidance of side effects. Gaucher fibroblasts treated with this compound showed up to a twofold enhancement in enzyme activity. *C*-Alkylation is a good alternative to *N*-alkylation in this case when looking for effective chaperones, as short *N*-alkyl compounds were found to be biologically inactive, and long *N*-alkyl chains made the compounds toxic.[68]

Isofagomine (1,2,5-trideoxy-2-hydroxymethyl-1,5-imino-D-xylitol, IFG) **1.29** is an artificial iminosugar. It was specifically designed to inhibit β-glucosidases.[69, 70] Similarly to the alkylated DNJ **1.25e**, it showed promise as a pharmacological chaperone for the treatment of Gaucher disease *via* inhibition of GCase.

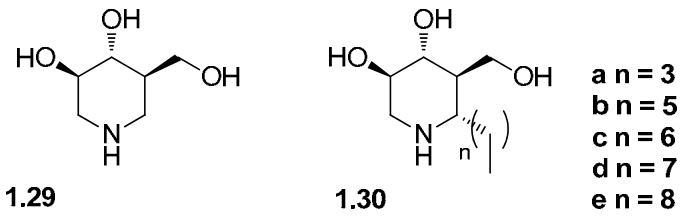


Figure 1.11 Isofagomine (afegostat, Plicera®) and alkylated derivatives.

Although isofagomine has not advanced beyond Phase II clinical trials,[71] there are other, more potent, *C*-alkylated derivatives of IFG that have not yet been trialled. Fan and coworkers synthesised derivatives **1.30a-e** that incorporated an alkyl chain between 4 and 9 carbon atoms long. The IC_{50} values for the alkylated compounds against GCase were in fact much better than for isofagomine itself; the 6-nonyl compound **130e** was 100 times more potent (0.6 nM compared to 56 nM).[72] Very recent studies in fibroblasts showed that 6-nonyl IFG **130e** approximately doubles the level of functional enzyme within the lysosome;[73] therefore this molecule may be a better drug candidate than the original parent compound.

1.6.3 DGJ derivatives as α-galactosidase inhibitors

Fan *et al.* researched DGJ derivatives applicable to the treatment of Fabry disease.[74, 75] As mentioned previously (**Section 1.5.1.2**), clinical trials are evaluating the use of DGJ **1.22** as a pharmacological chaperone for the treatment of this condition.

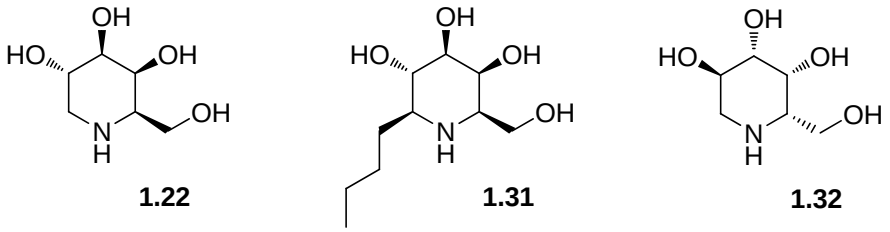


Figure 1.12 DGJ, its β-1-*C*-butyl derivative, and its enantiomer.

β-1-*C*-Butyl-DGJ **1.31** is a naturally-occurring alkylated derivative of DGJ. It was isolated from the roots of *Adenophora* species, and acts as a potent and selective α-galactosidase inhibitor.[76] In cell studies, the butyl compound **1.31** was one of four candidates found to increase levels of functional enzyme increased α-galactosidase A by acting as a chaperone. It increased the enzyme level 2.3-fold at 100 μM (6.3-fold at 1000 μM), which is a pharmacologically useful improvement. In another interesting recent development, DGJ and L-DGJ **1.32** have been shown to act as synergistic pharmacological chaperones for α-galactosidase A; DGJ is a competitive inhibitor while L-DGJ is a non-competitive inhibitor.[77]

1.6.4 L-Swainsonine naringinase inhibitors

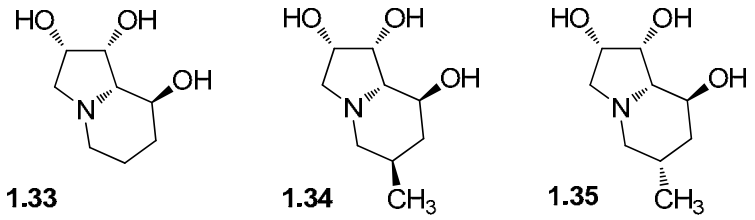
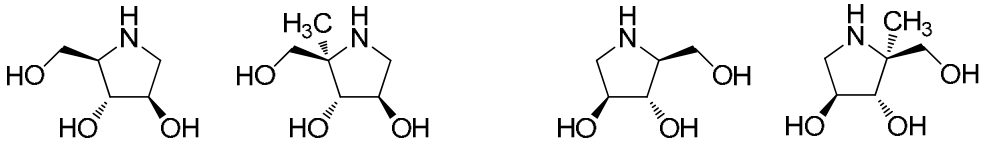


Figure 1.13 L-Swainsonine and its 6-*C*-methyl derivatives.

L-Swainsonine **1.33**, the mirror image of the natural iminosugar **1.15**, is an inhibitor of naringinase (a type of rhamnosidase).[78] The 6-*C*-methyl analogues of L-swainsonine **1.34** and **1.35** were synthesised by Håkansson *et al.*, and the (6*R*) compound **1.34** was found to be a better naringinase inhibitor than the parent compound by an order of magnitude.[79] Swainsonine and its substituted derivatives will be discussed further in **Chapter 2**.

1.6.5 Pyrrolidine glucosidase inhibitors



1.10 **1.36** **1.37** **1.38**

Figure 1.14 DAB, 4-*C*-methyl DAB, LAB, and 4-*C*-methyl LAB.

1,4-Dideoxy-1,4-imino-D-arabinitol (DAB) **1.10** is a naturally-occurring iminosugar. DAB and its synthetic enantiomer LAB **1.37** are both inhibitors of α-glucosidases. 4-*C*-Methyl DAB **1.36** and 4-*C*-methyl LAB **1.38** were also found to be potent and specific glucosidase inhibitors, particularly towards α-sucrase.[80] Modifications of pyrrolidine iminosugars will be discussed in **Chapter 4**.

1.7 Aims of project

This chapter has outlined the significance of carbohydrates and carbohydrate-processing enzymes in biology. Many iminosugars are potent glycosidase inhibitors, making them potential leads for the treatment of numerous diseases; some of their applications have been described. The following chapters explore the synthesis of new sugar and iminosugar compounds. **Chapter 2** describes the synthesis of new analogues of swainsonine, an α-mannosidase inhibitor that has generated much interest as a potential cancer treatment. **Chapter 3** details the synthesis of a branched sugar and an attempted route to di-branched iminosugars. **Chapter 4** describes the synthesis of pyrrolidine iminosugar amides; some of these compounds are potent β-*N*-acetylhexosaminidase inhibitors and a number of diastereomers were made in order to elucidate the properties needed for successful enzyme inhibition.

Chapter 2

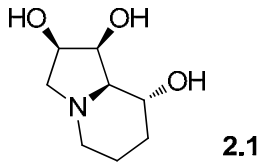
The Synthesis and Evaluation of 6-C-Methyl Swainsonines

2.1 Overview

This chapter describes the biological activity and therapeutic potential of the iminosugar swainsonine. Previous attempts to make biologically active swainsonine analogues are reviewed, and a synthetic route to novel 6-methyl compounds is described. Conformational information (**Section 2.6**) and enzyme assay results (**Section 2.7**) are also included.

2.2 Introduction to Swainsonine

D-(-)-Swainsonine **2.1** is a naturally-occurring bicyclic iminosugar.[81] It can also be classed as a trihydroxy indolizidine alkaloid.



2.1

Figure 2.1 D-(-)-Swainsonine

2.2.1 Natural occurrence

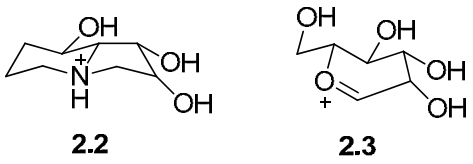
Swainsonine was first isolated in 1973 from the fungus *Rhizoctonia leguminicola*[82] and then in 1979 from the *Swainsona canescens* plant in Australia.[24] This substance has since been found in many other plants, and more rarely in certain other fungi. The biological activity of swainsonine

has long been noted: consumption of 'locoweeds', plants now known to contain the iminosugar, are a major cause of livestock poisoning, particularly *Oxytropis* and *Astragalus* species in North America[83-86] and *Swainsona* species in Australia.[85, 87]

2.2.2 Inhibition of α-mannosidases

Many iminosugars are excellent inhibitors of carbohydrate-processing enzymes. Swainsonine, for example, is a well-known inhibitor of α-mannosidases such as lysosomal α-mannosidase (LM)[84, 88] and Golgi α-mannosidase II (GMII)[88, 89] (which are both retaining mannosyl hydrolases). The IC_{50} value of swainsonine with jack bean α-mannosidase (JBM), which is a useful model for GMII, lies between 0.1 and 0.4 μM.[90-92] The neurological symptoms of locoism in livestock are caused by the accumulation of mannose-rich oligosaccharides in the lysosomes,[93] which leads to vacuolisation of cells, including neurons.

Swainsonine inhibits α-mannosidases competitively, and its affinity for these enzymes is explained by its action as a transition state mimic. Swainsonine is protonated at physiological pH, and in this form **2.2** it mimics the mannosyl oxocarbenium ion **2.3** (**Figure 2.2**).



2.2 **2.3**

Figure 2.2 Protonated SW as a mimic of the mannosyl oxocarbenium ion.

An X-ray crystal structure of swainsonine bound to the active site of drosophila Golgi α-mannosidase II (dGMII) has been obtained by van den Elsen *et al.* (**Figure 2.3**).[94] It is problematic to isolate and purify mammalian GMII in sufficient quantities for such studies; however, dGMII has high sequence identity with the human type, and comparable kinetic

properties and substrate specificity,[95, 96] and so provides the best available model for human GMII.

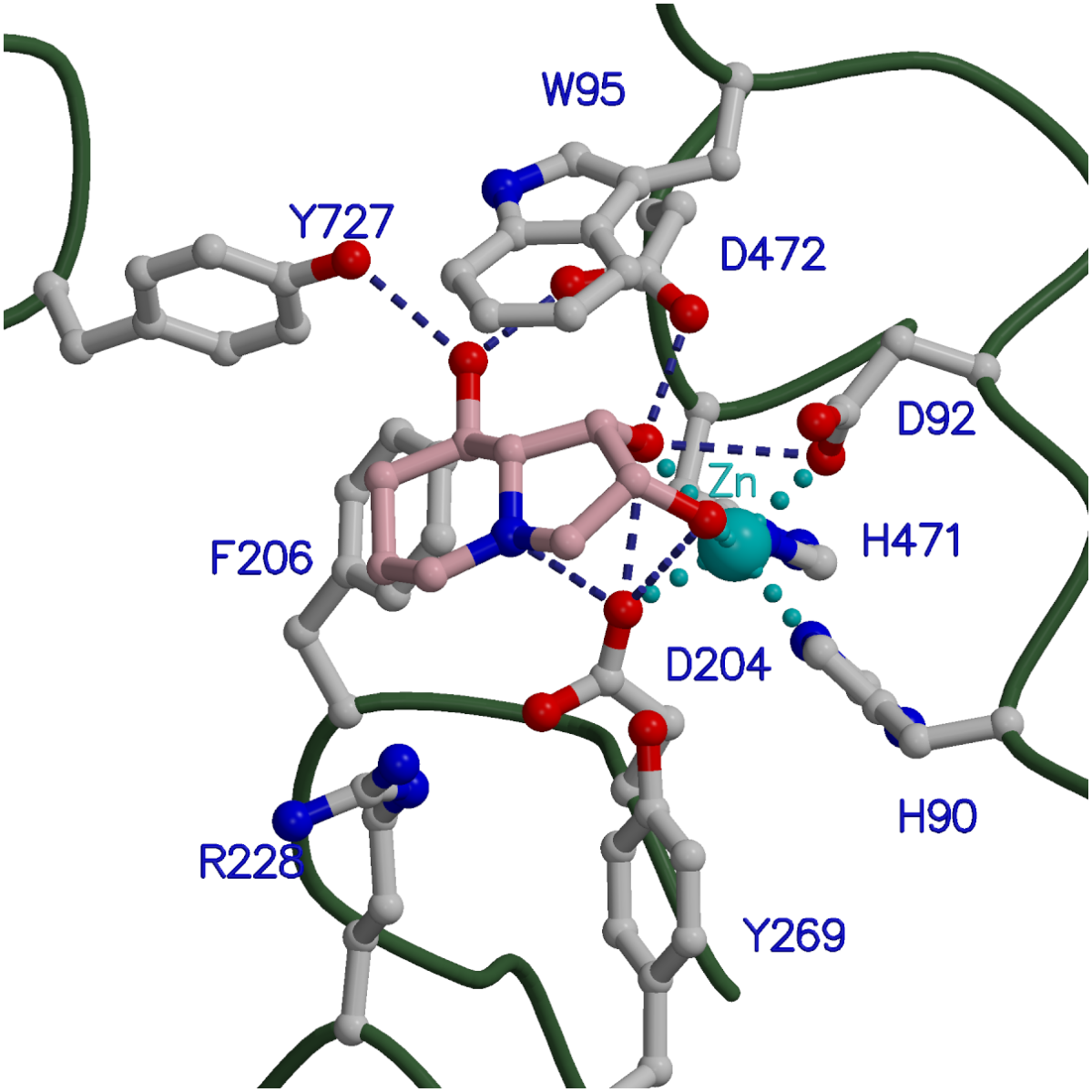


Figure 2.3 X-ray crystal structure of swainsonine (protonated) bound to the active site of drosophila GMII. Adapted by permission from Macmillan Publishers Ltd: *EMBO J*, J.M.H. van den Elsen et al., *20*, 3008, 2001. Abbreviations: D = aspartic acid, F = phenylalanine, H = histidine, R = arginine, W = tryptophan, Y = tyrosine.

Significant substrate-enzyme interactions include: hydrophobic interactions between the swainsonine ring and the aromatic residues (W 95, F 206, Y 727) of the enzyme cavity;

hydrogen bonds between the swainsonine hydroxyl groups and aspartate carboxylate groups (D 92 and D 472); a hydrogen bond between the zinc-coordinating oxygen of aspartate (D 204) and the protonated nitrogen of swainsonine; and coordination of the swainsonine's 2-hydroxyl group to the T_6-octahedral zinc ion.

2.2.3 Application in cancer treatment

Highly branched complex type glycans are important biochemical markers. In experimental systems, the presence of such glycans on the cell surface is necessary for expression of the metastatic phenotype, and their levels correlate with the degree of aggression in animal and human studies.[97-99] The presence of these glycans on the cells of malignant tumours has been well documented.[100-107] By inhibiting GMII, swainsonine prevents the formation and expression of such glycans. This is thought to be the major reason for swainsonine's anti-cancer activity: it has been shown to reduce *in vitro* proliferation of human colorectal carcinoma (CRC), renal carcinoma (RCC), and melanoma cell lines; it has also been shown to reduce tumour growth and metastasis *in vivo*.[108-114]

The inhibitory activity of swainsonine against GMII, along with its related role as a potential immunomodulator,[115, 116] made the compound a potential lead for the development of anti-cancer treatments. In the 1990s-2000s, it underwent Phase I,[117] Phase IB,[118] and Phase II[119, 120] clinical trials. In the first Phase II trial (involving forty advanced RCC and CRC patients), disease progression had stabilised in eight subjects and a complete response was seen in one patient. The side effects reported were generally mild, and there was no significant toxicity. However, the subsequent Phase II trial (involving seventeen advanced or metastatic RCC patients) did not live up to expectations; all participants discontinued treatment due to disease progression or toxicity.

2.3 Substituted swainsonine derivatives

Because swainsonine is a promising target for cancer treatment, but causes too many side effects to be used as a treatment in its natural form, research into substituted derivatives has been conducted with the aim of increasing either the absolute inhibition of GMII, or the inhibition of GMII relative to LM.[121] Varying the patterns of hydroxylation or changing the stereochemistry generally causes loss of activity, although certain epimers such as 8,8a-di-*epi* swainsonine retain some activity.[122] However, in certain cases the incorporation of a carbon substituent into the swainsonine skeleton has increased the activity of the molecule.

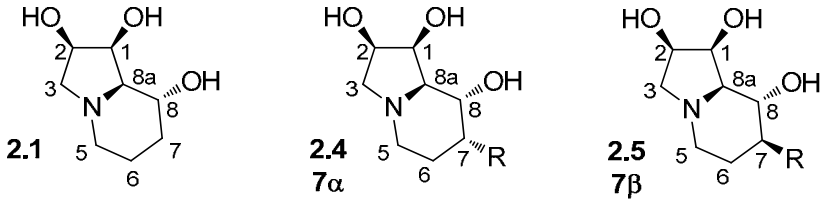


Figure 2.4 D-Swainsonine, numbered according to IUPAC rules for indolizidines; substituents can be α or β.

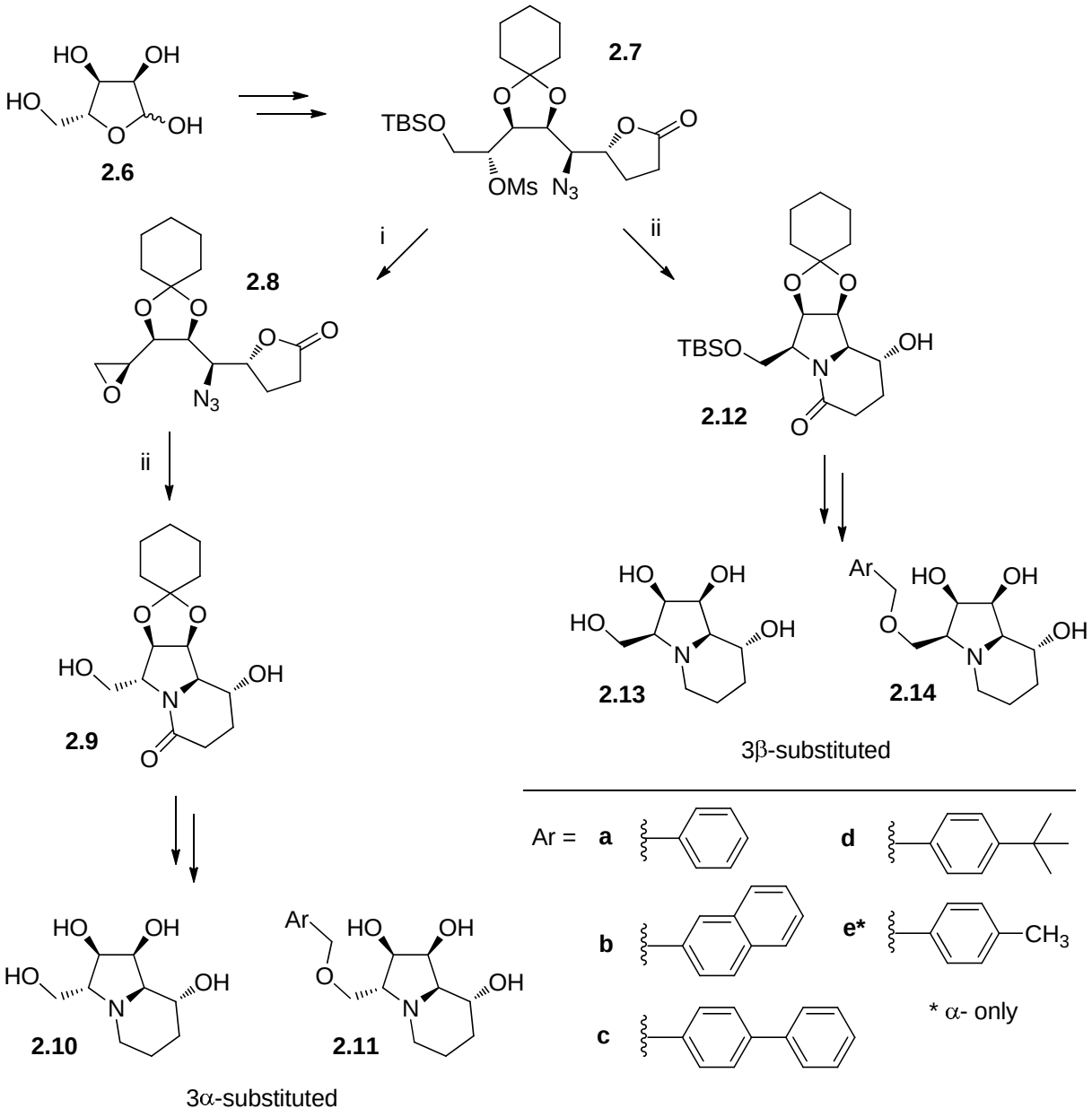
Substituents can be incorporated at different positions on the indolizidine ring (as numbered in **Figure 2.4** above).[123] Substituents can be in the α- or β-orientation (down or up, respectively, as drawn above). The synthesis and evaluation of these compounds will be described in the following section.

2.3.1 D-Swainsonine derivatives

2.3.1.1 3-Hydroxymethyl D-swainsonine derivatives

Substitution at the 3-position was investigated by Pearson and coworkers (**Scheme 2.1**).[91, 124] The hydroxymethyl group originated in the starting material, D-ribose **2.6**. The syntheses involved a Claisen rearrangement and a Sharpless osmylation to give the azidomesylate

intermediate **2.7**. This could be inverted at C-8 *via* the epoxide **2.8** to access the 3α-hydroxymethyl compounds, or reacted on to give 3β-hydroxymethyl derivatives.



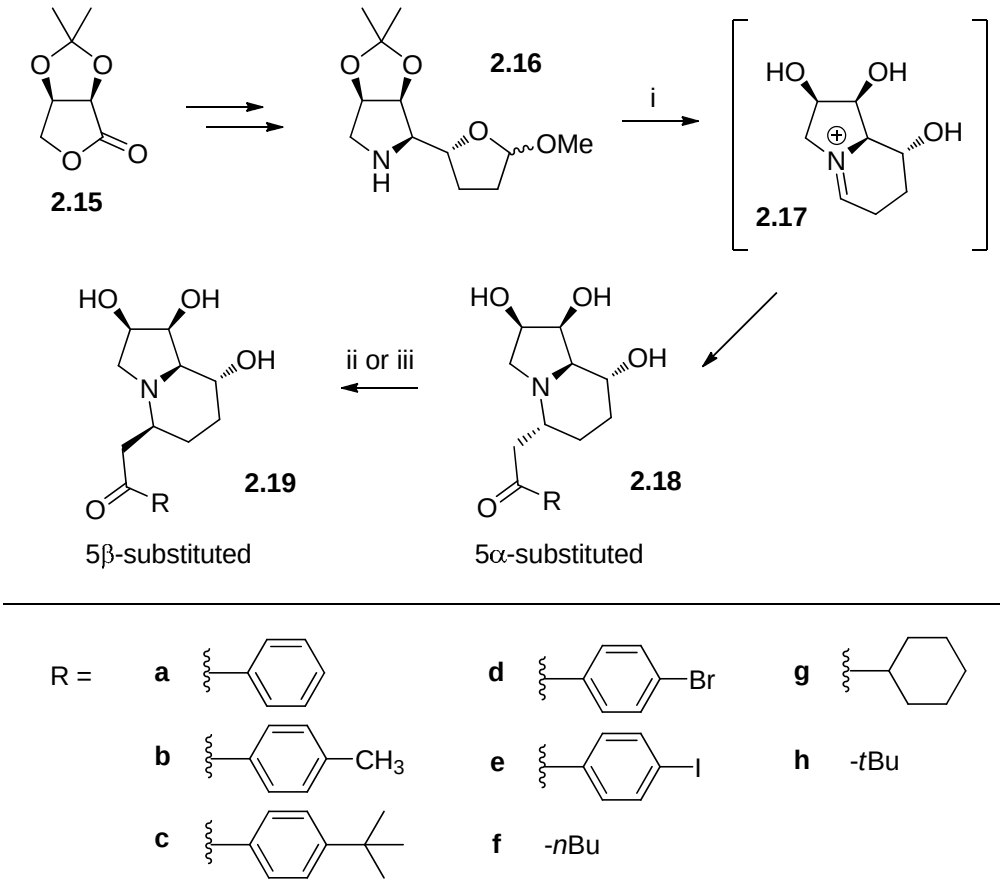
Selected reagents and conditions: (i) CsF, MeCN; (ii) $Pd(OH)_2/C$, H_2, then NaOMe, MeOH.

Scheme 2.1 Preparation of 3-substituted swainsonine analogues by Pearson and co-workers.

The presence of a 3α-hydroxymethyl group decreased the IC_{50} value for jack bean α-mannosidase, as did the presence of any 3β-substituent. However, the arylated 3α-derivatives **2.11a-e** showed comparable or enhanced activity against JBM compared to the parent compound. In the best case (**2.11d**), the IC_{50} of 0.05 μM compared to 0.1-0.4 μM for swainsonine. Subsequent X-ray studies[125] supported Pearson's proposal that the aromatic α-substituents acted as an effective mimic of a disaccharide unit present in the enzyme's natural substrate.

2.3.1.2 5-substituted D-swainsonine

Fujita *et al*. synthesised a number of 5-substituted swainsonine compounds (**Scheme 2.2**).[126]



Selected reagents and conditions: (i) $RCOCH_3$, HCl, various solvents, temperatures and reaction times; (ii) Dowex 1X8 OH^- form, 1:1, MeOH:H_2O; (iii) MeOH.

Scheme 2.2 Preparation of 5-substituted swainsonine analogues by Fujita *et al.*.

The amine acetal **2.16** was combined with a ketone *via* a stereoselective Mannich reaction. The 5α-substituted compounds **2.18** were prepared selectively by axial attack of the enol on the iminium intermediate **2.17**, and could then be epimerised to give the thermodynamically favoured 5β-substituted swainsonines **2.19**. The wide variety of ketones available allowed a number of different swainsonine analogues to be prepared.

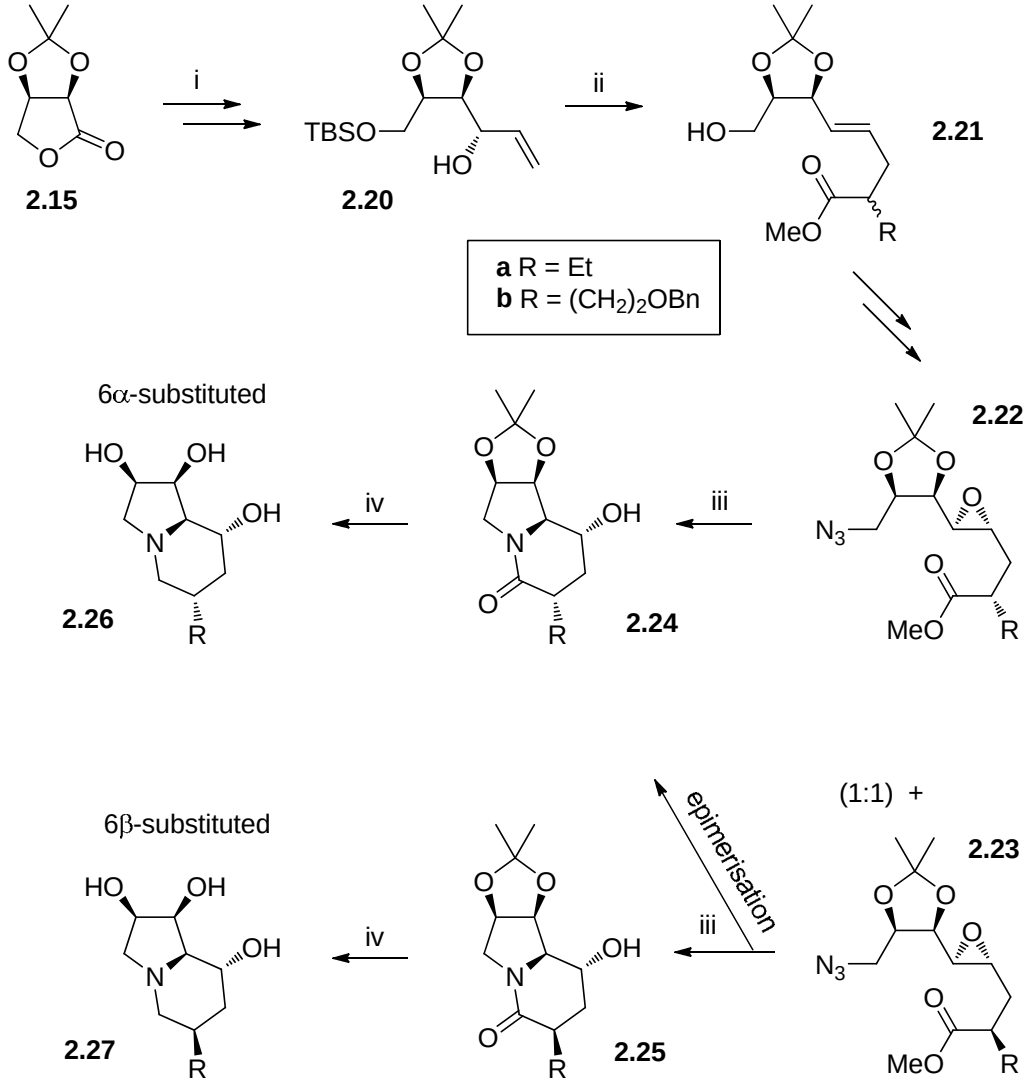
Among the 5α-compounds, the presence of an aromatic group did not markedly decrease the affinity of the compounds for JBM. The compound bearing the bulkiest aromatic group (**2.18c**) was the best inhibitor of JBM (IC_{50} 0.22 μM, compared to 0.25 μM for swainsonine). However, aliphatic substituents decreased the IC_{50} value in every case. All of the 5β-compounds demonstrated either or a lack of activity, or greatly reduced activity compared to their respective 5α-precursors, with inhibition decreasing with increasing steric bulk.

Recent X-ray studies[127] on the 5α-substituted swainsonines bound to dGMII elucidated the enzyme-substrate interactions: novel hydrophobic interactions between the 5α-aryl group and the active site, as well as hydrogen bonding through the carbonyl of the substituent, are crucial factors in the preserving the inhibitory activity of the swainsonine compounds.

2.3.1.3 Ethyl and (2-benzyloxy)ethyl substituents at C-6

Only one paper[128] concerns the synthesis of 6-substituted D-swainsonine analogues (**Scheme 2.3**). The use of two different orthoesters allowed four compounds **2.26-2.27** to be made. The allyl alcohol **2.20** underwent a Johnson orthoester Claisen rearrangement, giving a mixture of diastereomers **2.21** in each case. Following conversion to the separable azide epoxides **2.22** and **2.23**, reductive cyclisation gave lactams **2.24** and **2.25**, and finally the substituted swainsonine derivatives **2.26** and **2.27**. Control of stereochemistry was an issue in this synthesis; in both cases around 20% of the epoxide formed was the unwanted diastereomer, and the 6β-substituted

lactams underwent substantial epimerisation (ethyl **2.23a** gave 41% **2.25a** and 20% of the epimerised **2.24a**; aryl **2.23b** gave only 13% **2.25b** and 66% of the epimerised **2.24a**).



Selected reagents and conditions: (i) 3 steps;[129] (ii) $RCH_2C(OMe)_3$, cat. $EtCO_2H$, toluene, reflux, then TBAF, THF; (iii) $Pd(OH)_2/C$, H_2, MeOH, EtOAc; (iv) $BH_3{\cdot}SMe_2$, then 6N HCl/THF.

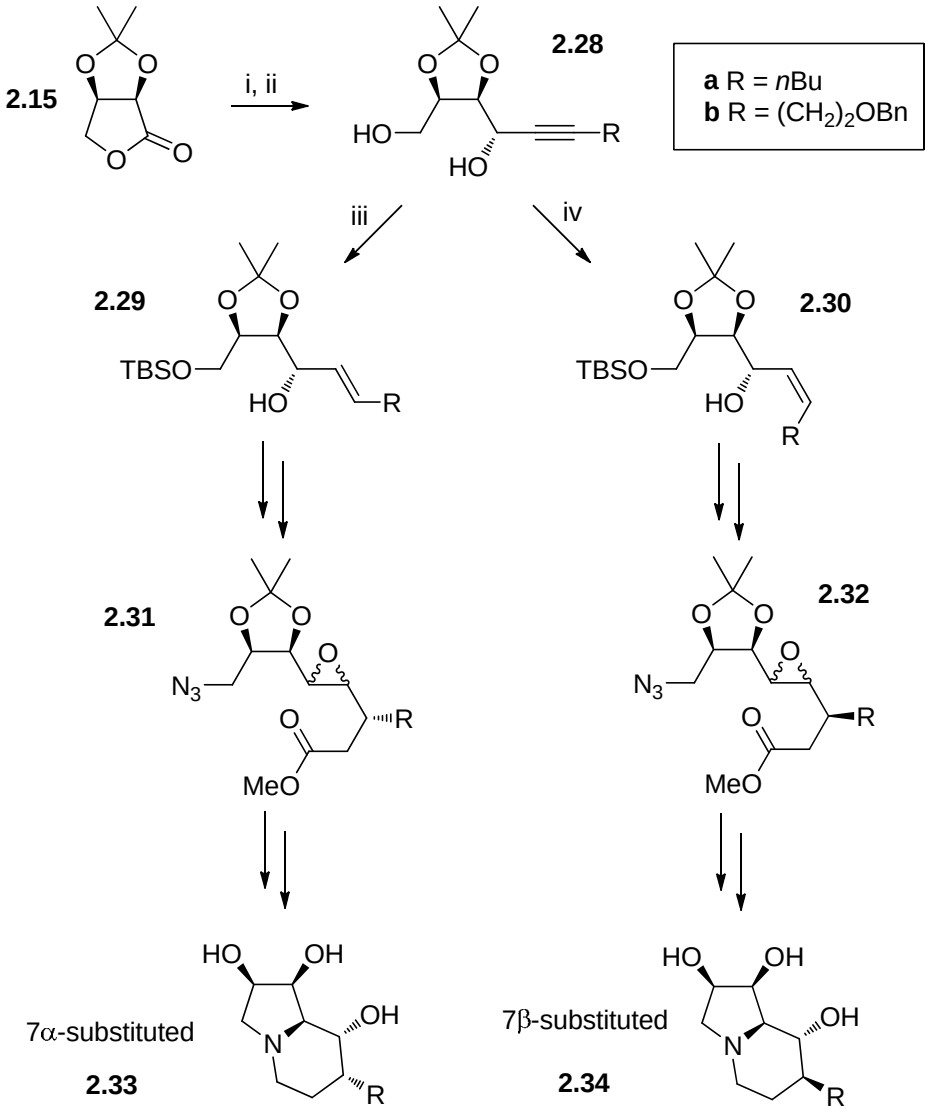
Scheme 2.3 6-Substituted analogues of swainsonine prepared by Pearson and Hembre.

The biological activity of the 6-substituted compounds showed the same pattern as the previously mentioned 3- and 5-substituted examples: the equatorially 6α-substituted compounds were much better inhibitors than the axially 6β-substituted ones. 6α-Ethyl swainsonine **2.26a**

bound more strongly to jack bean α-mannosidase (30 μM) than did the more bulky 6α-(2-benzyloxy) ethyl swainsonine **2.26b** (230 μM).

2.3.1.4 Substituents at C-7

The synthesis of 7-substituted swainsonine derivatives has also been investigated (**Scheme 2.4**).

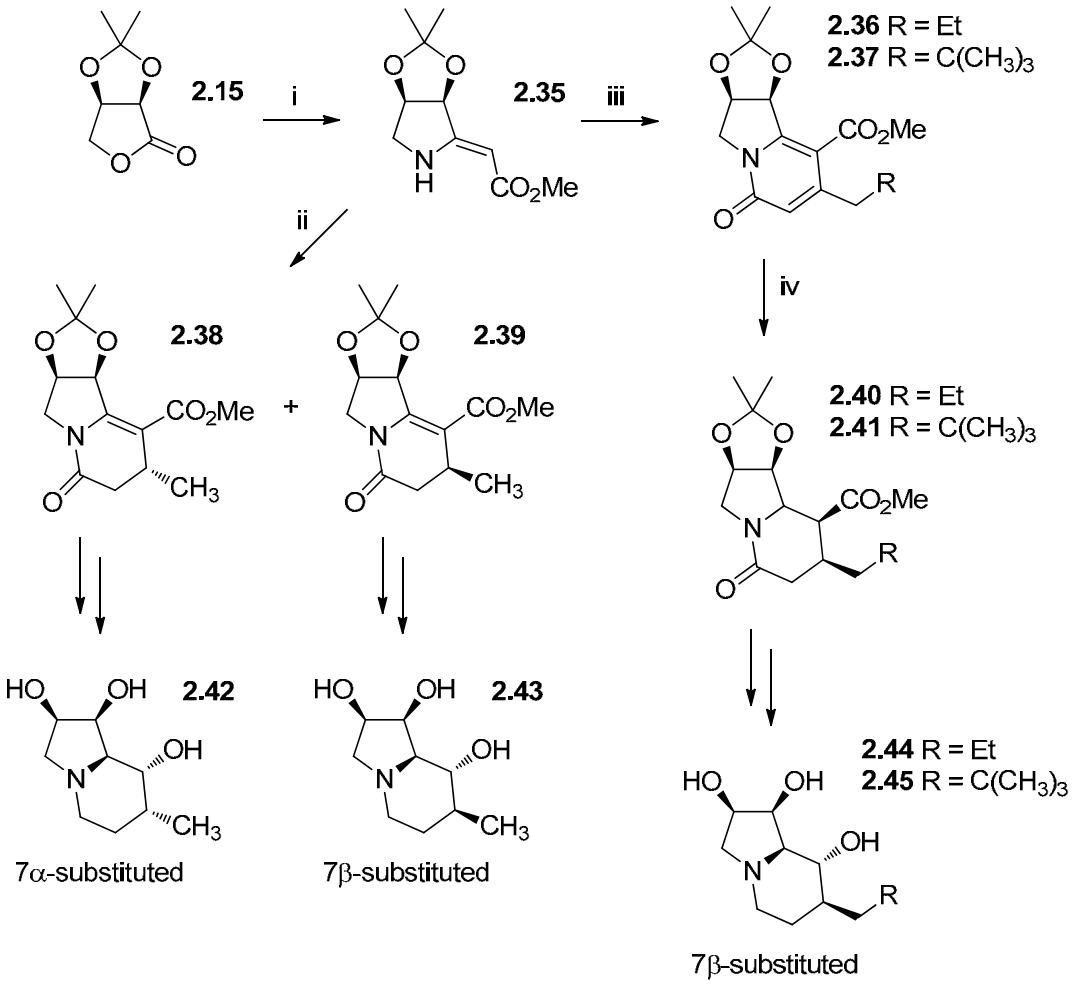


Selected reagents and conditions: (i) DIBAL-H; (ii) RC≡CMgBr; (iii) Red-Al®, then TBSCl, imid.; (iv) $Pd/BaSO_4$, H_2, then TBSCl, imid..

Scheme 2.4 7-Substituted analogues of swainsonine synthesised by Pearson and Hembre.

The use of two different Grignard reagents gave rise to four end products **2.33** and **2.34** (as well as the corresponding 8,8a-di-*epi* swainsonine derivatives, which arose from an unselective epoxidation step). When tested against jack bean α-mannosidase, substitution of *n*-butyl or (2-benzyloxy) ethyl at C-7 was found to cause complete loss of inhibitory activity, except for **2.33b** which was only weakly active. The 8,8a-di-*epi* compounds were inactive.

Shi *et al.* synthesised swainsonine derivatives with methyl, *n*-propyl, and *i*-butyl substituents at the 7-position (**Scheme 2.5**).[130]



Selected reagents and conditions: (i) 6 steps;[131] (ii) H_3C⟨CO$_2$Me, NaH/THF, 0-25°C; (iii) R⟨C⟩CO$_2$Me, NaH; (iv) Pd/C, H_2, 10-15 atm.

Scheme 2.5 7-Substituted swainsonine compounds prepared from a common chiral enamine.

The enaminoester intermediate **2.35** was treated with different biselectrophiles to give bicyclic intermediates **2.36-2.39**. The methyl-substituted intermediates **2.38** and **2.39**[132] were separable and were converted to both isomers of 7-methyl swainsonine. In the case of intermediates **2.36** and **2.37**, a highly selective hydrogenation gave the diastereomerically pure intermediates **2.40** and **2.41**, leading to the 7β-substituted swainsonine compounds **2.44** and **2.45**. No biological results for the four swainsonine compounds have been published.

2.3.2 L-Swainsonine derivatives

L-(+)-Swainsonine **2.46**, the mirror image of the natural compound, shows a different activity profile to D-(-)-swainsonine. Although inactive against α-D-mannosidases, L-swainsonine inhibits a different class of enzymes, α-L-rhamnosidases. Previous work in the Fleet group concerned the synthesis and evaluation of 6-methyl L-swainsonine (**Figure 2.5**).[79]

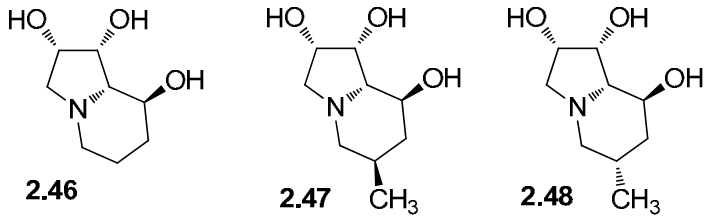


Figure 2.5 L-Swainsonine and its 6-methyl analogues.

The 6-methyl L-swainsonines were tested against a number of glycosidases.[133] (6*R*)-*C*-Methyl-L-swainsonine **2.47** was found to be a better inhibitor of naringinase (an α-L-rhamnosidase) than L-swainsonine itself. With an IC_{50} of 34 nM, this compound was found to be the best naringinase inhibitor yet reported.

2.4 New synthetic targets

In light of the biological activity of (6*R*)-methyl L-swainsonine **2.47**, and the promising results from certain α-substituted D-swainsonine derivatives, it was decided to synthesise the both epimers of 6-*C*-methyl D-swainsonine (**Figure 2.6**), and evaluate their activity against various glycosidases.

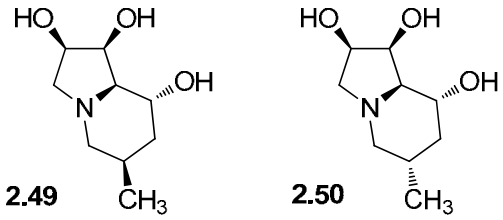
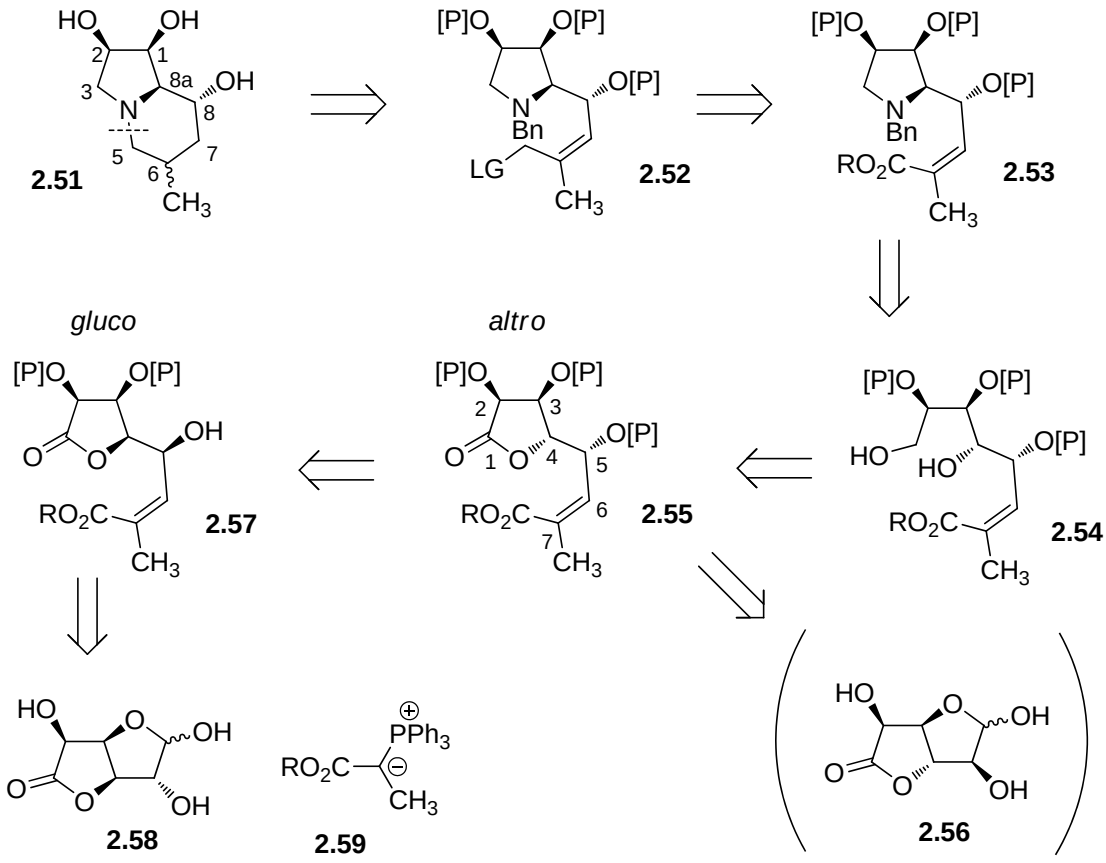


Figure 2.6 D-Swainsonine target compounds.

Introduction of a 6-*C*-methyl branch was expected to modify the inhibitory profile of swainsonine; compounds that show better selectivity for inhibition of Golgi α-mannosidase II over lysosomal α-mannosidase than the parent compound would be better prospects for the treatment of cancer.

2.4.1 Retrosynthesis

A synthesis from carbohydrate starting materials was desirable, as this would remove the need to create multiple stereocentres, which caused problems in the synthesis of related compounds.[128] The following retrosynthesis of 6-methyl swainsonine **2.51** was envisaged (**Scheme 2.6**).



Scheme 2.6 A retrosynthesis of 6-methyl swainsonine from glucuronolactone.

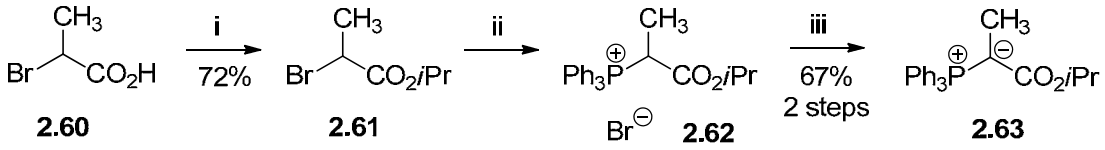
Disconnection between C-4 and nitrogen in the target compounds gives the pyrrolidine **2.52**, bearing a terminal leaving group. This intermediate could be made from the α,β-unsaturated ester **2.53**. The cyclic amine functionality could be made from an open chain diol **2.54**, which comes from the reduction of lactone **2.55**. The direct carbohydrate precursor to this intermediate, altrurono-lactone **2.56**, is unavailable, but inversion of stereochemistry at C-4 and C-5 gives the common *gluco* configuration. Glucuronolactone **2.58** is cheap and readily available, and its reaction with a branched ylid **2.59** would give the desired α,β-unsaturated ester **2.57**.

Wittig reactions have been employed in other syntheses of swainsonine from *gluco* starting materials. However, Ali's synthesis gave a yield of <1% from methyl α-D-glucoside,[134-136] and Suami's synthesis required the formation of a protected acetamido glucoside[137] followed by a

further 18 steps.[138-140] The synthetic route to 6-methyl swainsonine described above involved fewer steps and was expected to give better yields.

2.5 Synthesis of 6-C-methyl D-swainsonine

2.5.1 Ylid formation and Wittig reaction

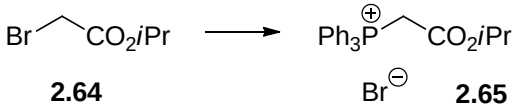


Reagents and conditions: (i) IPA, cat. *p*TSA·H_2O, reflux; (ii) PPh_3, toluene, reflux; (iii) 1 M NaOH (aq).

Scheme 2.7 Formation of novel branched Wittig reagent.

Esterification of 2-bromopropionic acid **2.60** according to a standard method,[141] followed by triphenylphosphine displacement of bromide, gave branched Wittig reagent **2.62** (**Scheme 2.7**). This substance was quite difficult to handle and to dry, being a sticky glass. However, treatment with aqueous sodium hydroxide gave the novel branched ylid **2.63** (67% from the ester **2.61**). The *iso*-propyl ester was chosen because the intermediates it produced were expected to be more stable to nucleophilic substitution, for example, than the methyl ester.

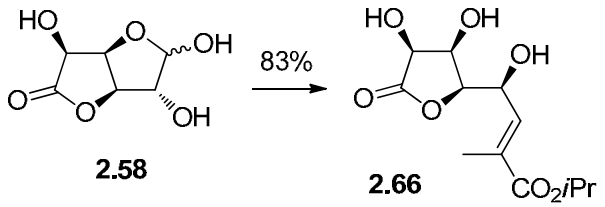
It was also decided to make the unbranched Wittig reagent **2.65**, in order to synthesise the analogous unbranched intermediates (**Scheme 2.8**).



Reagents and conditions: PPh_3, toluene, RT.

Scheme 2.8 Synthesis of the unbranched Wittig reagent.

Synthesis of the unbranched Wittig reagent was cleaner and simpler compared to the branched reagent. It therefore made sense to optimise reaction conditions by experimenting on the unbranched intermediates, before reacting the more valuable branched intermediates. From here onwards, only the reaction schemes for the branched intermediates are shown, provided the unbranched intermediates underwent the same reaction conditions and gave similar yields.



2.58 **2.66**

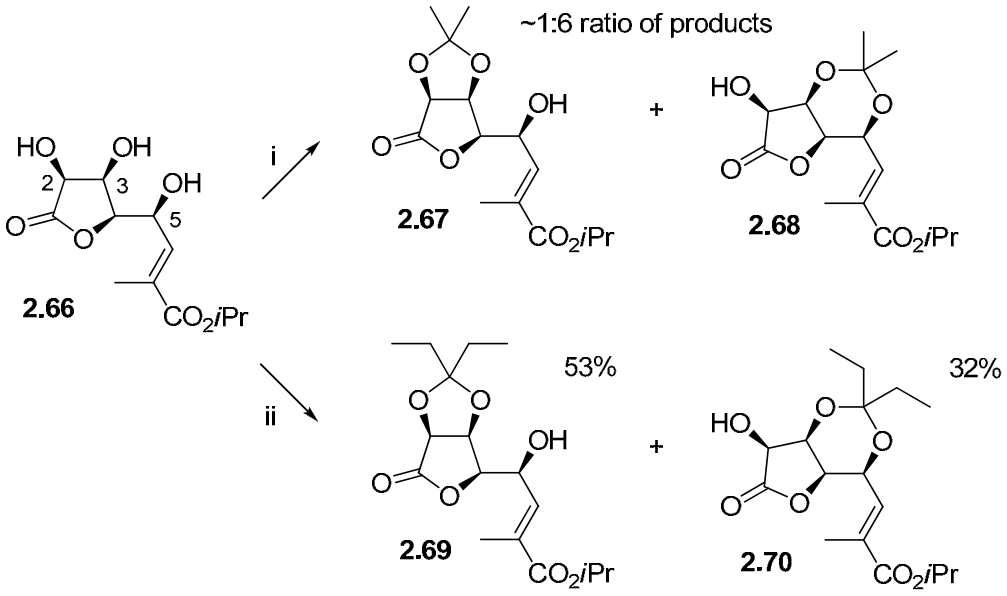
Reagents and conditions: $Ph_3P{=}C(CH_3)CO_2iPr$, dioxane, 50°C.

Scheme 2.9 Wittig reaction on glucuronolactone.

The Wittig reaction itself (**Scheme 2.9**) proceeded in 83% yield. Only the (*E*)-alkene **2.66**, the product expected from a stabilised ylid, was observed.

2.5.2 Protection, mesylation and double inversion

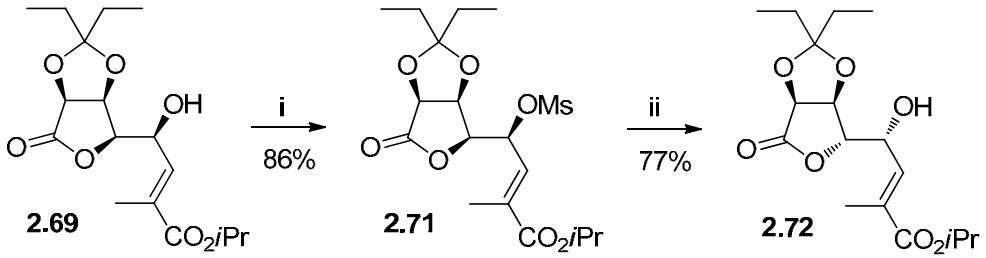
The Wittig product **2.66** required selective protection at the 2- and 3-positions, leaving the 5-hydroxyl free. It has been documented that in such systems, acetonide protection favours formation of the 6-membered ketal over the 5-membered ketal.[142-146] Treatment of triol **2.66** with acetone (**Scheme 2.10**, **step i**), followed by NMR analysis of the crude reaction mixture, indicated a product ratio of about 6:1 in favour of the unwanted product. However, treatment of the starting material with pentan-3-one (**Scheme 2.10**, **step ii**) gave the desired product **2.69** in 53% yield, along with 32% of the 3,5-protected compound **2.70**.



Reagents and conditions: (i) acetone, $CuSO_4$, cat. conc. H_2SO_4; (ii) pentan-3-one, $CuSO_4$, conc. H_2SO_4.

Scheme 2.10 Pentanone shows the opposite regioselectivity to acetone.

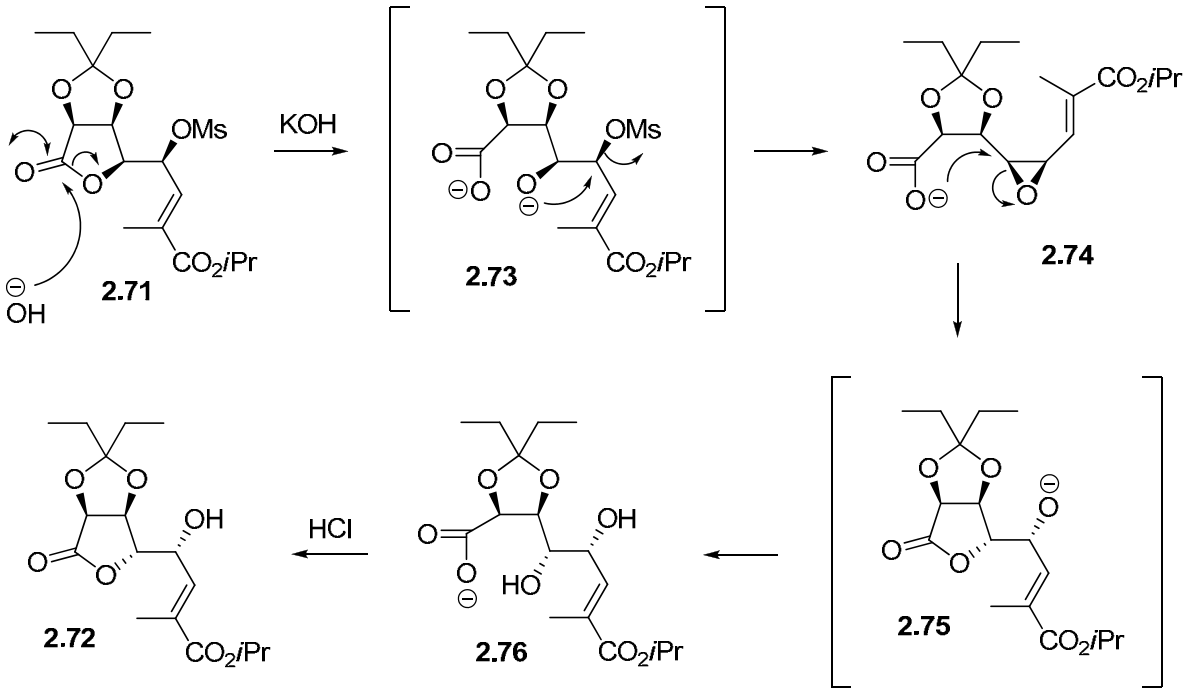
The regioisomers could be separated and the unwanted compound equilibrated. Thus most of the material was converted to the 2,3-protected lactone **2.69**, and was set up for the double inversion of stereochemistry at C-4 and C-5. This was achieved *via* mesylation followed by treatment of mesylate **2.71** with alkali (**Scheme 2.11**).



Reagents and conditions: (i) MsCl, Et_3N, DCM, 0°C; (ii) 2 M KOH (aq), dioxane, then HCl (aq).

Scheme 2.11 Inversion of stereochemistry at C-4 and C-5.

The double inversion proceeds *via* the following mechanism (**Scheme 2.12**):



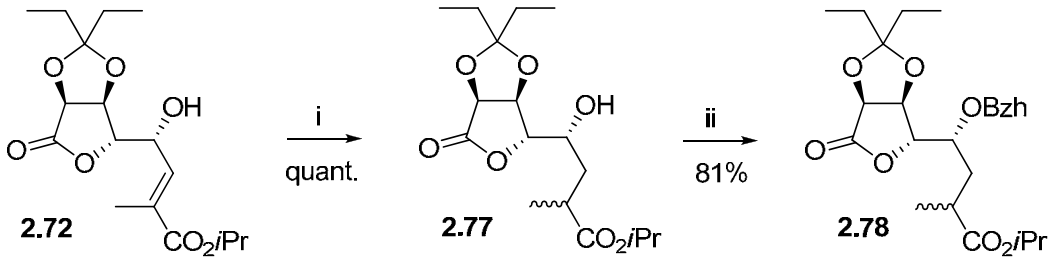
Scheme 2.12 Mechanism for base-catalysed double inversion.

Treatment with potassium hydroxide opened lactone **2.71**, whereupon the C-4 alkoxide immediately attacked C-5 in an S_N2 fashion, ejecting the mesylate and leading to inversion of configuration at C-5. The epoxide **2.74** formed very rapidly (< 5 min), and upon a second S_N2 attack by the carboxylate group, C-4 was inverted and the resulting lactone **2.75** reopened by the hydroxide ion. The progress of the reaction could not be monitored by t.l.c., but mass spectroscopy enabled detection of the intermediates **2.74** and **2.76**. Subsequent acidification of the diol carboxylate **2.76** effected closure to the 1,4-lactone **2.72**.

In the best case, the double inversion proceeded cleanly in 77% yield. However, the reaction proved unreliable, sometimes giving a complex mixture of compounds containing very little of the desired product. The pentylidene protecting group was robust and not removed under these reaction conditions, but it is possible that hydrolysis of the *iso*-propyl ester led to unwanted side reactions.

2.5.3 Hydrogenation and protection of lactone

In order to discount the possibility of the alkene functionality participating in unwanted side-reactions (see **Section 2.5.3.1**), the lactone **2.72** was hydrogenated (**Scheme 2.13**). This occurred in quantitative yield to give a 1:1 inseparable mixture of diastereomers **2.77**. Because both 6-methyl epimers of the final product were needed for biological testing, there was no problem with performing an unselective hydrogenation initially. It was hoped that the diastereomers would be separable at a later stage in the synthesis; if they remained inseparable, then asymmetric hydrogenation methods (*e.g.* using other metals) would be investigated.



Reagents and conditions: (v) Pd/C, H_2, dioxane; (vi) Ph_2CN_2, toluene, reflux.

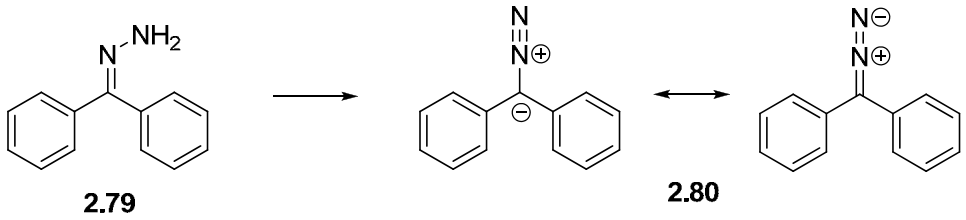
Scheme 2.13 Hydrogenation and protection of intermediates.

Following hydrogenation, the remaining free hydroxyl at C-5 was protected as a benzhydryl (diphenylmethyl) ether **2.78**.

2.5.3.1 Benzhydryl protecting group

The most common protecting groups for carbohydrates are silyl or benzyl ethers,[2] but there are problems associated with the use of these groups. They are generally introduced under basic conditions, which can lead to epimerisation and/or elimination in the molecule. Benzylation can be performed under non-basic conditions *via* the trichloroacetimidate,[147] but this is a more involved process which often gives lower yields.[148] Additionally, silyl ethers are prone to

migration under basic conditions, which are employed later on in this synthesis. Benzhydryl was chosen as a protecting group in this synthesis because it is readily introduced under neutral conditions, and is stable to base.[149] The benzhydryl reagent, diphenyldiazomethane **2.80**, was prepared from benzophenone hydrazone **2.79** (**Scheme 2.14**).[150]



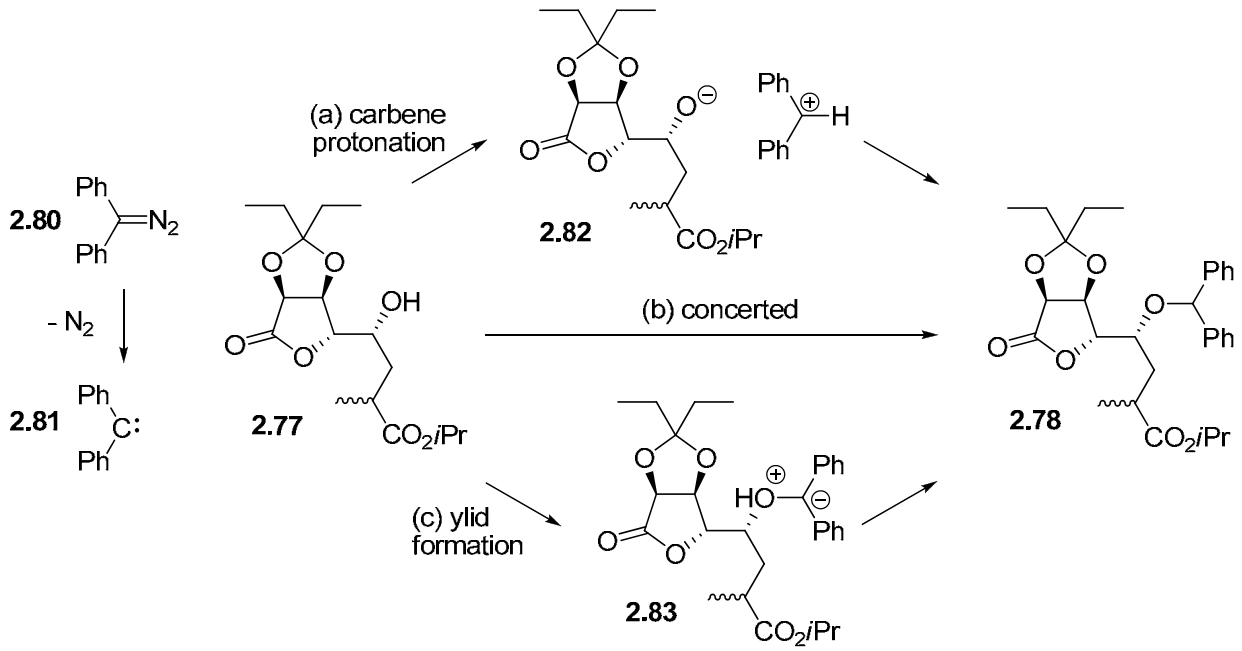
2.79 **2.80**

Reagents and conditions: yellow mercuric oxide, saturated ethanolic KOH, anhydrous $MgSO_4$, MTBE.

Scheme 2.14 Preparation of the benzhydryl reagent.

The mechanism of the benzhydrylation has been studied.[151-155] Loss of N_2 through thermal decomposition of the reagent gives the active diphenylcarbene **2.81** (**Scheme 2.15**). The previous hydrogenation step was performed to discount the possibility that the carbene would react with the alkene.

There are three possible mechanisms for the subsequent formation of benzhydryl ether **2.78**: (a) protonation of the carbene;[151, 153] (b) concerted insertion of the carbene into the O-H bond;[152] and (c) ylid formation.[154, 155] There is evidence for all of these mechanisms from the different kinetic and spectroscopic studies, so the reaction may proceed by more than one route. Protected compound **2.78** was obtained in 81% yield.

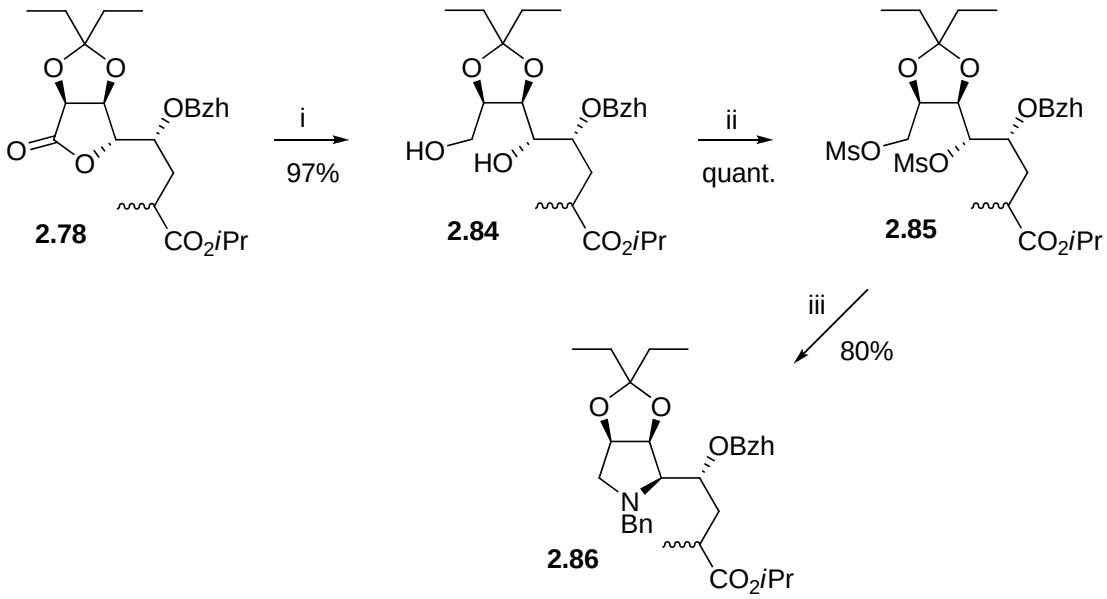


Reagents and conditions: Ph_2CN_2, toluene, reflux.

Scheme 2.15 Proposed mechanisms for the protection of the hydroxyl group.

2.5.4 Nitrogen heterocycle formation

With the fully protected compound **2.78** in hand, the lactone was converted to the cyclic benzyl amine **2.86** in three steps (**Scheme 2.16**). The lactone was fully reduced to the diol **2.84** in 97% yield, and the diol mesylated at both free positions. Treatment with benzylamine led to double displacement of the mesylate groups to give the cyclic benzyl amine **2.86** in 80% yield. This resulted in inversion of configuration at C-4, giving rise to the correct stereochemistry for the final swainsonine products.



Reagents and conditions: (vii) $NaBH_4$, MeOH, 0°C → RT; (viii) MsCl, Et_3N, DCM, 0°C; (ix) $BnNH_2$, reflux.

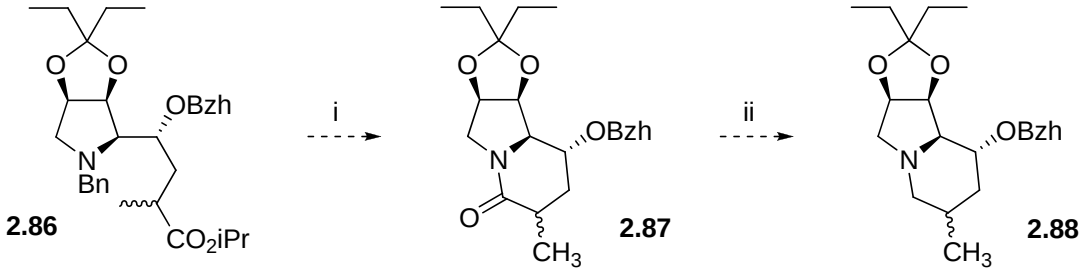
Scheme 2.16 Formation of the monocyclic amine intermediate.

2.5.5 Indolizidine formation

To close the six-membered ring, three different options were considered.

2.5.5.1 Lactam formation

The first method considered was the direct displacement of the *iso*-propyl group to give lactam **2.87** (**Scheme 2.17**), as this method had appeared frequently in the literature.[156-161]



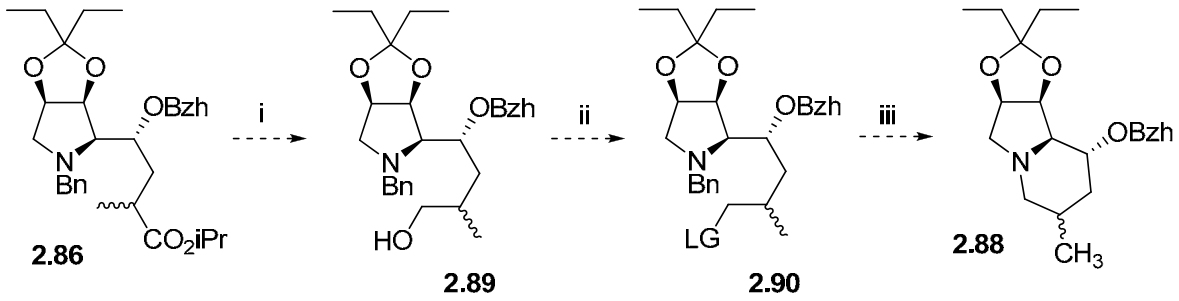
Reagents and conditions: (i) Pd/C, H_2, MeOH; (ii) $BH_3{\cdot}SMe_2$, THF.

Scheme 2.17 Indolizidine formation *via* closure of the lactam followed by reduction.

However, it was expected that the *iso*-propyl ester in this instance would be more difficult to displace than the methyl esters found elsewhere. Another potential problem with this approach, when trying to prepare equal amounts the pure epimers for biological testing, was the possibility of epimerisation at C-6. Therefore an alternative method was attempted.

2.5.5.2 Nucleophilic displacement of terminal leaving group

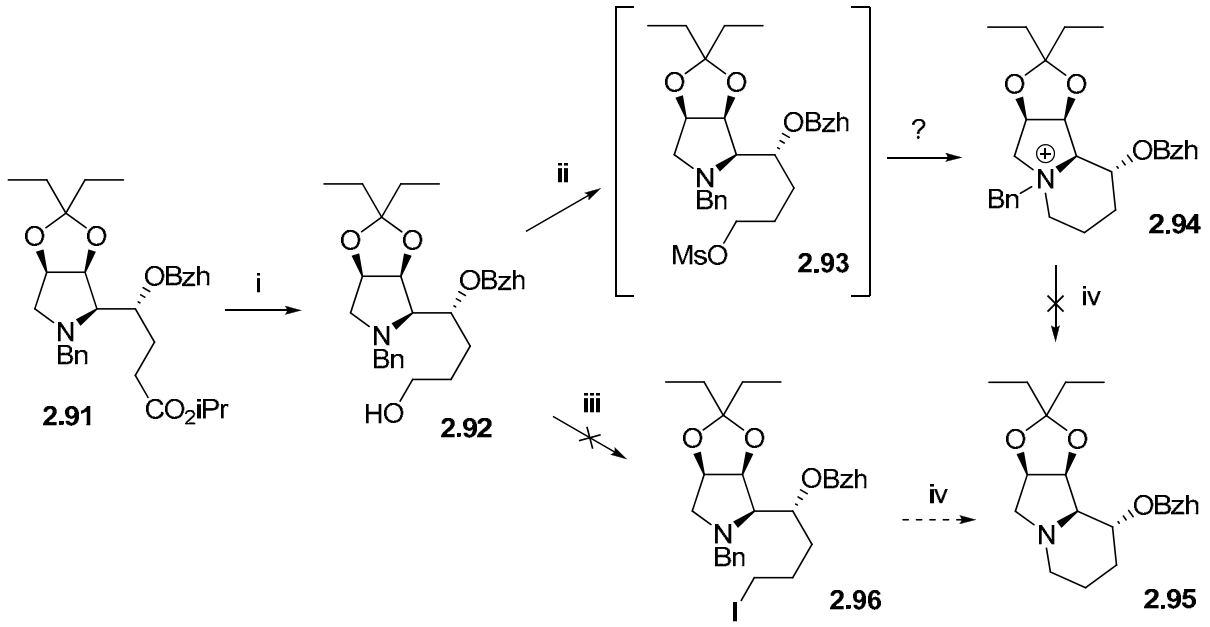
An alternative method would be reduction of the ester to the alcohol **2.89**, followed by activation of the alcohol to a leaving group (**Scheme 2.18**).



Selected reagents and conditions: (i) $LiAlH_4$, THF, -20°C; (ii) activation of the alcohol; (iii) Pd/C, H_2, MeOH.

Scheme 2.18 Indolizidine formation *via* by nucleophilic displacement of the activated alcohol.

Hydrogenation of the benzyl group would then allow nucleophilic displacement of the leaving group to effect the second ring closure. This strategy was attempted using the unbranched cyclic amine intermediate **2.91** (**Scheme 2.19**).



Reagents and conditions: (i) $LiAlH_4$, THF, -20°C; (ii) MsCl, Et_3N, DCM, 0°C; (iii) PPh_3, I_2, imid., toluene, 85°C; (iv) Pd/C, H_2, MeOH.

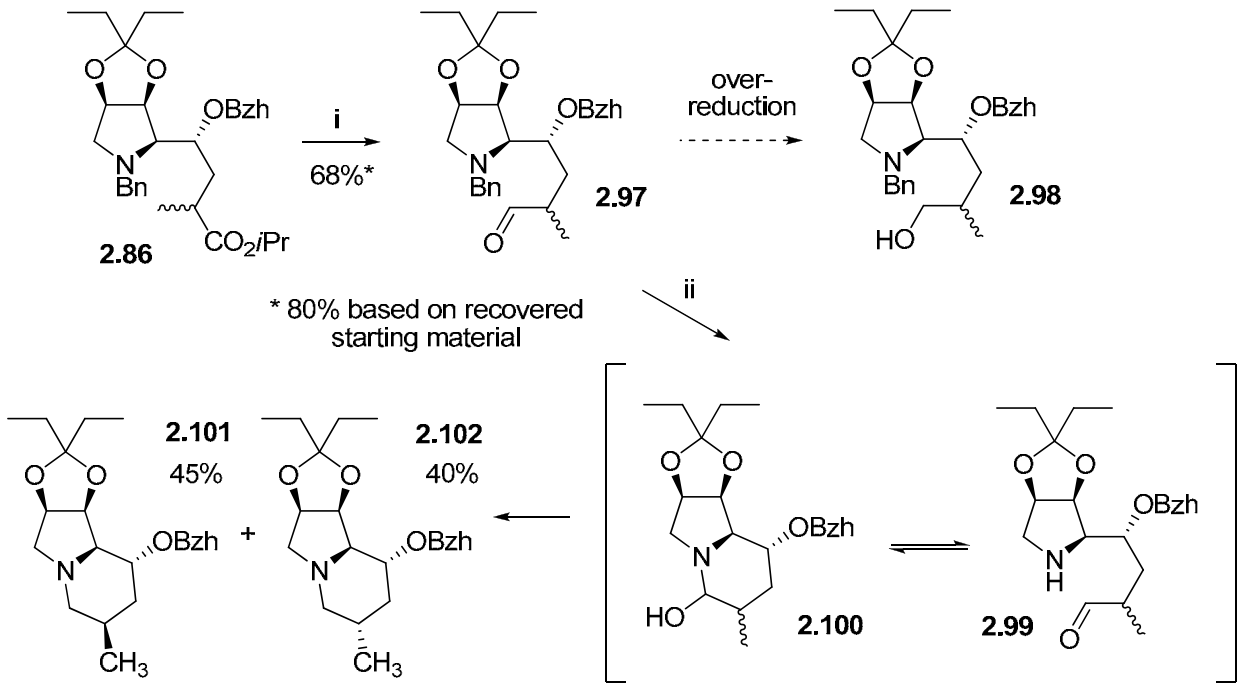
Scheme 2.19 Attempts to activate and displace the alcohol group.

The ester was fully reduced to the linear alcohol **2.92**. The first attempt to activate the alcohol was *via* a mesylation. The mesylate **2.93**, while detected in the crude reaction mixture, did not survive the aqueous workup. It was thought that immediate displacement of mesylate may have resulted in the quaternary ammonium species **2.94**, so the mesylation was repeated and the crude product hydrogenated without prior purification. However, this did not result in the formation of the swainsonine derivative **2.95**. The attempted conversion of the alcohol to an iodide *via* an Appel reaction was also unsuccessful, perhaps for the same reason.

Beyond this stage in the synthesis, only the methyl branched compounds were used.

2.5.5.3 Reductive amination

The chosen route for closure of the six-membered ring was reduction of the ester to the aldehyde **2.97**, followed by reductive amination to give the indolizidine ring structure (**Scheme 2.20**).



Reagents and conditions: (i) DIBAL-H, DCM, -78°C; (ii) Pd/C, H_2, MeOH.

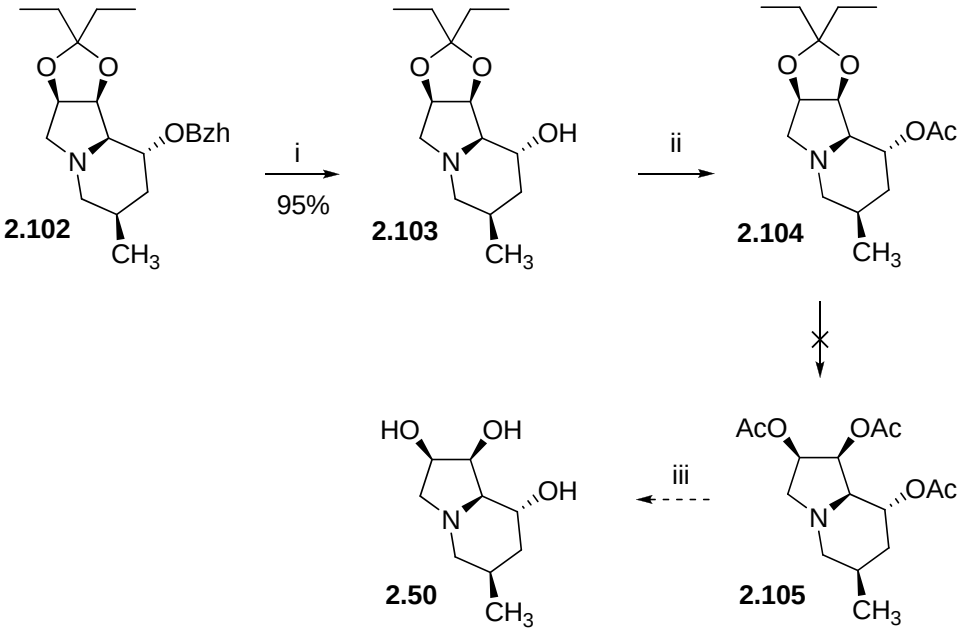
Scheme 2.20 The chosen synthetic route to protected 6-methyl swainsonines.

Only 1.1 equivalents of reducing agent were used due to the potential for over-reduction to the alcohol **2.98**. As a result, some starting material remained unreacted, but this could be recovered and used again. By this method a maximum yield of 68% (80% based on recovered starting material) of the aldehyde was achieved.

Hydrogenation of the benzyl amine **2.97** produced the secondary amine **2.99**, which existed in equilibrium with the hemiaminal **2.100**. The hemiaminal was directly reduced to give the desired protected swainsonines. Following the second ring closure, it was possible to separate the diastereomers by column chromatography. The yields for the reductive amination were 45% for (6*R*)-methyl swainsonine **2.101** and 40% for (6*S*)-methyl swainsonine **2.102**.

2.5.6 Deprotection of separated compounds

The benzhydryl group was removed readily under acidic conditions (33% aqueous TFA at 35°C), but the diethylketal protecting group was very robust (**Scheme 2.21**).



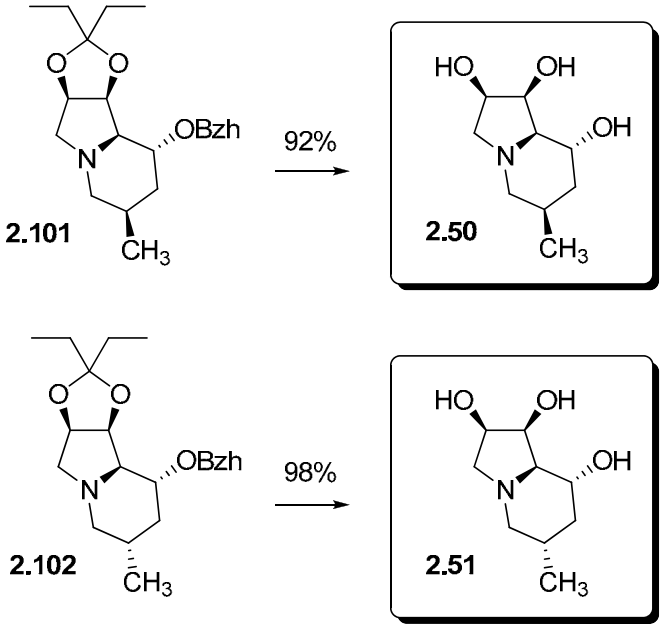
Reagents and conditions: (i) 1:2, TFA:H_2O, 35°C; (ii) $BF_3{\cdot}OEt_2$, Ac_2O, 0°C → RT; (iii) K_2CO_3 (aq), MeOH.

Scheme 2.21 Partial deprotection of the (6*R*)-methyl swainsonine derivative.

The partially deprotected intermediate **2.103** was treated with boron trifluoride in acetic anhydride in an attempt to obtain the triacetyl swainsonine **2.105**, which could then be deacetylated to give the target compound **2.50**. However, treatment with the Lewis acid only led to acetylation of the alcohol without removal of the ketal group.

The products of reductive amination **2.101** and **2.102** were fully deprotected only when treated with 90% aqueous TFA at reflux for 6 hours (**Scheme 2.22**). There are many other examples of molecules for which similarly harsh conditions were required for the removal of this group.[162-165] In terms of sterics, pentanone is more bulky than acetone, and both of the protected hydroxyl

groups are secondary. Under the reaction conditions the nitrogen is immediately protonated, so another positive charge on molecule is not favoured.



Reagents and conditions: 9:1, TFA:H_2O, reflux.

Scheme 2.22 Deprotection of benzhydryl and pentylidene groups.

The overall combined yield of 6-*C*-methyl-D-swainsonine from D-glucuronolactone was 11% over twelve steps.

2.6 NMR Characterisation of products

The target compounds **2.50** and **2.51** were characterised fully and the data compared to that of their enantiomers. In each case, vicinal couplings in the proton NMR spectrum provided information about the conformation of the molecule (**Table 2.1**, overleaf).

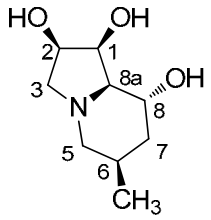
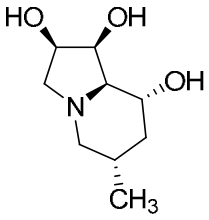
		
H,H coupling	(6*R*)-*C*-Methyl-D-swainsonine **2.50**	(6*S*)-*C*-Methyl-D-swainsonine **2.51**
5,6	1.0	[3.0]
5',6	3.6	10.6
6,Me	7.3	6.3
6,7	3.2	4.1
6,7'	5.2	11.8
7,7'	12.6	12.1
7,8	3.9	4.4
7',8	10.8	11.4
8,8a	9.3	9.5

Table 2.1 Comparison of proton coupling constants (in Hz). [ ]: value estimated by simulation of the 1D spectrum.

*2.6.1 Conformation of (6*R*)-methyl-D-swainsonine*

In the (6*R*) compound **2.50**, the couplings between H-6 and its four neighbours are all quite small (between 1.5 and 5.2 Hz). The large couplings between H-7' and H-8 (10.8 Hz) and between H-8 and H-8a (9.3 Hz) suggest that these protons are approximately axial. The data indicates that the six-membered ring adopts a chair-like conformation (**Figure 2.7**), in which H-6 is equatorial and 6-CH_3 axial.

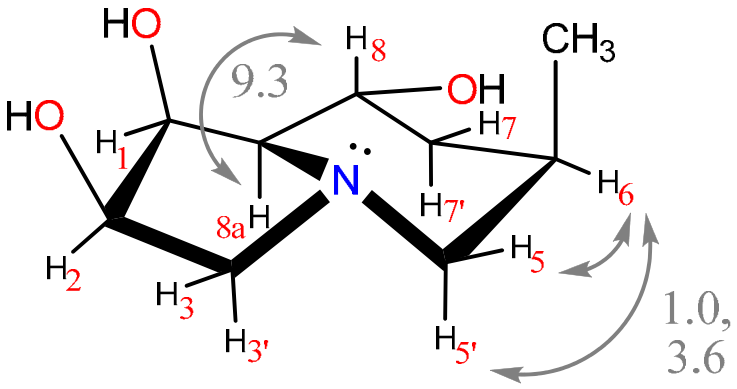


Figure 2.7 Suggested conformation of (6*R*)-*C*-methyl D-swainsonine **2.50**.

The crystal structure of the enantiomer 6-*C*-methyl-L-swainsonine (**Figure 2.8**), which has been published previously,[166] confirms this.

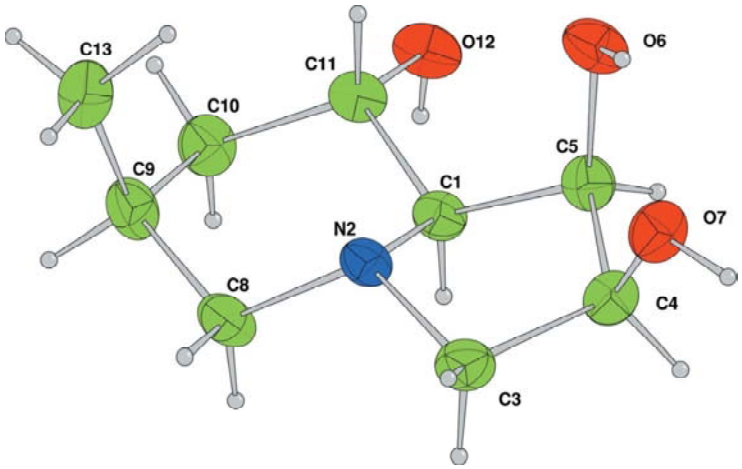


Figure 2.8 X-ray crystal structure obtained from (6*S*)-*C*-methyl L-swainsonine. Reproduced by permission from the International Union of Crystallographers: *Acta Crystallographica Section E*, A. E. Håkansson *et al*., *63*, 210, 2007.

2.6.2 Conformation of (6S)-methyl-D-swainsonine

In the (6*S*) compound **2.51**, a series of strong couplings between neighbouring protons (H-5' – H-6 – H-7' – H-8 – H-8a) indicates that these protons are all approximately axial. This is consistent with the six-membered ring adopting a chair-like conformation (**Figure 2.9**), in which H-6 is axial and 6-CH_3 is equatorial.

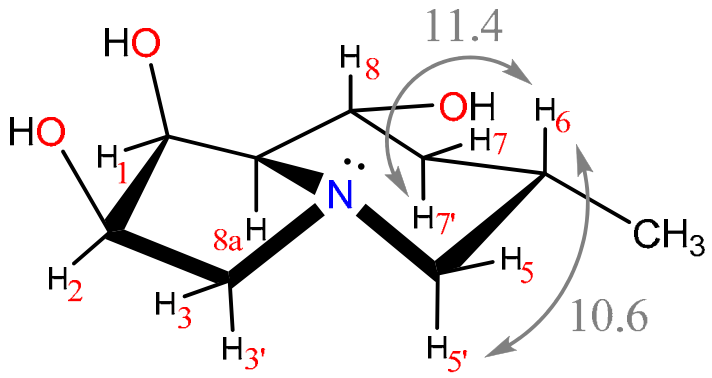


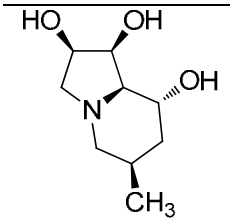
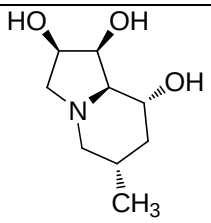
Figure 2.9 Suggested conformation of (6*S*)-*C*-methyl-D-swainsonine **2.51**.

(6*S*)-*C*-Methyl-D-swainsonine **2.51** is predicted to be a better α-mannosidase inhibitor than the (6*R*) compound **2.50**. The X-ray structure of the dGMII active site shows that the presence of an axial methyl group would lead to an unfavourable steric clash with the F 206 residue. On the other hand, the presence of an equatorial methyl group would appear to be more easily accommodated in the active site, without disrupting the enzyme-substrate interactions to the same degree.

2.7 Results of Biological Testing

The methyl swainsonines were tested against a range of glycosidases (**Table 2.2**, overleaf). The enzyme assays were carried out by Prof. Atsushi Kato at the University of Toyama.

Concentration of iminosugars giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)  **2.49**	 **2.50**
α-Glucosidase		
Rice	NI[a] (19.3%)[b]	NI (12.3%)
Yeast	NI (1.2%)	NI (0.8%)
Rat intestinal maltase	NI (23.3%)	NI (9.5%)
β-Glucosidase		
Almond	NI (4.1%)	NI (0%)
Bovine liver	NI (10.2%)	NI (23.6%)
α-Galactosidase		
Coffee beans	NI (3.5%)	NI (3.7%)
Human lysosome	NI (0%)	NI (0%)
β-Galactosidase		
Bovine liver	NI (6.4%)	NI (10.1%)
Rat intestinal lactase	NI (8.1%)	NI (8.6%)
α-Mannosidase		
Jack bean	14	3.8
β-Mannosidase		
Snail	NI (2.2%)	NI (1.5%)
α-L-Rhamnosidase		
P. decumbens	NI (5.7%)	NI (12.0%)
α-L-Fucosidase		
Bovine epididymis	NI (0%)	NI (0%)
Trehalase		
Rat intestinal trehalase	NI (1.3%)	NI (0%)

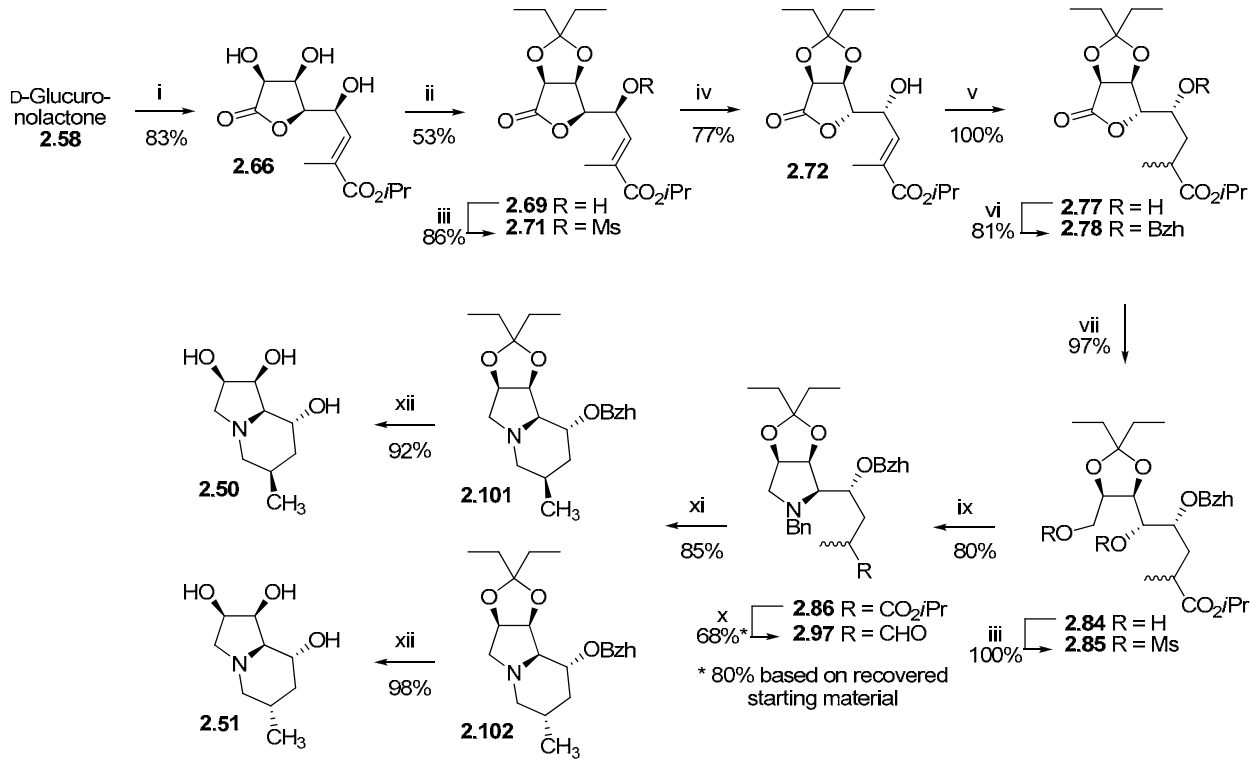
[a] NI: No inhibition (less than 50% at 1000 μM); [b] ( ): inhibition % at 1000 μM.

Table 2.2 Results of the methyl swainsonine enzyme assays.

Both compounds were found to be good selective inhibitors of jack bean α-mannosidase. As expected, (6*R*)-*C*-methyl swainsonine **2.50** is a weaker inhibitor than the (6*S*) compound **2.51**. Both methyl compounds are better JBM inhibitors than any of the 6-substituted swainsonine compounds previously made (*e.g.* 30 μM for 6α-ethyl swainsonine **2.26a**; 275 μM for 6β-benzyl-oxyethyl swainsonine **2.27b**).[128] However, swainsonine itself is still a stronger inhibitor than (6*S*)-*C*-methyl swainsonine.

2.8 Conclusion and Future Work

This chapter has described the synthesis of two novel swainsonine analogues, along with information on their conformations and biological activities. Although the compounds were weaker inhibitors of jack bean α-mannosidase than swainsonine itself, an important factor in their suitability as a cancer treatment is their selectivity for human Golgi α-mannosidase II over human lysosomal α-mannosidase. The relative IC_{50} values of different swainsonine analogues against JBM do not necessarily predict their relative activities against dGMII, or indeed their selectivities for Golgi α-mannosidase over lysosomal α-mannosidase.[127] More detailed enzyme assays, along with *in vivo* studies, could provide further insight into the biological activity of these compounds.



Reagents and conditions: (i) $Ph_3P{=}C(CH_3)CO_2iPr$, dioxane, 50°C; (ii) pentan-3-one, $CuSO_4$, conc. H_2SO_4; (iii) MsCl, Et_3N, DCM, 0°C; (iv) 2M KOH (aq), dioxane; then HCl (aq); (v) Pd/C, H_2, dioxane; (vi) Ph_2CN_2, toluene, reflux; (vii) $NaBH_4$, MeOH, 0°C; (ix) $BnNH_2$, reflux; (x) DIBAL-H, DCM, -78°C; (xi) Pd/C, H_2, MeOH; (xii) 9:1, TFA:H_2O, reflux.

Scheme 2.23 The synthesis of 6-*C*-methyl D-swainsonine from glucuronolactone**.**

2.9 Experimental

2.9.1 General Experimental

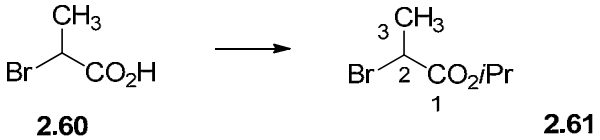
Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations were recorded on a Perkin Elmer 241 polarimeter with a path length of 1 dm. Concentrations are recorded in g/100 mL. Infrared spectra were recorded on a Bruker Tensor 27 Fourier Transform spectrophotometer using thin films on NaCl plates, or KBr discs, with absorption maxima given in wavenumbers (cm^{-1}). The following abbreviations are used to describe infra-red absorption

bands: br, broad; s, strong; w, weak. Only signals representing functional groups are reported; C-H absorptions and the fingerprint region are not listed. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker DPX400 (400 MHz), a Bruker DQX400 (400 MHz), or by Dr B. Odell or Dr T. Claridge on a Bruker AVC500 (500 MHz) spectrometer, at the frequencies specified. Proton spectra were assigned using COSY. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker DQX400 (100.7 MHz) or by Dr B. Odell or Dr T. Claridge on a Bruker AVC500 (127.8 MHz) spectrometer, at the frequencies specified. Spectra were assigned using HMQC; multiplicities were assigned using DEPT sequence. All chemical shifts are quoted in the δ-scale in ppm using residual solvent as the internal standard. Scalar coupling constants (*J*) are measured in Hertz. Low resolution mass spectra were recorded on a Micromass Platform 1 spectrometer using electrospray ionisation. High resolution mass spectra were recorded by Mr R. Proctor using a Walters 2790-Micromass LCT electrospray ionisation using chemical ionisation (CI) (NH_3, Cl) techniques as stated. *m/z* values are reported in Daltons and followed by percentage abundance in parentheses. Analytical thin layer chromatography (t.l.c.) was carried out on Merck Kieselgel $60F_{254}$ pre-coated aluminium-backed plates. Visualisation of t.l.c. plates was achieved using an ultraviolet lamp (λ_{max} = 254 or 365 nm), phosphomolybdic acid/ceric sulfate (2.5%/1% in 1 M H_2SO_4), and/or potassium permanganate (0.5% in 1 M NaOH). Flash column chromatography was carried out using Sorbsil C60 40/60 silica. All reactions using anhydrous conditions were performed using dry solvents and flame dried apparatus under an atmosphere of argon. Solvents were dried under pressure over activated alumina columns prior to use. Where available other anhydrous solvents were purchased from Fluka and stored over molecular sieves. All other solvents were used as supplied (analytical or HPLC grade) without prior purification. Water was deionised. Reagents

were used as supplied without prior purification. All aqueous solutions were saturated unless otherwise stated.

2.9.2 Experimental

(±)-*iso*-Propyl 2-bromopropionate

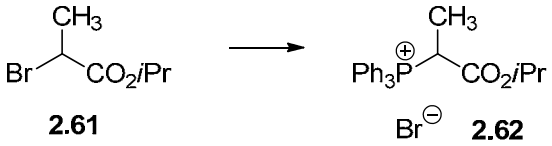


2.60 **2.61**

(±)-2-Bromopropionic acid **2.60** (16.8 mL, 0.186 mol, 1 eq) was dissolved in *iso*-propyl alcohol (300 mL) under an atmosphere of argon, and *para*-toluenesulfonic acid monohydrate (0.392 g, cat.) was added. The stirred solution was heated to reflux (≈85°C) and left for 24 h, whereupon t.l.c. (9:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.38) and formation of a major product (R_f 0.76). The reaction mixture was concentrated *in vacuo* and the residue redissolved in dichloromethane. The organic layer was washed with $NaHCO_3$ (aq) (3 × 25 mL) followed by water (25 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield (±)-*iso*-propyl 2-bromopropionate **2.61** (26.1 g, 72%) as a pale yellow liquid.

IR ν_{max} (thin film): 1736 (s, C=O), 680 (s, C-Br); δ_H (400 MHz, $CDCl_3$): 5.02 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.30 (q, $J_{2,3}$ 6.9, 1H; H-2), 1.78 (d, $J_{2,3}$ 7.0, 3H; 3 × H-3), 1.26 (d, J_{iPr} 6.3, 6H; CH(C*H*$_3)_2$); δ_C (100 MHz, $CDCl_3$): 168.7 (C-1), 69.5 (*C*H$(CH_3)_2$), 40.6 (C-2), 21.5, 21.4 (CH(*C*H$_3)_2$), 21.3 (C-3); HRMS *m/z* (Probe ESI/FI$^+$) found: 193.9946 $[M]^+$; $C_6H_{11}BrO_2$ requires 193.9942.

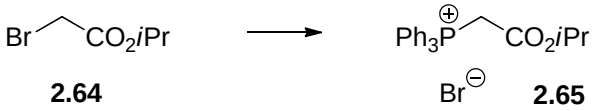
(±)-*iso*-Propyl (2-triphenylphosphonium bromide)-propionate



Triphenylphosphine (6.95 g, 26.5 mmol, 1 eq) was dissolved in toluene (100 mL), and *iso*-propyl 2-bromopropionate **2.61** (5.17 g, 26.5 mmol, 1 eq) was added. The stirred solution was heated to reflux and stirred for 3 h, whereupon an orange syrup had formed. The solvent was decanted and the residue concentrated *in vacuo* to yield *iso*-propyl (2-triphenylphosphonium bromide)-propionate **2.62** (8.08 g, 67%) as an orange glass.

IR ν_{max} (thin film): 1732 (s, C=O), 1485 (P-Ph), 1375 (s, $C(CH_3)_2$); δ_H (400 MHz, $CDCl_3$): 7.99-7.87 (m, 6H; 6 × *o/m*-Ar-H), 7.79-7.71 (m, 3H; 3 × *p*-Ar-H), 7.70-7.62 (m, 6H; 6 × *o/m*-Ar-H), 6.57, 6.53 (2 × q, $J_{2,3}$ 7.1, 1H; H-2), 4.78 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 1.67, 1.62 (2 × d, $J_{2,3}$ 7.1, 3H; 3 × H-3), 1.02, 0.87 (2 × d, J_{iPr} 6.3, 6H; CH(C*H*$_3)_2$); δ_C (100 MHz, $CDCl_3$): 167.2 (C-1), 135.0, 134.4, 134.3, 130.3, 130.2 (15 × *o/m/p*-C_6H_5), 118.2, 117.4 (3 × *ipso*-C_6H_5), 71.4 (*C*H$(CH_3)_2$), 36.5 (C-2), 21.1, 21.4 (CH(*C*H$_3)_2$), 13.1 (C-3); LRMS *m/z* (ESI^+): 377 (100) $[M - Br]^+$; HRMS *m/z* (ESI^+) found: 377.1665 $[M - Br]^+$; $C_{24}H_{26}O_2P$ requires 377.1665.

***iso*-Propyl (2-triphenylphosphonium bromide)-acetate**

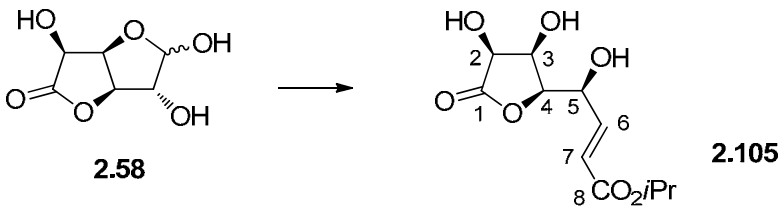


This compound was prepared according to a known procedure.[167] Triphenylphosphine (27.5 g, 105 mmol, 1.3 eq) was dissolved in toluene (250 mL), and *iso*-propyl bromoacetate **2.64** (10.5 mL, 81.1 mmol, 1 eq) was added to the stirred solution. After 18 h, the resulting

suspension was filtered to afford *iso*-propyl-(2-triphenylphosphonium bromide)-acetate **2.65** (35.8 g, 100%) as a white solid.

m.p. 146-148°C; IR ν_{max} (thin film): 1727 (s, C=O), 1587 (aromatic C=C); δ_H (400 MHz, $CDCl_3$): 7.96-7.87 (m, 6H; 6 × *o/m*-Ar-H), 7.83-7.75 (m, 3H; 3 × *p*-Ar-H), 7.73-7.64 (m, 6H; 6 × *o/m*-Ar-H), 5.53 (d, $J_{2,2'}$ 14.2, 2H; H-2 and H-2') 4.83 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 1.03 (d, J_{iPr} 6.1, 6H; CH(C*H*$_3)_2$); δ_C (100 MHz, $CDCl_3$): 175.4 (C-1), 135.1, 134.1, 130.2 (15 × *o/m/p*-C_6H_5), 117.7 (2 × *ipso*-C_6H_5), 49.7 (*C*H$(CH_3)_2$), 34.2 (C-2), 21.4 (CH(*C*H$_3)_2$); LRMS *m/z* (ESI^+): 364.2 (100) $[M + H - Br]^+$; HRMS *m/z* (ESI^+): found 363.1510 $[M - Br]^+$; $C_{23}H_{24}O_2P$ requires 363.1514.

iso-Propyl (_E_)-6,7-dideoxy-L-_gulono_-1,4-lactono-oct-6-enoate

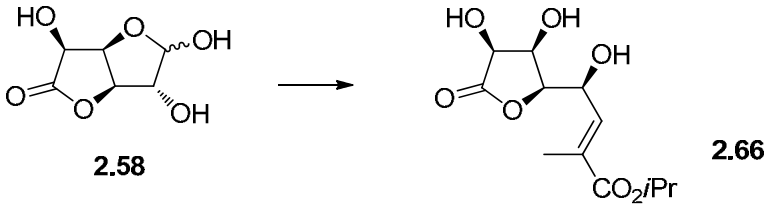


iso-Propyl (2-triphenylphosphonium bromide)-acetate **2.65** (3.50 g, 7.90 mmol, 1.1 eq) was dissolved in dichloromethane (50 mL) and shaken with 1 M NaOH (aq) (25 mL) for 10 min. The organic layer was concentrated *in vacuo* to yield *iso*-propyl-(triphenylphosphoranylidene)-acetate, which was redissolved in 1,4-dioxane (60 mL). D-Glucurono-6,3-lactone **2.58** (1.26 g, 7.18 mmol, 1 eq) was added to the stirred solution at room temperature under an atmosphere of nitrogen. After 5 h, t.l.c. (3:1, $CHCl_3$:MeOH) showed consumption of starting material (R_f 0.32) and formation of a major product (R_f 0.84). The reaction mixture was concentrated *in vacuo* and the residue partitioned between water (100 mL) and dichloromethane (100 mL). The organic layer was washed with water (3 × 100 mL) and the combined aqueous fractions concentrated *in*

vacuo. The crude was purified by flash column chromatography on silica gel (19:1 → 9:1, $CHCl_3$:MeOH) to afford *iso*-propyl (*E)*-6,7-dideoxy-L-*gulono*-1,4-lactono-oct-6-enoate **2.106** (1.60 g, 85%) as a white crystalline solid.

m.p. 112-114°C; $[\alpha]_D^{22}$ -17.4 (*c* 1.30, MeOH); IR ν_{max} (KBr disc): 3410 (br, O-H), 1795 (s, C=O, C-1), 1713 (s, C=O, C-8); δ_H (500 MHz, $(CD_3)_2CO$): 7.11 (dd, $J_{5,6}$ 4.1, $J_{6,7}$ 15.8, 1H; H-6), 6.27 (d, $J_{6,7}$ 15.8, 1H; H-7), 5.08 (d, $J_{2,OH}$ 6.1, 1H; 2-OH), 5.04 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.85 (d, $J_{5,OH}$ 4.4, 1H; 5-OH), 4.78-4.73 (m, 2H; H-5 and 3-OH), 4.62 (dd, $J_{2,3}$ 4.7, $J_{2,OH}$ 7.3, 1H; H-2), 4.50 (a-dt, $J_{3,4} \approx J_{3,OH}$ 2.9, $J_{2,3}$ 4.6, 1H; H-3), 4.26 (dd, $J_{3,4}$ 2.8, $J_{4,5}$ 7.6, 1H; H-4), 1.26 (d, J_{iPr} 6.3, 6H; $CH(CH_3)_2$); δ_C (125 MHz, $(CD_3)_2CO$): 175.7 (C-1), 166.1 (C-8), 145.7 (C-6), 123.9 (C-7), 82.7 (C-4), 71.8 (C-2), 70.7 (C-3), 70.3 (C-5), 68.1 ($CH(CH_3)_2$), 22.0 ($CH(CH_3)_2$); LRMS *m/z* (ESI^-): 520 (100) $[2M - H]^-$; HRMS *m/z* (ESI^+): found 283.0788 $[M + Na]^+$; $C_{11}H_{16}NaO_7$ requires 283.0794.

iso-Propyl (_E_)-6,7-dideoxy-7-_C_-methyl-L-_gulono_-1,4-lactono-oct-6-enoate

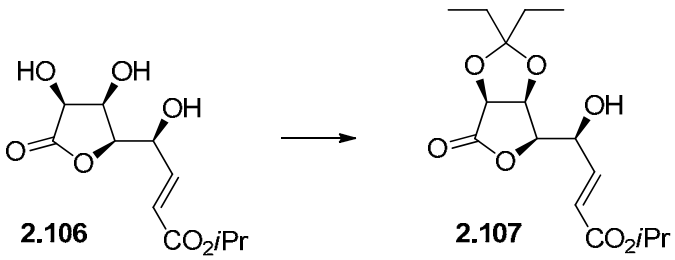


iso-Propyl-(2-triphenylphosphonium bromide)-propionate **2.62** (36.5 g, 80.0 mmol, 1.5 eq) was dissolved in dichloromethane (150 mL) and shaken with 1 M NaOH (aq) (100 mL) for 10 min. The organic layer was concentrated *in vacuo* to yield *iso*-propyl-2-(triphenylphosphoranylidene)-propionate **2.63**, which was redissolved in 1,4-dioxane (300 mL). The solution was warmed to 50°C and D-glucurono-6,3-lactone **2.58** (9.23 g, 53.3 mmol, 1 eq) was added to the stirred solution. After 16 h, t.l.c. (3:1, $CHCl_3$:MeOH) indicated consumption of starting material

(R_f 0.32) and formation of a major product (R_f 0.73). The reaction mixture was concentrated *in vacuo* and the residue partitioned between water (50 mL) and EtOAc (50 mL). The organic layer was washed with water (3 × 150 mL) and the aqueous fractions combined and concentrated *in vacuo*. Some of the product was crystallised from the aqueous washes, and the remaining crude was purified by flash column chromatography on silica gel (1:1, cyclohexane:EtOAc → EtOAc). *iso*-Propyl (*E*)-6,7-dideoxy-7-*C*-methyl-L-*gulono*-1,4-lactono-oct-6-enoate **2.66** was obtained (total yield 12.1 g, 83%) as a white crystalline solid.

m.p. 140-142°C; $[\alpha]_D^{22}$ -7.4 (*c* 1.0, acetone); IR ν_{max} (KBr disc): 3374 (br, O-H), 1772 (s, C=O, C-1), 1683 (s, C=O, C-8); δ_H (400 MHz, $(CD_3)_2CO$): 6.65 (dq, *J* 1.5, $J_{5,6}$ 9.2, 1H; H-6), 5.03 (d, $J_{2,OH}$ 7.5, 1H; 2-OH), 5.02 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.89 (ddd, $J_{5,OH}$ 4.3, $J_{4,5}$ 7.5, $J_{5,6}$ 9.2, 1H; H-5), 4.63 (dd, *J* 0.9, $J_{3,OH}$ 3.1, 1H; 3-OH), 4.61 (d, $J_{5,OH}$ 4.3, 1H; 5-OH), 4.59 (dd, $J_{2,3}$ 4.4, $J_{2,OH}$ 7.3, 1H; H-2), 4.41 (ddd, *J* 0.8, $J_{3,4}$ 2.9, $J_{4,5}$ 7.3, 1H; H-4), 4.37 (dt, $J_{3,4}$ 3.1, $J_{3,OH}$ 3.1, $J_{2,3}$ 4.4, 1H; H-3), 1.95 (d, *J* 1.5, 3H; 7-CH_3), 1.27 (d, J_{iPr} 6.3, 6H; CH(C*H*$_3)_2$); δ_C (100 MHz, $(CD_3)_2CO$): 175.4 (C-1), 167.2 (C-8), 132.2, (C-6) 129.1 (C-7), 82.0 (C-4), 71.4 (C-2), 70.5 (C-3), 68.1 (C-5), 67.3 (*C*H$(CH_3)_2$), 21.5 (CH(*C*H$_3)_2$), 13.0 (7-CH_3); LRMS *m/z* (ESI^-): 548 (100) $[2M - H]^-$; HRMS *m/z* (ESI^-) found: 273.0978 $[M - H]^-$; $C_{12}H_{17}O_7$ requires 273.0980.

iso-Propyl (_E_)-6,7-dideoxy-2,3-_O_-diethylketal-L-_gulono_-1,4-lactono-oct-6-enoate



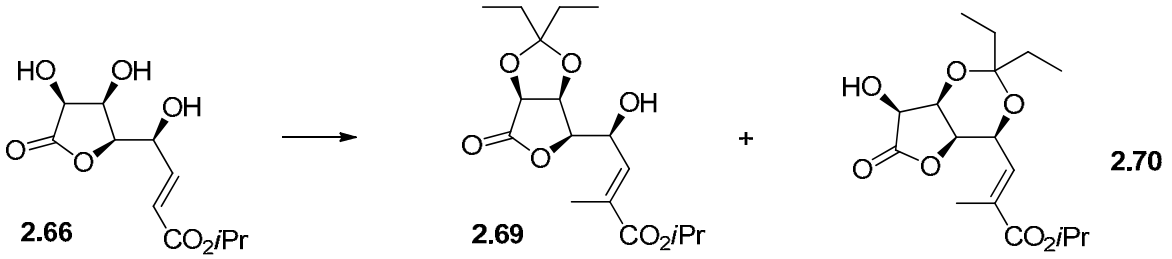
2.106 **2.107**

iso-Propyl (*E)*-6,7-dideoxy-L-*gulono*-1,4-lactono-oct-6-enoate **2.106** (1.08 g, 4.17 mmol) was dissolved in pentan-3-one (20 mL) under an atmosphere of nitrogen. Anhydrous copper (II)

sulfate (1.05 g, 6.56 mmol, 1.6 eq) and concentrated sulfuric acid (0.2 mL, cat.) were added to the stirred solution. After 3 h, t.l.c. (EtOAc) showed consumption of starting material (R_f 0.49) and formation of a major product (R_f 0.86). Excess solid sodium bicarbonate was added and the mixture stirred for 30 min, whereupon all the acid had been neutralised. The mixture was filtered through Celite®, concentrated *in vacuo*, and the crude purified by flash column chromatography on silica gel (2:1, cyclohexane:EtOAc) to afford *iso*-propyl (*E)*-6,7-dideoxy-2,3-*O*-diethylketal-L-*gulono*-1,4-lactono-oct-6-enoate **2.107** (0.725 g, 53%) as a colourless syrup.

$[\alpha]_D^{22}$ -6.0 (*c* 1.10, $CHCl_3$); IR ν_{max} (thin film): 3444 (br, O-H), 1789 (s, C=O, C-1), 1713 (s, C=O, C-8); δ_H (400 MHz, $CDCl_3$): 6.99 (dd, $J_{5,6}$ 4.3, $J_{6,7}$ 15.7, 1H; H-6), 6.32 (dd, J 1.4, $J_{6,7}$ 15.5, 1H; H-7), 5.07 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.89 (d, $J_{2,3}$ 5.3, 1H; H-2), 4.81 (dd, $J_{3,4}$ 3.7, $J_{2,3}$ 5.4, 1H; H-3), 4.79-4.73 (m, 1H; H-5), 4.30 (dd, $J_{3,4}$ 3.5, $J_{4,5}$ 7.8, 1H; H-4), 3.17 (br d, $J_{5,OH}$ 2.8, 1H; 5-OH), 1.71 (dq, J 3.4, J_{Et} 7.5, 2H; C$H_2$$CH_3$), 1.65 (q, J_{Et} 7.5, 2H; C$H_2$$CH_3$), 1.27 (d, J_{iPr} 6.3, 6H; CH(CH_3)$_2$), 0.90, 0.89 (2 × t, J_{Et} 7.5, 6H; 2 × CH_2CH_3); δ_C (100 MHz, $CDCl_3$): 173.1 (C-1), 165.7 (C-8), 142.0 (C-6), 124.5 (C-7), 118.8 (*C*Et_2), 81.5 (C-4), 76.6 (C-3), 76.0 (C-5), 70.0 (C-2), 68.2 (*C*H$(CH_3)_2$), 29.7, 29.3 (2 × *C*$H_2$$CH_3$), 21.8 (CH(*C*$H_3$)$_2$), 8.2, 7.6 (2 × CH_2*C*H_3); LRMS *m/z* (ESI^+): 351 (100) $[M + Na]^+$; HRMS *m/z* (ESI^+): found 351.1410 $[M + Na]^+$; $C_{16}H_{24}NaO_7$ requires 351.1420.

iso-Propyl (_E_)-6,7-dideoxy-2,3-_O_-diethylketal-7-_C_-methyl-L-_gulono_-1,4-lactono-oct-6-enoate and _iso_-propyl (_E_)-6,7-dideoxy-3,5-_O_-diethylketal-7-_C_-methyl-L-_gulono_-1,4-lactono-oct-6-enoate



iso-Propyl 6,7-dideoxy-7-_C_-methyl-L-_gulono_-1,4-lactono-oct-6-enoate **2.66** (3.02 g, 11.0 mmol) was dissolved in pentan-3-one (100 mL) under an atmosphere of nitrogen. Anhydrous copper (II) sulfate (2.80 g, 17.6 mmol, 1.6 eq) and concentrated sulfuric acid (1 mL, cat.) were added to the stirred solution. After 3 h, t.l.c. (EtOAc) indicated consumption of starting material (R_f 0.39) and formation of a major product (R_f 0.81) and a minor product (R_f 0.56). Excess solid sodium bicarbonate was added and the mixture stirred for 1 h, whereupon all the acid had been neutralised. The mixture was filtered and concentrated *in vacuo* and the crude purified by flash column chromatography on silica gel (2:1 → 3:2→ 1:1, cyclohexane:EtOAc) to afford _iso_-propyl (_E_)-6,7-dideoxy-2,3-_O_-diethylketal-7-_C_-methyl-L-_gulono_-1,4-lactono-oct-6-enoate **2.69** (1.51 g, 40%) (53% obtained from 1.4 g) and _iso_-propyl (_E_)-6,7-dideoxy-3,5-_O_-diethylketal-7-_C_-methyl-L-_gulono_-1,4-lactono-oct-6-enoate **2.70** (1.13 g, 30%) (32% obtained from 1.4 g) both as white crystalline solids. The compounds were assigned by the ^{13}C chemical shifts for the quaternary carbons, and coupling between H-2 and 2-OH in **2.70**.

Data for (_E_)-6,7-dideoxy-2,3-_O_-diethylketal-_iso_-propyl-L-_gulono_-1,4-lactono-oct-6-enoate **2.69**

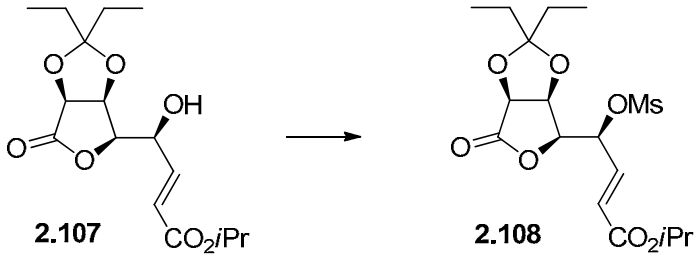
m.p. 72-74°C; $[\alpha]_D^{22}$ -7.9 (_c_ 0.81, $CHCl_3$); IR ν_{max} (thin film): 3473 (br, 5-O-H), 1790 (s, C=O, C-1), 1710 (s, C=O, C-8); δ_H (400 MHz, $CDCl_3$): 6.60 (dd, _J_ 1.5, $J_{5,6}$ 9.3, 1H; H-6), 5.07 (sept,

J_{iPr} 6.2, 1H; C*H*(CH_3)$_2$), 4.90 (dd, $J_{4,5}$ 8.0, $J_{5,6}$ 9.2, 1H; H-5), 4.87 (d, $J_{2,3}$ 5.3, 1H; H-2), 4.70 (dd, $J_{3,4}$ 3.4, $J_{2,3}$ 5.3, 1H; H-3), 4.44 (dd, $J_{3,4}$ 3.4, $J_{4,5}$ 7.9, 1H; H-4), 2.88 (br s, 1H; 5-OH), 1.95 (d, *J* 1.5, 3H; 7-CH_3), 1.72 (dq, *J* 2.7, J_{Et} 7.5, 2H; C*H*$_2$$CH_3$), 1.60 (dq, *J* 1.4, J_{Et} 7.6, 2H; C*H*$_2$$CH_3$), 1.27 (d, J_{iPr} 6.1, 6H; CH(C*H*$_3$)$_2$), 0.90, 0.84 (2 × t, J_{Et} 7.4, 2 × 3H; 2 × CH_2C*H*$_3$); δ_C (100 MHz, $CDCl_3$): 173.5 (C-1), 167.1 (C-8), 134.2 (C-6), 133.7 (C-7), 118.5 (*C*Et$_2$), 81.1 (C-4), 76.7, 76.2 (C-2 and C-3), 68.5 (*C*H(CH_3)$_2$), 67.9 (C-5), 29.5, 29.2 (2 × *C*$H_2$$CH_3$), 21.8 (CH(*C*$H_3$)$_2$), 13.4 (7-$CH_3$), 8.2, 7.6 (2 × CH_2*C*H_3); LRMS *m/z* (ESI^+): 707 (100) $[2M + Na]^+$; HRMS *m/z* (ESI^+) found: 365.1571 $[M + Na]^+$; $C_{17}H_{26}NaO_7$ requires 365.1571.

Data for (*E*)-6,7-dideoxy-3,5-diethylketal-*iso*-propyl-L-*gulono*-1,4-lactono-oct-6-enoate **2.70**

m.p. 135-137°C; $[\alpha]_D^{22}$ +55.5 (*c* 0.90, $CHCl_3$); IR ν_{max} (KBr disc): 3223 (br, 2-O-H), 1791 (s, C=O, C-1), 1708 (s, C=O, C-8); δ_H (400 MHz, $CDCl_3$): 6.76 (dd, *J* 1.5, $J_{5,6}$ 7.6, 1H; H-6), 5.07 (sept, J_{iPr} 6.3, 1H; C*H*(CH_3)$_2$), 4.86 (dd, $J_{4,5}$ 1.9, $J_{5,6}$ 7.7, 1H; H-5), 4.64 (dd, $J_{3,4}$ 2.2, $J_{2,3}$ 4.1, 1H; H-3); 4.48 (br dd, $J_{2,3}$ 3.6, $J_{2,OH}$ 9.2, 1H; H-2); 4.13 (a-t, $J_{3,4} \approx J_{4,5}$ 2.1, 1H; H-4), 2.84 (br d, $J_{2,OH}$ 10.2, 1H; 2-OH), 2.03-1.82 (m, 5H; 7-CH_3 and C*H*$_2$$CH_3$), 1.75-1.64 (m, 2H; C*H*$_2$$CH_3$), 1.28 (d, J_{iPr} 6.1, 6H; CH(C*H*$_3$)$_2$), 0.93, 0.91 (2 × t, J_{Et} 7.4, 6H; 2 × CH_2C*H*$_3$); δ_C (100 MHz, $CDCl_3$): 174.9 (C-1), 166.7 (C-8), 134.0 (C-6), 132.3 (C-7), 102.4 (*C*Et$_2$), 71.6 (C-5), 70.3 (C-4), 68.6 (C-2), 67.2 (C-3), 64.9 (*C*H(CH_3)$_2$), 22.5, 21.8 (2 × *C*$H_2$$CH_3$), 21.8 (CH(*C*$H_3$)$_2$), 13.4 (7-$CH_3$), 8.0, 6.6 (2 × CH_2*C*H_3); LRMS *m/z* (ESI^-): 683 (100) $[2M - H]^-$; HRMS *m/z* (ESI^+) found: 365.1575 $[M + Na]^+$; $C_{17}H_{26}NaO_7$ requires 365.1571.

iso-Propyl (_E_)-6,7-dideoxy-2,3-_O_-diethylketal-5-_O_-methanesulfonyl--L-_gulono_-1,4-lactono-oct-6-enoate

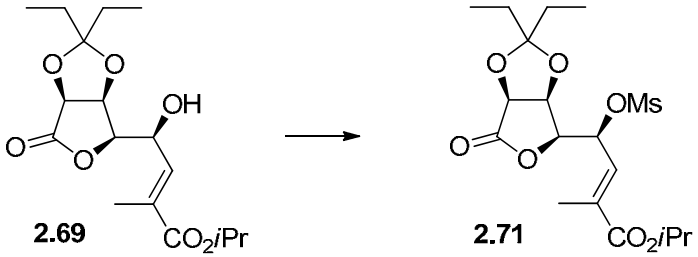


iso-Propyl (*E)*-6,7-dideoxy-2,3-*O*-diethylketal-L-*galactono*-1,4-lactono-oct-6-enoate **2.107** (0.571 g, 1.74 mmol, 1 eq) was dissolved in anhydrous dichloromethane (20 mL) under an atmosphere of argon. Triethylamine (0.73 mL, 5.2 mmol, 3 eq) was added to the stirred solution and the mixture cooled to -5°C. Methanesulfonyl chloride (0.40 mL, 5.2 mmol, 3 eq) was slowly added such that the temperature did not exceed 0°C. The solution was left for 2 h, whereupon t.l.c. (1:1, cyclohexane:EtOAc) showed consumption of starting material (R_f 0.53) and formation of a major product (R_f 0.78). The mixture was allowed to warm to room temperature and concentrated *in vacuo*. The residue was partitioned between EtOAc (25 mL) and water (25 mL) and the aqueous layer extracted with EtOAc (2 × 25 mL). The combined organic fractions were concentrated *in vacuo* and the crude purified by flash column chromatography on silica gel (2:1 → 1:1, cyclohexane:EtOAc) to afford *iso*-propyl (*E)*-6,7-dideoxy-2,3-*O*-diethylketal-5-*O*-methanesulfonyl-L-*galactono*-1,4-lactono-oct-6-enoate **2.108** (0.498 g, 71%) as a white crystalline solid.

m.p. 86-88°C; $[\alpha]_D^{22}$ +22.4 (*c* 1.00, $CHCl_3$); IR ν_{max} (thin film): 1797 (s, C=O, C-1), 1716 (s, C=O, C-8), 1665 (w, C=C), 1366, 1175 (O-SO_2); δ_H (400 MHz, $CDCl_3$): 6.97 (dd, $J_{5,6}$ 4.8, $J_{6,7}$ 15.6, 1H; H-6), 6.37 (dd, *J* 1.7, $J_{6,7}$ 15.8, 1H; H-7), 5.52 (ddd, *J* 1.7, $J_{5,6}$ 4.7, $J_{4,5}$ 9.1, 1H; H-5), 5.08 (sept, J_{iPr} 6.3, 1H; C*H*(CH_3)$_2$), 4.91 (d, $J_{2,3}$ 5.4, 1H; H-2), 4.79 (dd, $J_{3,4}$ 3.6, $J_{2,3}$ 5.4, 1H; H-3), 4.46 (dd, $J_{3,4}$ 3.5, $J_{4,5}$ 9.1, 1H; H-4), 3.16 (s, 3H; OSO_2CH_3), 1.76 (q, J_{Et} 7.4, 2H; C$H_2$$CH_3$),

1.72-1.62 (m, 2H; CH_2CH$_3$), 1.28 (d, J_{iPr} 6.2, 6H; CH(CH_3)$_2$), 0.99-0.83 (m, 6H; 2 × CH$_2$CH_3); δ_C (125 MHz, $CDCl_3$): 171.9 (C-1), 164.6 (C-8), 136.8 (C-6), 127.3 (C-7), 119.4 (*C*Et$_2$), 79.0 (C-5), 78.5 (C-2), 76.3 (C-4), 75.2 (C-3), 68.7 (*C*H(CH$_3$)$_2$), 39.0 (OSO$_2$CH$_3$), 29.8, 29.3 (2 × *C*H$_2$CH$_3$), 21.8 (CH(*C*H$_3$)$_2$), 8.2, 7.6 (2 × CH$_2$*C*H$_3$); LRMS *m/z* (ESI$^+$): 424 (100) [M + NH$_4$]$^+$; HRMS *m/z* (ESI$^+$): found 429.1185 [M + Na]$^+$; $C_{17}H_{26}NaO_9S$ requires 429.1190.

iso-Propyl (*E*)-6,7-dideoxy-2,3-*O*-diethylketal-5-*O*-methanesulfonyl-7-*C*-methyl-L-*gulono*-1,4-lactono-oct-6-enoate

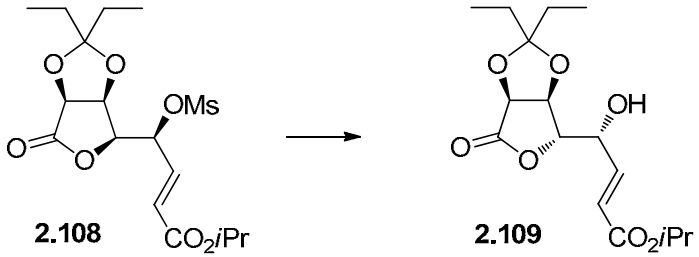


iso-Propyl (*E*)-6,7-dideoxy-2,3-*O*-diethylketal-7-*C*-methyl-L-*gulono*-1,4-lactono-oct-6-enoate **2.69** (3.05 g, 8.92 mmol, 1 eq) was dissolved in anhydrous dichloromethane (50 mL) under an atmosphere of argon. Triethylamine (3.73 mL, 26.7 mmol, 3 eq) was added to the stirred solution and the mixture cooled to -5°C. Methanesulfonyl chloride (2.07 mL, 26.7 mmol, 3 eq) was slowly added such that the temperature did not exceed 0°C. The solution was left for 1 h, whereupon t.l.c. (1:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.53) and formation of a major product (R_f 0.69). The mixture was diluted with additional dichloromethane (100 mL) and water (200 mL). The phases were separated and the aqueous layer extracted with dichloromethane (2 × 100 mL). The organic fractions were combined and concentrated *in vacuo* and the crude purified by flash column chromatography on silica gel (3:1 → 5:2, cyclohexane:EtOAc) to afford *iso*-propyl (*E)*-6,7-dideoxy-2,3-*O*-diethylketal-5-*O*-

methanesulfonyl-7-*C*-methyl-L-*gulono*-1,4-lactono-oct-6-enoate **2.71** (2.84 g, 76%) (86% obtained from 1.1 g) as a white crystalline solid.

m.p. 87-89°C; $[\alpha]_D^{22}$ +6.9 (*c* 0.68, $CHCl_3$); IR ν_{max} (KBr disc): 1796 (s, C=O, C-1), 1714 (s, C=O, C-8), 1174 (O-SO_2); δ_H (400 MHz, $CDCl_3$): 6.58 (dd, *J* 1.5, $J_{5,6}$ 10.1, 1H; H-6), 5.66 (dd, $J_{4,5}$ 9.1, $J_{5,6}$ 10.1, 1H; H-5), 5.09 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.88 (d, $J_{2,3}$ 5.1, 1H; H-2), 4.67 (dd, $J_{3,4}$ 3.5, $J_{2,3}$ 5.2, 1H; H-3), 4.62 (dd, $J_{3,4}$ 3.4, $J_{4,5}$ 9.1, 1H; H-4), 3.12 (s, 3H; OSO_2CH_3), 2.03 (d, *J* 1.5, 3H; 7-CH_3), 1.74 (q, J_{Et} 7.5, 2H; CH_2CH_3), 1.61 (dq, *J* 4.7, J_{Et} 7.4, 2H; CH_2CH_3), 1.30 (d, J_{iPr} 6.1, 6H; CH(C$H_3)_2$), 0.94 (t, J_{Et} 7.5, 3H; CH_2CH_3), 0.84 (t, J_{Et} 7.4, 3H; CH_2CH_3); δ_C (100 MHz, $CDCl_3$): 172.5 (C-1), 166.2 (C-8), 137.4 (C-7), 128.3 (C-6), 118.8 (*C*Et_2), 78.1 (C-5), 77.2 (C-2), 76.4 (C-3), 75.6 (C-4), 69.1 (*C*H$(CH_3)_2$), 39.1 (OSO_2CH_3), 29.7, 29.1 (2 × *C*H_2CH_3), 21.7 (CH(*C*$H_3)_2$), 13.5 (7-CH_3), 8.2, 7.6 (2 × CH_2CH_3); LRMS *m/z* (ESI^+): 438 (100) $[M + NH_4]^+$; HRMS *m/z* (ESI^+) found: 443.1345 $[M + Na]^+$; $C_{18}H_{28}NaO_9S$ requires 443.1346.

iso-Propyl (*E*)-6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-oct-6-enoate

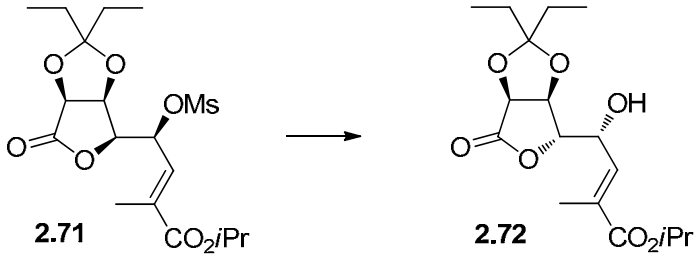


iso-Propyl (*E*)-6,7-dideoxy-2,3-*O*-diethylketal-5-*O*-methanesulfonyl-L-*gulono*-1,4-lactono-oct-6-enoate **2.108** (0.120 g, 0.295 mmol, 1 eq) was dissolved in 1,4-dioxane (10 mL). 2 M KOH (aq) (0.3 mL, 0.6 mmol, 2 eq) was added to the stirred solution. After 1 h the mixture was acidified with 1 M HCl (aq) (0.6 mL, 1.2 mmol, 4 eq), and t.l.c. (9:1, $CHCl_3$:MeOH) showed consumption of starting material (R_f 0.88) and formation of a major product (R_f 0.32). The mixture was concentrated *in vacuo* and the residue partitioned between dichloromethane (20 mL) and water

(20 mL). The aqueous layer was extracted with dichloromethane (2 × 20 mL) and the organic layers combined and concentrated *in vacuo*. The crude was purified by flash column chromatography on silica gel (19:1, $CHCl_3$:MeOH) to afford *iso*-propyl (*E*)-6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-oct-6-enoate **2.109** (71 mg, 73%) as an off-white solid.

m.p. 81-83°C; $[\alpha]_D^{22}$ -21.4 (*c* 0.84, $CHCl_3$); IR ν_{max} (thin film): 3419 (br, O-H), 1785 (s, C=O, C-1), 1713 (s, C=O, C-8); δ_H (500 MHz, $(CD_3)_2CO$): 7.00 (dd, $J_{5,6}$ 4.7, $J_{6,7}$ 15.8, 1H; H-6), 6.10 (d, $J_{6,7}$ 15.8, 1H; H-7), 5.16 (d, $J_{3,4}$ 5.4, 1H; H-4), 5.03 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.96 (d, $J_{3,4}$ 5.7, 1H; H-3), 4.85 (d, $J_{2,3}$ 5.7, 1H; H-2), 4.79-4.75 (m, 2H; H-5 and 5-OH), 1.66, 1.65 (2 × q, J_{Et} 7.3, 2 × 2H; 2 × C$H_2$$CH_3$), 1.26 (d, J_{iPr} 6.3, 6H; CH(CH_3)$_2$), 0.90, 0.85 (2 × t, J_{Et} 7.6, 2 × 3H; 2 × CH_2CH_3); δ_C (125 MHz, $(CD_3)_2CO$): 174.5 (C-1), 165.8 (C-8), 146.5 (C-6), 123.6 (C-7), 117.3 (*C*Et_2), 84.5 (C-4), 80.1 (C-2), 76.5 (C-3), 70.9 (C-5), 68.2 (*C*H$(CH_3)_2$), 28.9 (2 × *C*H_2CH_3), 22.0 (CH(*C*H_3)$_2$), 8.6, 7.5 (2 × CH_2*C*H_3); LRMS *m/z* (ESI^+): 346 (100) $[M + NH_4]^+$; HRMS *m/z* (ESI^+) found 351.1411 $[M + Na]^+$; $C_{16}H_{24}NaO_7$ requires 351.1420.

iso-Propyl (_E_)-6,7-dideoxy-2,3-_O_-diethylketal-7-_C_-methyl-D-_talono_-1,4-lactono-oct-6-enoate

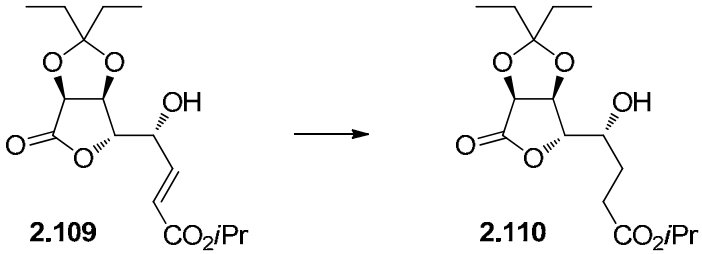


iso-Propyl (*E*)-6,7-dideoxy-2,3-*O*-diethylketal-5-*O*-methanesulfonyl-7-*C*-methyl-L-*gulono*-1,4-lactono-oct-6-enoate **2.71** (1.21 g, 2.89 mmol, 1 eq) was dissolved in 1,4-dioxane (20 mL). 2 M KOH (aq) (4.32 mL, 8.66 mmol, 3 eq) was added to the stirred solution. The reaction was monitored by mass spectroscopy, and after 2.5 h the mixture was acidified to pH 2.5-3.0 with 2 M HCl (aq). After a further 30 minutes t.l.c. (3:1, cyclohexane:EtOAc) indicated the formation

of a new product (R_f 0.23). The reaction mixture was concentrated *in vacuo* and the crude purified by flash column chromatography on silica gel (9:1 → 6:1 → 3:1, cyclohexane:EtOAc) to afford *iso*-propyl (*E)*-6,7-dideoxy-2,3-*O*-diethylketal-7-*C*-methyl-D-*talono*-1,4-lactono-oct-6-enoate **2.72** (0.857 g, 65%) (77% obtained from 0.50 g) as a white crystalline solid.

m.p. 74-76°C; $[\alpha]_D^{22}$ -35.3 (*c* 0.78, $CHCl_3$); IR ν_{max} (thin film): 3461 (br, O-H), 1790 (s, C=O, C-1), 1711 (s, C=O, C-8); δ_H (400 MHz, $CDCl_3$): 6.70 (dd, *J* 1.5, $J_{5,6}$ 8.7, 1H; H-6), 5.05 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.86 (d, $J_{2,3}$ 5.8, 1H; H-3), 4.82 (d, $J_{2,3}$ 5.8, 1H; H-2), 4.69 (ddd, $J_{4,5}$ 1.4, $J_{5,OH}$ 4.1, $J_{5,6}$ 8.6, 1H; H-5), 4.56 (br d, $J_{4,5}$ 1.5, 1H; H-4), 2.43 (br d, $J_{5,OH}$ 4.3, 1H; 5-OH), 1.93 (d, *J* 1.4, 3H; 7-CH_3), 1.72-1.61 (m, 4H; 2 × C*H*$_2$CH$_3$), 1.27 (d, J_{iPr} 6.3, 6H; CH(C*H*$_3$)$_2$), 0.91 (t, J_{Et} 7.5, 3H; CH_2C*H*$_3$), 0.87 (t, J_{Et} 7.4, 3H; CH_2C*H*$_3$); δ_C (100 MHz, $CDCl_3$): 174.4 (C-1), 166.7 (C-8), 135.7 (C-6), 132.8 (C-7), 117.6 (*C*Et$_2$), 84.1 (C-4), 78.7 (C-2), 75.6 (C-3), 68.8 (*C*H$(CH_3)_2$), 67.9 (C-5), 29.9, 29.3 (2 × *C*H$_2$CH$_3$), 21.8 (CH(*C*H$_3$)$_2$), 13.2 (7-CH_3), 8.2, 7.3 (2 × CH_2*C*H$_3$); LRMS *m/z* (ESI^+): 702 (100) $[2M + NH_4]^+$; HRMS *m/z* (ESI^+) found: 365.1572 $[M + Na]^+$; $C_{17}H_{26}NaO_7$ requires 365.1571.

iso-Propyl 6,7-dideoxy-2,3-_O_-diethylketal-D-_talono_-1,4-lactono-octanoate

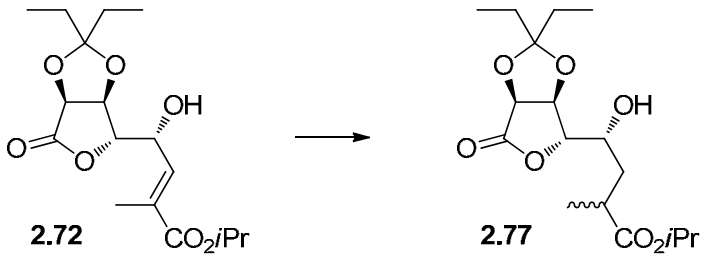


iso-Propyl (*E)*-6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-oct-6-enoate **2.109** (71 mg, 0.216 mmol, 1 eq) was dissolved in 1,4-dioxane (5 mL) under an atmosphere of argon. 10% Pd/C (10% by mass, 10 mg) was added to the stirred solution. The reaction vessel was degassed and the reaction mixture placed under an atmosphere of hydrogen. After 4 h, mass spectroscopy

did not detect starting material (329: $[M + H]^+$) and detected product (331: $[M + H]^+$), whereupon the reaction mixture was filtered through Celite®. The filtrate was concentrated *in vacuo* and the crude purified by flash column chromatography on silica gel (2:1 → 1:1, cyclohexane:EtOAc) to afford *iso*-propyl 6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-octanoate **2.110** (71 mg, 99%) as an off-white crystalline solid.

m.p. 80-82°C; $[\alpha]_D^{22}$ +11.1 (*c* 0.94, $CHCl_3$); IR ν_{max} (thin film): 3443 (br, O-H), 1781 (s, C=O); δ_H (400 MHz, $CDCl_3$): 5.00 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.85 (d, $J_{2,3}$ 5.6, 1H; H-3), 4.75 (d, $J_{2,3}$ 5.8, 1H; H-2), 4.48 (d, $J_{4,5}$ 0.9, 1H; H-4), 3.87 (br dt, $J_{5,6} \approx J_{5,OH}$ 3.5, $J_{5,6'}$ 8.9, 1H; H-5), 3.69 (br d, $J_{5,OH}$ 3.8, 1H; 5-OH), 2.59 (ddd, $J_{6,7}$ 4.8, $J_{6,7'}$ 7.7, $J_{7,7'}$ 17.1, 1H; H-7), 2.45 (ddd, $J_{6,7'}$ 5.0, $J_{6',7'}$ 8.5, $J_{7,7'}$ 17.1, 1H; H-7'), 2.07-1.95, 1.93-1.84 (2 × m, 2 × 1H; H-6 and H-6'), 1.68 (q, J_{Et} 7.4, 2H; CH_2CH$_3$), 1.63 (q, J_{Et} 7.5, 2H; CH_2CH$_3$), 1.24 (dd, *J* 2.3, J_{iPr} 6.2, 6H; CH(CH_3)$_2$), 0.90, 0.87 (2 × t, J_{Et} 7.5, 2 × 3H; 2 × CH$_2$CH_3); δ_C (100 MHz, $CDCl_3$): 174.7, 174.1 (C-1 and C-8), 117.2 (*C*Et$_2$), 84.8 (C-4), 79.3 (C-2), 75.5 (C-3), 71.2 (C-5), 68.8 (*C*H$(CH_3)_2$), 31.3 (C-7), 29.8, 29.7 (2 × *C*H$_2$CH$_3$), 28.0 (C-6), 21.7 (CH(*C*H$_3$)$_2$), 8.2, 7.3 (2 × CH$_2$*C*H$_3$); LRMS *m/z* (ESI^+): 348 (100) $[M + NH_4]^+$; HRMS *m/z* (ESI^+) found: 353.1573 $[M + Na]^+$; $C_{16}H_{26}NaO_7$ requires 353.1571.

iso-Propyl 6,7-dideoxy-2,3-_O_-diethylketal-7-_C_-methyl-D-_talono_-1,4-lactono-octanoate



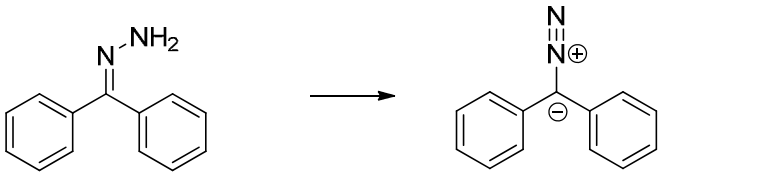
iso-Propyl (*E)*-6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-oct-6-enoate **2.72** (1.09 g, 3.19 mmol, 1 eq) was dissolved in 1,4-dioxane (40 mL) under an atmosphere of argon. 10% Pd/C

(10% by mass, 0.104 g) was added to the stirred solution. The reaction vessel was degassed and the reaction mixture placed under an atmosphere of hydrogen. After 12 h, t.l.c. (2:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.41, UV active) and formation of product (R_f 0.45, UV inactive), and mass spectroscopy detected product (367: $[M + Na]^+$) and no starting material. The reaction mixture was filtered through Celite®, the filtrate concentrated *in vacuo*, and the crude purified by flash column chromatography on silica gel (4:1 → 3:1, cyclohexane:EtOAc) to afford *iso*-propyl 6,7-dideoxy-2,3-*O*-diethylketal-7-*C*-methyl-D-*talono*-1,4-lactono-octanoate **2.77** (1.10 g, 100%) as a waxy white solid in a 1:1 mixture of diastereomers.

m.p. 55-57°C; $[\alpha]_D^{22}$ +9.4 (*c* 4.0, $CHCl_3$); IR ν_{max} (thin film): 3472 (br, O-H), 1788 (s, C=O, C-1), 1726 (s, C=O, C-8); δ_H (400 MHz, $CDCl_3$): 4.97 (sept, J_{iPr} 6.2, 2H; C*H*$(CH_3)_2$$^{\mathbf{A}}$ and C*H*$(CH_3)_2$$^{\mathbf{B}}$), 4.81 (d, $J_{2,3}$ 5.8, 2H; H-2$^{\mathbf{A}}$ and H-2$^{\mathbf{B}}$), 4.73 (d, $J_{2,3}$ 5.8, 1H; H-3$^{\mathbf{A}}$), 4.72 (d, $J_{2,3}$ 5.8, 1H; H-3$^{\mathbf{B}}$), 4.47 (a-br s, 1H; H-4$^{\mathbf{A}}$), 4.44 (d, $J_{4,5}$ 1.0, 1H; H-4$^{\mathbf{B}}$), 3.92-3.80 (m, 2H; H-5$^{\mathbf{A}}$ and H-5$^{\mathbf{B}}$), 3.60 (br d, $J_{5,OH}$ 5.6, 1H; 5-OH$^{\mathbf{B}}$), 3.40 (br d, $J_{5,OH}$ 5.3, 1H; 5-OH$^{\mathbf{A}}$), 2.70 (ddq, $J_{6',7}$ 4.0, $J_{7,Me}$ 7.2, $J_{6,7}$ 9.2, 1H; H-7$^{\mathbf{B}}$), 2.54 (ddq, $J_{6',7}$ 5.3, $J_{7,Me}$ 7.1, $J_{6,7}$ 9.1, 1H; H-7$^{\mathbf{A}}$), 2.05 (dt, $J_{6,7}$ 9.1, $J_{5,6}$ 9.1, $J_{6,6'}$ 14.5, 1H; H-6$^{\mathbf{A}}$), 1.89 (ddd, $J_{5,6}$ 4.0, $J_{6,7}$ 9.8, $J_{6,6'}$ 14.5, 1H; H-6$^{\mathbf{B}}$), 1.80 (ddd, $J_{6',7}$ 3.4, $J_{5,6}$ 9.2, $J_{6,6'}$ 14.5, 1H; H-6'$^{\mathbf{B}}$), 1.70-1.55 (m, 9H; 2 × C*H*$_2$CH$_3$$^{\mathbf{A}}$, 2 × C*H*$_2CH_3$$^{\mathbf{B}}$, and H-6'$^{\mathbf{A}}$), 1.24-1.17 (m, 18H; CH(C*H*$_3$)$_2$$^{\mathbf{A}}$, CH(C*H*$_3$)$_2$$^{\mathbf{B}}$, 7-CH$_3$$^{\mathbf{A}}$, and 7-CH$_3$$^{\mathbf{B}}$), 0.87 (t, J_{Et} 7.5, 6H; 2 × CH$_2$C*H*$_3$$^{\mathbf{A}}$), 0.86, 0.86 (2 × t, J_{Et} 7.5, 6H; 2 × CH$_2$C*H*$_3$$^{\mathbf{B}}$); δ_C (100 MHz, $CDCl_3$): 177.0 (C-1$^{\mathbf{A}}$ and C-1$^{\mathbf{B}}$), 172.8 (C-8$^{\mathbf{A}}$ and C-8$^{\mathbf{B}}$), 117.2 (*C*Et$_2$$^{\mathbf{A}}$ and *C*Et$_2$$^{\mathbf{B}}$), 85.3 (C-4$^{\mathbf{B}}$), 84.8 (C-4$^{\mathbf{A}}$), 79.4, 79.2 (C-3$^{\mathbf{A}}$ and C-3$^{\mathbf{B}}$), 75.6, 75.5 (C-2$^{\mathbf{A}}$ and C-2$^{\mathbf{B}}$), 70.5 (C-5$^{\mathbf{A}}$ and C-5$^{\mathbf{B}}$), 68.7, 68.5 (*C*H(CH$_3$)$_2$$^{\mathbf{A}}$ and *C*H(CH$_3$)$_2$$^{\mathbf{B}}$), 37.6 (C-7$^{\mathbf{A}}$), 36.8 (C-6$^{\mathbf{B}}$), 36.2 (C-7$^{\mathbf{B}}$), 36.0 (C-6$^{\mathbf{A}}$), 29.8, 29.3 (2 × *C*H$_2CH_3$$^{\mathbf{A}}$ and 2 × *C*H$_2$CH$_3$$^{\mathbf{B}}$), 21.7 (CH(*C*H$_3$)$_2$$^{\mathbf{A}}$ and CH(*C*H$_3$)$_2$$^{\mathbf{B}}$), 18.0 (7-CH$_3$$^{\mathbf{A}}$), 17.2 (7-CH$_3$$^{\mathbf{B}}$), 8.2 (2 ×

$CH_2CH_3^{B}$), 7.3 (2 × $CH_2CH_3^{A}$); LRMS *m/z* (ESI^+): 711 (100) $[2M + Na]^+$; HRMS *m/z* (ESI^+) found: 367.1731 $[M + Na]^+$; $C_{17}H_{28}NaO_7$ requires 367.1727.

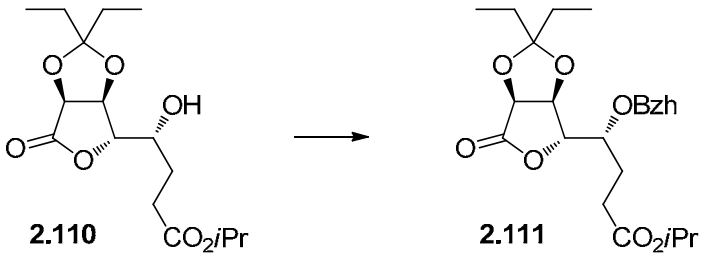
Diphenyldiazomethane



2.79 **2.80**

This compound was prepared according to a known procedure.[150] Benzophenone hydrazone **2.79** (34.4 g, 175 mmol, 1 eq) was dissolved in methyl *tert*-butyl ether (500 mL). Yellow mercuric oxide (95.0 g, 438 mmol, 2.5 eq), saturated ethanolic potassium hydroxide (17.5 mL), and anhydrous $MgSO_4$ (45 g) were added to the stirred solution. After 5 h t.l.c. (10:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.25), whereupon the suspension was filtered and the filtrate concentrated *in vacuo*. The crude was redissolved in *n*-hexane, filtered and recrystallised at -25 to -50 °C. The mixture was filtered to give diphenyldiazomethane **2.80** (20.4 g, 61%) as a purple crystalline solid.

***iso*-Propyl 5-*O*-benzhydryl-6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-octanoate**



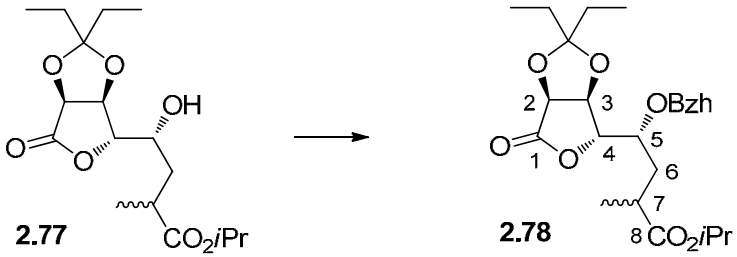
2.110 **2.111**

iso-Propyl 6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-octanoate **2.110** (0.160 g, 0.484 mmol, 1 eq) was dissolved in toluene (20 mL) and heated to reflux (≈110°C).

Diphenyldiazomethane **2.80** (0.140 g, 0.726 mmol, 1.5 eq) was added to the stirred solution. After 1 h, the solution had turned from purple to yellow and t.l.c. (3:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.25) and formation of a major product (R_f 0.60). The reaction mixture was concentrated *in vacuo*, and the crude purified by flash column chromatography on silica gel (19:1 → 4:1, cyclohexane:EtOAc) to afford *iso*-propyl 5-*O*-benzhydryl-6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-octanoate **2.111** (0.195 g, 81%) as a colourless oil.

$[\alpha]_D^{22}$ -38.0 (*c* 5.0, $CHCl_3$); IR ν_{max} (thin film): 3088, 3063 (w, Ar-H), 3030 (m, Ar-H), 2978 (s, Ar-H), 2942, 2883 (m, Ar-H), 1791 (s, C=O, C-1), 1727 (s, C=O, C-8); δ_H (400 MHz, $CDCl_3$): 7.41-7.18 (m, 10H; 2 × C_6H_5), 5.55 (s, 1H; C*H*Ph_2); 5.00 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.73 (d, $J_{2,3}$ 5.6, 1H; H-2), 4.52 (d, $J_{4,5}$ 1.2, 1H; H-4), 3.97 (d, $J_{2,3}$ 5.6, 1H; H-3), 3.70 (ddd, $J_{4,5}$ 1.3, $J_{5,6}$ 5.2, $J_{5,6'}$ 8.0, 1H; H-5), 2.46-2.27 (m, 2H; H-7 and H-7'), 2.16-2.00 (m, 2H; H-6 and H-6'), 1.63 (q, J_{Et} 7.4, 2H; C*H*$_2CH_3$), 1.46 (q, J_{Et} 7.5, 2H; C*H*$_2CH_3$), 1.23 (d, J_{iPr} 6.3, 6H; CH(C*H*$_3)_2$), 0.85 (t, J_{Et} 7.4, 3H; CH_2C*H*$_3$), 0.77 (t, J_{Et} 7.5, 3H; CH_2C*H*$_3$); δ_C (100 MHz, $CDCl_3$): 174.1, 172.3 (C-1 and C-8), 142.4, 141.0 (2 × *ipso*-Ar), 128.8, 128.6, 128.5, 128.3, 127.4, 126.1 (10 × *o/m/p*-C_6H_5), 117.2 (*C*Et_2), 82.6 (C-4), 81.1 (*C*HPh_2), 79.5 (C-3), 75.7 (C-2), 74.2 (C-5), 68.1 (*C*H$(CH_3)_2$), 29.8 (C-7), 29.7, 29.4 (2 × *C*H_2CH_3), 24.3 (C-6), 21.8 (CH(*C*$H_3)_2$), 8.1, 7.3 (2 × CH_2*C*H_3); LRMS *m/z* (ESI^+): 514 (100) $[M + NH_4]^+$; HRMS *m/z* (ESI^+) found: 519.2356 $[M + Na]^+$; $C_{29}H_{36}NaO_7$ requires 519.2353.

iso-Propyl 5-_O_-benzhydryl-6,7-dideoxy-2,3-_O_-diethylketal-7-_C_-methyl-D-_talono_-1,4-lactono-octanoate

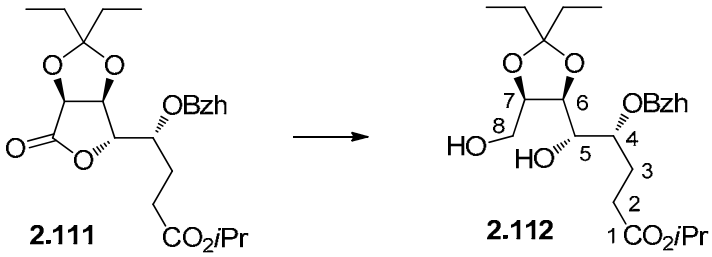


iso-Propyl 6,7-dideoxy-2,3-*O*-diethylketal-7-*C*-methyl-D-*talono*-1,4-lactono-octanoate **2.77** (1.06 g, 3.09 mmol, 1 eq) was dissolved in toluene (35 mL) and heated to reflux (≈110°C). Diphenyldiazomethane **2.79** (0.766 g, 3.94 mmol, 1.3 eq) was added to the stirred solution. After 1 h, the solution had turned from purple to yellow and t.l.c. (3:1, cyclohexane:EtOAc) indicated incomplete consumption of starting material (R_f 0.28) and formation of two products (R_f 0.66, R_f 0.62). Further diphenyldiazomethane (0.195 g, 1.00 mmol, 0.23 eq) was added, and after a further 50 min the reaction had gone to completion. The reaction mixture was concentrated *in vacuo*, and the crude purified by flash column chromatography on silica gel (19:1 → 9:1 → 7:1, cyclohexane:EtOAc) to afford *iso*-propyl 5-*O*-benzhydryl-6,7-dideoxy-2,3-*O*-diethylketal-7-*C*-methyl-D-*talono*-1,4-lactono-octanoate **2.78** (1.18 g, 75%) as a white solid in a 1:1 mixture of diastereomers.

m.p. 62-64°C; $[\alpha]_D^{22}$ -51.6 (*c* 1.0, $CHCl_3$); IR ν_{max} (thin film): 2978 (s, Ar-H), 1793 (s, C=O, C-1), 1724 (s, C=O, C-8), 1494, 1455 (s, C=C, Ph); δ_H (400 MHz, $CDCl_3$): 7.43-7.16 (m, 20H; 2 × $C_6H_5^{\mathbf{A}}$ and 2 × $C_6H_5^{\mathbf{B}}$), 5.54 (s, 1H; C*H*$Ph_2^{\mathbf{A}}$), 5.49 (s, 1H; C*H*$Ph_2^{\mathbf{B}}$), 5.03 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2^{\mathbf{A}}$), 4.99 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2^{\mathbf{B}}$), 4.76 (d, $J_{2,3}$ 5.6, 1H; H-$2^{\mathbf{A}}$), 4.68 (d, $J_{2,3}$ 5.6, 1H; H-$2^{\mathbf{B}}$), 4.57 (d, $J_{4,5}$ 1.2, 1H; H-$4^{\mathbf{A}}$), 4.48 (d, $J_{4,5}$ 1.4, 1H; H-$4^{\mathbf{B}}$), 3.92 (d, $J_{2,3}$ 5.6, 1H; H-$3^{\mathbf{A}}$), 3.88 (d, $J_{2,3}$ 5.6, 1H; H-$3^{\mathbf{B}}$), 3.71 (a-dt, $J_{4,5}$ 1.5, $J_{5,6} \approx J_{5,6'}$ 6.4, 1H; H-$5^{\mathbf{B}}$), 3.56 (ddd, $J_{4,5}$ 1.5, $J_{5,6}$ 3.5, $J_{5,6'}$ 10.5, 1H; H-$5^{\mathbf{A}}$), 2.53-2.41 (m, 2H; H-$7^{\mathbf{A}}$ and H-$7^{\mathbf{B}}$), 2.19-2.06 (m, 2H; H-$6^{\mathbf{A}}$ and H-$6^{\mathbf{B}}$),

1.93-1.78 (m, 2H; H-6'^{A} and H-6'^{B}), 1.68-1.57 (m, 4H; CH_2CH$_3^{A}$ and CH_2CH$_3^{B}$), 1.47 (q, J_{Et} 7.5, 2H; CH_2CH$_3^{A}$), 1.44 (q, J_{Et} 7.5, 2H; CH_2CH$_3^{B}$), 1.26-1.17 (m, 15H; CH(CH_3)$_2^{A}$, CH(CH_3)$_2^{B}$, and 7-CH$_3^{A}$), 1.14 (d, $J_{7,Me}$ 7.0, 3H; 7-CH$_3^{B}$), 0.86 (t, J_{Et} 7.4, 3H; CH$_2$CH_3^{A}), 0.84 (t, J_{Et} 7.4, 3H; CH$_2$CH_3^{B}), 0.77 (t, J_{Et} 7.4, 3H; CH$_2$CH_3^{A}), 0.76 (t, J_{Et} 7.4, 3H; CH$_2$CH_3^{B}); δ_C (100 MHz, $CDCl_3$): 175.3 (C-1^{B}), 175.3 (C-1^{A}), 174.2 (C-8^{A}), 174.1 (C-8^{B}), 141.5, 141.2, 141.0 (2 × *ipso*-Ar^{A} and 2 × *ipso*-Ar^{B}), 128.9, 128.7, 128.7, 128.6, 128.6, 128.4, 128.3, 128.2, 127.4, 127.3, 126.2, 126.1 (10 × *o/m/p*-C$_6$H$_5^{A}$ and 10 × *o/m/p*-C$_6$H$_5^{B}$), 117.2 (*C*Et$_2^{A}$), 117.2 (*C*Et$_2^{B}$), 83.4 (C-4^{B}), 81.9 (C-4^{A}), 81.9, 80.5 (*C*HPh$_2^{A}$ and *C*HPh$_2^{B}$), 79.6 (C-3^{A}), 79.3 (C-3^{B}), 75.8 (C-2^{A}), 75.7 (C-2^{B}), 73.9 (C-5^{B}), 73.5 (C-5^{A}), 68.0 (*C*H(CH$_3$)$_2^{B}$), 68.0 (*C*H(CH$_3$)$_2^{A}$), 36.2, 35.9 (C-7^{A} and C-7^{B}), 33.8, 32.5 (C-6^{A} and C-6^{B}), 29.8 (*C*H$_2$CH$_3^{A}$), 29.7 (*C*H$_2$CH$_3^{B}$), 29.5 (*C*H$_2$CH$_3^{A}$), 29.4 (*C*H$_2$CH$_3^{B}$), 21.8, 21.8, 21.7 (CH(*C*H$_3$)$_2^{A}$ and CH(*C*H$_3$)$_2^{B}$), 18.6 (7-CH$_3^{A}$), 17.8 (7-CH$_3^{B}$), 8.3 (CH$_2$*C*H$_3^{A}$ and CH$_2$*C*H$_3^{B}$), 7.3 (CH$_2$*C*H$_3^{A}$ and CH$_2$*C*H$_3^{B}$); LRMS *m/z* (ESI$^+$): 533 (100) [M + Na]$^+$; HRMS *m/z* (ESI$^+$) found: 533.2489 [M + Na]$^+$; $C_{29}H_{38}NaO_7$ requires 533.2510.

iso-Propyl 4-_O_-benzhydryl-2,3-dideoxy-6,7-_O_-diethylketal-D-_erythro_-D-_gluco_-octoate

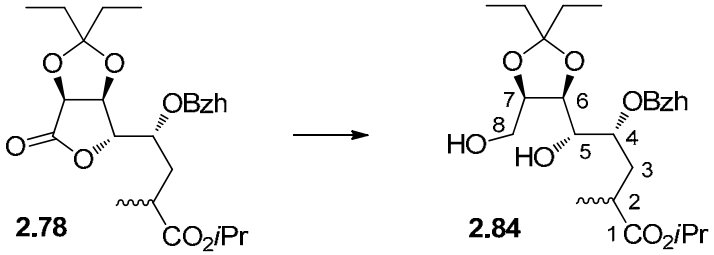


iso-Propyl 5-*O*-benzhydryl-6,7-dideoxy-2,3-*O*-diethylketal-D-*talono*-1,4-lactono-octanoate **2.111** (0.153 g, 0.309 mmol, 1 eq) was dissolved in anhydrous methanol (15 mL) and cooled to -5°C. Sodium borohydride (15 mg, 0.397 mmol, 1 eq) was added portionwise to the stirred solution over a period of 30 min, such that the temperature did not exceed 0°C. The mixture was stirred for 1 h while being allowed to warm to room temperature, whereupon another portion of sodium

borohydride (15 mg, 0.397 mmol, 1 eq) was added. After a further 1 h t.l.c. (3:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.55) and formation of a major product (R_f 0.19). The reaction mixture was quenched with NH_4Cl (aq) (10 mL) and stirred for 1 h. The reaction mixture was filtered and concentrated *in vacuo*, and the crude purified by flash column chromatography on silica gel (4:1, cyclohexane:EtOAc) to afford *iso*-propyl 4-*O*-benzhydryl-2,3-dideoxy-6,7-*O*-diethylketal-D-*erythro*-D-*gluco*-octoate **2.112** (0.138 g, 89%) as a colourless syrup.

$[\alpha]_D^{22}$ -16.8 (*c* 0.96, $CHCl_3$); IR ν_{max} (thin film): 3427 (br, O-H), 3063, 3029 (m, Ar-H), 2977, 2939 (s, Ar-H), 1729 (s, C=O), 1600 (w, C=C, Ph), 1494 (m, C=C, Ph), 1455 (s, C=C, Ph); δ_H (400 MHz, $CDCl_3$): 7.41-7.21 (m, 10H; 2 × C_6H_5), 5.65 (s, 1H; C*H*Ph_2), 4.94 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.38-4.31 (m, 1H; H-7), 4.09 (dd, $J_{5,6}$ 6.6, $J_{6,7}$ 9.9, 1H; H-6), 3.94-3.89 (m, 1H; H-4), 3.85 (dd, $J_{7,8}$ 7.8, $J_{8,8'}$ 11.6, 1H; H-8), 3.74 (br dd, $J_{7,8'}$ 3.9, $J_{8,8'}$ 11.2, 1H; H-8'), 3.64 (br d, $J_{4,5}$ 8.8, 1H; H-5), 3.16 (br s, 1H; 8-OH), 2.97 (br s, 1H; 5-OH), 2.28-2.10 (m, 1H; H-2 and H-2'), 2.02-1.91 (m, 2H; H-3 and H-3'), 1.62-1.49 (m, 4H; 2 × C*H*$_2CH_3$), 1.19 (dd, *J* 1.8, J_{iPr} 6.3, 6H; CH(C*H*$_3)_2$), 0.86, 0.83 (2 × t, J_{Et} 7.5, 6H; 2 × CH_2C*H*$_3$); δ_C (100 MHz, $CDCl_3$): 172.8 (C-1), 142.2, 141.6 (2 × *ipso*-Ar), 128.5, 128.4, 127.7, 127.7, 127.3, 127.0 (10 × *o/m/p*-C_6H_5), 112.1 (*C*Et_2), 82.4 (*C*HPh_2), 77.5 (C-7), 76.0 (C-6), 74.7 (C-4), 70.9 (C-5), 67.8 (*C*H$(CH_3)_2$), 60.9 (C-8), 30.2 (C-2), 29.6, 26.2 (2 × *C*H_2CH_3), 28.5 (C-3), 21.8 (CH(*C*$H_3)_2$), 8.8, 7.9 (2 × CH_2*C*H_3); LRMS *m/z* (ESI^+): 501 (100) $[M + H]^+$, 518 (80) $[M + NH_4]^+$, 523 (74) $[M + H]^+$; HRMS *m/z* (ESI^+) found: 501.2840 $[M + Na]^+$; $C_{29}H_{41}O_7$ requires 501.2847.

iso-Propyl 4-_O_-benzhydryl-2,3-dideoxy-6,7-_O_-diethylketal-2-_C_-methyl-D-_erythro_-D-_gluco_-octoate

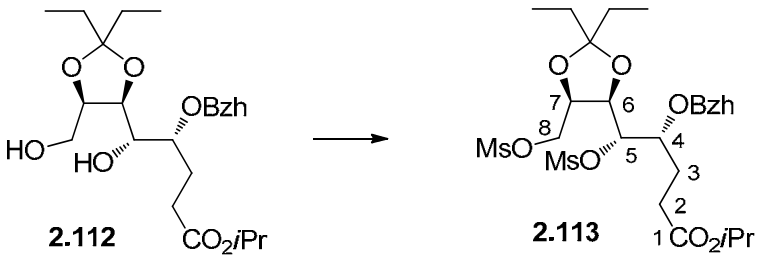


iso-Propyl 5-_O_-benzhydryl-6,7-dideoxy-2,3-_O_-diethylketal-7-_C_-methyl-D-_talono_-1,4-lactono-octanoate **2.78** (1.18 g, 2.31 mmol, 1 eq) was dissolved in anhydrous methanol (40 mL) and cooled to -5°C. Sodium borohydride (94 mg, 2.49 mmol, 1.1 eq) was added portionwise to the stirred solution over a period of 30 min, such that the temperature did not exceed 0°C. The mixture was stirred for 1.25 h, whereupon t.l.c. (3:1, cyclohexane:EtOAc) indicated incomplete consumption of starting material (R_f 0.68, R_f 0.65) and formation of a major product (R_f 0.31). Additional portions of sodium borohydride (totalling 0.387 g, 10.2 mmol, 4.4 eq) were added at approximately hourly intervals until the reaction was complete. The reaction mixture was quenched with NH_4Cl (aq) (10 mL), stirred for 20 min, concentrated *in vacuo*, and the crude purified by flash column chromatography on silica gel (9:1 → 5:1 → 3:1, cyclohexane:EtOAc) to afford _iso_-propyl 4-_O_-benzhydryl-2,3-dideoxy-6,7-_O_-diethylketal-2-_C_-methyl-D-_erythro_-D-_gluco_-octoate **2.84** (1.15 g, 97%) as a colourless syrup in a 1:1 mixture of diastereomers.

m.p. 76-78°C; $[\alpha]_D^{22}$ +27.0 (_c_ 0.39, MeOH); IR ν_{max} (thin film): 3422 (br, O-H), 2975, 2935 (s, Ar-H), 1726 (s, C=O); δ_H (400 MHz, $CDCl_3$): 7.41-7.21 (m, 20H; 2 × $C_6H_5^{\mathbf{A}}$ and 2 × $C_6H_5^{\mathbf{B}}$), 5.66 (s, 1H; C*H*$Ph_2^{\mathbf{A}}$), 5.63 (s, 1H; C*H*$Ph_2^{\mathbf{B}}$), 4.92 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2^{\mathbf{A}}$), 4.88 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2^{\mathbf{B}}$), 4.38-4.31 (m, 2H; H-$7^{\mathbf{A}}$ and H-$7^{\mathbf{B}}$), 4.12 (dd, $J_{6,7}$ 6.5, $J_{5,6}$ 9.9, 1H; H-$6^{\mathbf{A}}$), 4.06 (dd, $J_{6,7}$ 6.3, $J_{5,6}$ 9.9, 1H; H-$6^{\mathbf{B}}$), 3.92 (ddd, $J_{4,5}$ 0.8, $J_{3,4}$ 5.4, $J_{3',4}$ 7.3, 1H; H-$4^{\mathbf{A}}$), 3.89-3.80 (m, 3H; H-$4^{\mathbf{B}}$, H-$8^{\mathbf{A}}$, and H-$8^{\mathbf{B}}$), 3.75 (ddd, $J_{7,8'}$ 1.4, $J_{8,8'}$ 4.9, $J_{8',OH}$ 8.7, 1H; H-$8'^{\mathbf{A}}$), 3.72 (ddd,

$J_{7,8'}$ 1.3, $J_{8,8'}$ 5.0, $J_{8',OH}$ 8.7, 1H; H-8'^{B}), 3.63 (a-dt, $J_{4,5}$ 0.9, $J_{5,OH} \approx J_{5,6}$ 9.8, 1H; H-5^{A}), 3.60 (a-dt, $J_{4,5}$ 0.9, $J_{5,OH} \approx J_{5,6}$ 9.4, 1H; H-5^{B}), 3.18 (br dd, $J_{8,OH}$ 4.4, $J_{8',OH}$ 8.7, 1H; 8-OH^{A}), 3.14 (br dd, $J_{8,OH}$ 4.8, $J_{8',OH}$ 8.7, 1H; 8-OH^{B}), 2.95 (br d, $J_{5,OH}$ 9.1, 1H; 5-OH^{B}), 2.86 (br d, $J_{5,OH}$ 9.7, 1H; 5-OH^{A}), 2.38-2.22 (m, 2H; H-2^{A} and H-2^{B}), 2.17-2.05 (m, 2H; H-3^{A} and H-3^{B}), 1.71-1.60 (m, 2H; H-3'^{A} and H-3'^{B}), 1.60-1.45 (m, 8H; 2 × CH_2CH$_3{}^{A}$ and 2 × CH_2CH$_3{}^{B}$), 1.18 (d, J_{iPr} 6.3, 6H; CH(CH_3)$_2{}^{A}$ and CH(CH_3)$_2{}^{B}$), 1.16 (d, J_{iPr} 6.3, 3H; CH(CH_3)$_2{}^{A}$), 1.12 (d, J_{iPr} 6.3, 3H; CH(CH_3)$_2{}^{B}$), 0.99 (d, $J_{2,Me}$ 7.0, 3H; 2-CH$_3{}^{B}$), 0.98 (d, $J_{2,Me}$ 7.0, 3H; 2-CH$_3{}^{A}$), 0.86 (t, J_{Et} 7.5, 3H; CH$_2$C$H_3{}^{A}$), 0.83 (t, J_{Et} 7.4, 3H; CH$_2$C$H_3{}^{B}$), 0.82 (t, J_{Et} 7.4, 3H; CH$_2$C$H_3{}^{A}$), 0.82 (t, J_{Et} 7.5, 3H; CH$_2$C$H_3{}^{B}$); δ_C (100 MHz, $CDCl_3$): 176.0, 175.9 (C-1^{A} and C-1^{B}), 142.3, 142.2, 141.8, 141.6 (2 × *ipso*-Ar^{A} and 2 × *ipso*-Ar^{B}), 131.3, 128.5, 128.4, 127.7, 127.6, 127.3, 127.3, 127.1, 127.0, 126.4 (10 × *o/m/p*-C$_6$H$_5{}^{A}$ and 10 × *o/m/p*-C$_6$H$_5{}^{B}$), 112.1 (*C*Et$_2{}^{A}$ and *C*Et$_2{}^{B}$), 82.7 (*C*HPh$_2{}^{B}$), 81.9 (*C*HPh$_2{}^{A}$), 77.5, 77.5 (C-7^{A} and C-7^{B}), 76.0, 75.9 (C-6^{A} and C-6^{B}), 74.1 (C-4^{A}), 73.2 (C-4^{B}), 70.9 (C-5^{A} and C-5^{B}), 67.7, 67.6 (*C*H(CH$_3$)$_2{}^{A}$ and *C*H(CH$_3$)$_2{}^{B}$), 61.0, 60.9 (C-8^{A} and C-8^{B}), 36.0, 36.0 (C-2^{A} and C-2^{B}), 34.8, 34.5 (C-3^{A} and C-3^{B}), 29.6, 29.5, 28.5, 28.5 (2 × *C*H$_2$CH$_3{}^{A}$ and 2 × *C*H$_2$CH$_3{}^{B}$), 21.7, 21.6 (CH(*C*H$_3$)$_2{}^{A}$ and CH(*C*H$_3$)$_2{}^{B}$), 17.8 (2-CH$_3{}^{A}$), 17.7 (2-CH$_3{}^{B}$), 8.8, 7.9 (2 × CH$_2$*C*H$_3{}^{A}$ and 2 × CH$_2$*C*H$_3{}^{B}$); LRMS *m/z* (ESI$^+$): 1052 (100) [2M + Na]$^+$; HRMS *m/z* (ESI$^+$) found: 537.2823 [M + Na]$^+$; $C_{29}H_{42}NaO_7$ requires 537.2823.

iso-Propyl 4-_O_-benzhydryl-2,3-dideoxy-6,7-_O_-diethylketal-5,8-di-_O_-methanesulfonyl-D-_erythro_-D-_gluco_-octoate

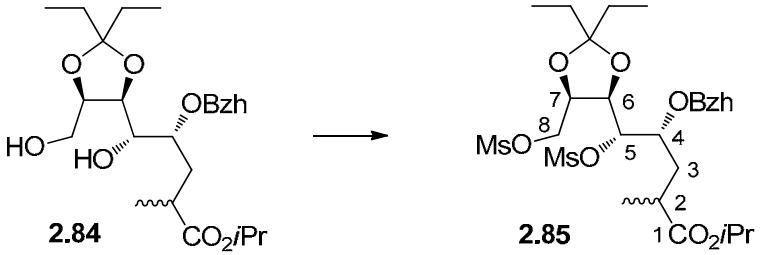


iso-Propyl 4-*O*-benzhydryl-2,3-dideoxy-6,7-*O*-diethylketal-D-*erythro*-D-*gluco*-octoate **2.112** (0.126 g, 0.251 mmol, 1 eq) was dissolved in anhydrous dichloromethane (20 mL) under an atmosphere of argon. Triethylamine (0.18 mL, 1.3 mmol, 5 eq) was added to the stirred solution and the mixture cooled to 0°C. Methanesulfonyl chloride (0.10 mL, 1.3 mmol, 5 eq) was slowly added such that the temperature did not exceed 5°C. The mixture left for 1 h, whereupon mass spectroscopy detected product (691: $[M + Cl]^-$) and no starting material. (The starting material and product co-spotted on t.l.c..) The mixture was allowed to warm to room temperature and concentrated *in vacuo*. The residue was partitioned between EtOAc (20 mL) and water (20 mL) and the aqueous layer extracted with EtOAc (3 × 20 mL). The organic fractions were combined and concentrated *in vacuo*, and the crude was purified by flash column chromatography on silica gel (4:1 → 2:1, cyclohexane:EtOAc) to afford *iso*-propyl 4-*O*-benzhydryl-2,3-dideoxy-6,7-*O*-diethylketal-5,8-di-*O*-methanesulfonyl-D-*erythro*-D-*gluco*-octoate **2.113** (0.134 g, 82%) as a white crystalline solid.

m.p. 112-114°C; $[\alpha]_D^{22}$ +32.6 (*c* 0.91, $CDCl_3$); IR ν_{max} (thin film): 3032 (m, Ar-H), 2978, 2939 (s, Ar-H), 2885 (m, Ar-H), 1725 (s, C=O), 1358, 1175 (s, O-SO_2); δ_H (400 MHz, $CDCl_3$): 7.39-7.24 (m, 10H; 2 × C_6H_5), 5.62 (s, 1H; C*H*Ph_2), 5.04 (dd, $J_{4,5}$ 3.3, $J_{5,6}$ 4.8, 1H; H-5), 4.94 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.57-4.49 (m, 1H; H-8), 4.36-4.26 (m, 2H; H-7 and H-8'), 4.17 (dd, $J_{5,6}$ 4.9, $J_{6,7}$ 5.7, 1H; H-6), 3.91 (a-dt, $J_{4,5}$ 3.1, $J_{3,4} \approx J_{3',4}$ 6.5, 1H; H-4), 3.12, 3.00 (2 × s, 2 × 3H; 2 × OSO_2CH_3), 2.32-2.24 (m, 2H; H-2 and H-2'), 2.03-1.94 (m, 2H; H-3 and H-3'), 1.75-1.67, 1.58-1.48 (2 × m, 2 × 2H; 2 × C*H*$_2$$CH_3$), 1.20 (d, J_{iPr} 6.3, 6H; CH(C*H*$_3$)$_2$), 0.93 (t, J_{Et} 7.5, 3H; CH_2C*H*$_3$), 0.83 (t, J_{Et} 7.6, 3H; CH_2C*H*$_3$); δ_C (100 MHz, $CDCl_3$): 172.4 (C-1), 141.4, 141.3 (2 × *ipso*-Ar), 128.5, 128.5, 128.0, 127.8, 127.5, 127.3 (10 × *o/m/p*-C_6H_5), 112.9 (*C*Et_2), 82.4 (*C*HPh_2), 78.9 (C-5), 75.6 (C-6), 75.3, 75.3 (C-4 and C-7), 70.0 (C-8), 68.0 (*C*H$(CH_3)_2$), 39.1,

37.5 (2 × OSO_2CH_3), 30.1 (CH_2CH_3), 29.6 (C-2), 27.9 (CH_2CH_3), 24.9 (C-3), 21.8, 21.8 ($CH(CH_3)_2$), 8.5, 8.5 (2 × CH_2CH_3); LRMS *m/z* (ESI^-): 691 (100) $[M + Cl]^-$; HRMS *m/z* (ESI^+) found 679.2211 $[M + Na]^+$; $C_{31}H_{42}NaO_{11}S_2$ requires 679.2217.

iso-Propyl 4-_O_-benzhydryl-2,3-dideoxy-6,7-_O_-diethylketal-5,8-di-_O_-methanesulfonyl-2-_C_-methyl-D-_erythro_-D-_gluco_-octoate

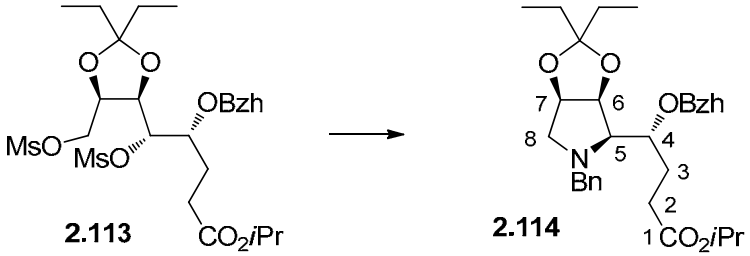


iso-Propyl 4-*O*-benzhydryl-2,3-dideoxy-6,7-*O*-diethylketal-2-*C*-methyl-D-*erythro*-D-*gluco*-octoate **2.84** (0.440 g, 0.854 mmol, 1 eq) was dissolved in anhydrous dichloromethane (20 mL) under an atmosphere of argon. Triethylamine (0.60 mL, 4.3 mmol, 5 eq) was added to the stirred solution and the mixture cooled to -5°C. Methanesulfonyl chloride (0.33 mL, 4.3 mmol, 5 eq) was slowly added such that the temperature did not exceed 0°C. After 3 h, mass spectroscopy detected product (693: $[M + Na]^+$) and no starting material. (The starting material and product co-spotted on t.l.c..) The mixture was allowed to warm to room temperature and concentrated *in vacuo*. The residue was partitioned between EtOAc (30 mL) and water (30 mL) and the aqueous layer extracted with EtOAc (2 × 20 mL). The organic fractions were combined and concentrated *in vacuo*, and the crude purified by flash column chromatography on silica gel (3:1, cyclohexane:EtOAc) to afford *iso*-propyl 4-*O*-benzhydryl-2,3-dideoxy-6,7-*O*-diethylketal-5,8-di-*O*-methanesulfonyl-2-*C*-methyl-D-*erythro*-D-*gluco*-octoate **2.85** (0.473 g, 100%) as a white crystalline solid in a 1:1 mixture of diastereomers.

m.p. 107-109°C; $[\alpha]_D^{25}$ +27.5 (*c* 0.69, $CHCl_3$); IR ν_{max} (thin film): 3032, 2977, 2941, 2883 (s, Ar-H), 1722 (s, C=O), 1358, 1177 (O-SO_2); δ_H (400 MHz, $CDCl_3$): 7.40-7.23 (m, 20H; 2 × $C_6H_5^{\mathbf{A}}$ and 2 × $C_6H_5^{\mathbf{B}}$), 5.62 (s, 1H; C*H*$Ph_2^{\mathbf{A}}$), 5.57 (s, 1H; C*H*$Ph_2^{\mathbf{B}}$), 5.10 (dd, $J_{4,5}$ 2.6, $J_{5,6}$ 3.9, 1H; H-5$^{\mathbf{A}}$), 4.99 (dd, $J_{4,5}$ 3.6, $J_{5,6}$ 5.1, 1H; H-5$^{\mathbf{B}}$), 4.98 (sept, J_{iPr} 6.2, 1H; C*H*$(CH_3)_2^{\mathbf{A}}$), 4.86 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2^{\mathbf{B}}$), 4.58 (dd, $J_{7,8}$ 2.1, $J_{8,8'}$ 9.7, 1H; H-8$^{\mathbf{A}}$), 4.50 (dd, $J_{7,8}$ 2.8, $J_{8,8'}$ 10.5, 1H; H-8$^{\mathbf{B}}$), 4.37 (ddd, $J_{7,8}$ 2.7, $J_{6,7}$ 6.0, $J_{7,8'}$ 7.8, 1H; H-7$^{\mathbf{B}}$), 4.34-4.23 (m, 4H; H-7$^{\mathbf{A}}$, H-8'$^{\mathbf{A}}$, H-8'$^{\mathbf{B}}$, and H-6$^{\mathbf{B}}$), 4.02 (dd, $J_{5,6}$ 4.1, $J_{6,7}$ 5.8, 1H; H-6$^{\mathbf{A}}$), 3.92 (ddd, $J_{4,5}$ 3.5, $J_{3,4}$ 4.4, $J_{3',4}$ 7.9, 1H; H-4$^{\mathbf{B}}$), 3.78 (ddd, $J_{4,5}$ 2.7, $J_{3,4}$ 5.0, $J_{3',4}$ 8.7, 1H; H-4$^{\mathbf{A}}$), 3.15 (s, 3H; $OSO_2CH_3^{\mathbf{B}}$), 3.10 (s, 3H; $OSO_2CH_3^{\mathbf{A}}$), 3.01 (s, 3H; $OSO_2CH_3^{\mathbf{B}}$), 3.00 (s, 3H; $OSO_2CH_3^{\mathbf{A}}$), 2.53-2.39 (m, 2H; H-2$^{\mathbf{A}}$ and H-2$^{\mathbf{B}}$), 2.11 (ddd, $J_{3,4}$ 4.7, $J_{2,3}$ 9.7, $J_{3,3'}$ 14.1, 1H; H-3$^{\mathbf{A}}$), 2.08 (ddd, $J_{3,4}$ 4.6, $J_{2,3}$ 9.3, $J_{3,3'}$ 14.0, 1H; H-3$^{\mathbf{B}}$), 1.81-1.61 (m, 6H; H-3'$^{\mathbf{A}}$, H-3'$^{\mathbf{B}}$, C*H*$_2$CH$_3^{\mathbf{A}}$, and C*H*$_2$CH$_3^{\mathbf{B}}$), 1.59-1.44 (m, 4H; C*H*$_2$CH$_3^{\mathbf{A}}$ and C*H*$_2$CH$_3^{\mathbf{B}}$), 1.22 (d, J_{iPr} 6.3, 3H; CH(C*H*$_3$)$_2^{\mathbf{A}}$), 1.18 (d, J_{iPr} 6.3, 3H; CH(C*H*$_3$)$_2^{\mathbf{B}}$), 1.16 (d, J_{iPr} 6.3, 6H; CH(C*H*$_3$)$_2^{\mathbf{A}}$ and CH(C*H*$_3$)$_2^{\mathbf{B}}$), 1.09 (d, $J_{2,Me}$ 7.2, 3H; 2-CH$_3^{\mathbf{B}}$), 1.04 (d, $J_{2,Me}$ 7.0, 3H; 2-CH$_3^{\mathbf{A}}$), 0.93, 0.92 (2 × t, J_{Et} 7.5, 6H; CH$_2$C*H*$_3^{\mathbf{A}}$ and CH$_2$C*H*$_3^{\mathbf{B}}$), 0.85 (t, J_{Et} 7.5, 3H; CH$_2$C*H*$_3^{\mathbf{B}}$), 0.80 (t, J_{Et} 7.4, 3H; CH$_2$C*H*$_3^{\mathbf{A}}$); δ_C (100 MHz, $CDCl_3$): 175.7 (C-1$^{\mathbf{B}}$), 175.3 (C-1$^{\mathbf{A}}$), 141.6, 141.5, 141.4, 141.1 (2 × *ipso*-Ar$^{\mathbf{A}}$ and 2 × *ipso*-Ar$^{\mathbf{B}}$), 128.5, 128.5, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.4, 127.3, 127.0 (10 × *o/m/p*-$C_6H_5^{\mathbf{A}}$ and 10 × *o/m/p*-$C_6H_5^{\mathbf{B}}$), 113.1 (*C*Et$_2^{\mathbf{A}}$), 112.7 (*C*Et$_2^{\mathbf{B}}$), 82.9 (*C*HPh$_2^{\mathbf{B}}$), 81.7 (*C*HPh$_2^{\mathbf{A}}$), 79.1 (C-5$^{\mathbf{A}}$), 78.6 (C-5$^{\mathbf{B}}$), 76.3 (C-6$^{\mathbf{A}}$), 75.4 (C-7$^{\mathbf{B}}$), 75.2, 75.0 (C-6$^{\mathbf{B}}$ and C-7$^{\mathbf{A}}$), 74.8 (C-4$^{\mathbf{B}}$), 74.3 (C-4$^{\mathbf{A}}$), 70.3 (C-8$^{\mathbf{A}}$), 69.7 (C-8$^{\mathbf{B}}$), 67.9 (*C*H$(CH_3)_2^{\mathbf{A}}$), 67.8 (*C*H$(CH_3)_2^{\mathbf{B}}$), 39.2, 39.1 (2 × $OSO_2CH_3^{\mathbf{B}}$), 37.5, 37.5 (2 × $OSO_2CH_3^{\mathbf{A}}$), 35.6, 35.5 (C-2$^{\mathbf{A}}$ and C-2$^{\mathbf{B}}$), 34.2 (C-3$^{\mathbf{B}}$), 32.9 (C-3$^{\mathbf{A}}$), 30.2, 30.0 (*C*H$_2$CH$_3^{\mathbf{A}}$ and *C*H$_2$CH$_3^{\mathbf{B}}$), 27.9, 27.7 (*C*H$_2$CH$_3^{\mathbf{A}}$ and *C*H$_2$CH$_3^{\mathbf{B}}$), 21.7, 21.6 (CH(*C*H$_3$)$_2^{\mathbf{A}}$ and CH(*C*H$_3$)$_2^{\mathbf{B}}$), 18.1 (2-CH$_3^{\mathbf{B}}$), 17.7 (2-CH$_3^{\mathbf{A}}$), 8.5, 8.5, 8.4,

8.4 (2 × $CH_2CH_3^{A}$ and 2 × $CH_2CH_3^{B}$); LRMS *m/z* (ESI^+): 688 (100) $[M + NH_4]^+$; HRMS *m/z* (ESI^+) found: 688.2813 $[M + NH_4]^+$; $C_{32}H_{50}NO_{11}S_2$ requires 688.2823.

***iso*-Propyl 4-*O*-benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octoate**

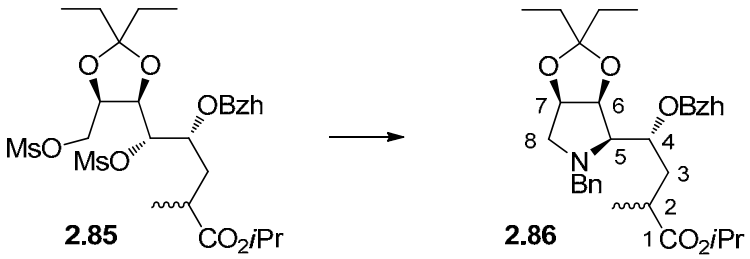


iso-Propyl 4-*O*-benzhydryl-2,3-dideoxy-6,7-*O*-diethylketal-5,8-di-*O*-methanesulfonyl-D-*erythro*-D-*gluco*-octoate **2.113** (0.132 g, 0.202 mmol) was dissolved in benzylamine (10 mL) under an atmosphere of argon, and the stirred solution was heated to reflux (≈155°C). After 25 minutes, t.l.c. (3:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.20) and formation of a major product (R_f 0.76). The solution was concentrated *in vacuo* and the excess benzylamine removed with azeotroping with *m*-xylene (5 × 5 mL). The crude was purified by flash column chromatography on silica gel (19:1 → 9:1, cyclohexane:EtOAc) to afford *iso*-propyl 4-*O*-benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octoate **2.114** (75 mg, 65%) as a colourless syrup.

$[\alpha]_D^{22}$ -65.1 (*c* 0.66, $CHCl_3$); IR ν_{max} (thin film): 3062, 3030 (w, Ar-H), 2976, 2939 (s, Ar-H), 2883 (w, Ar-H), 2793 (m, N-CH_2) 1728 (s, C=O); δ_H (400 MHz, $CDCl_3$): 7.43-7.21 (m, 15H; 3 × C_6H_5), 5.57 (s, 1H; C*H*Ph_2), 4.99 (sept, J_{iPr} 6.3, 1H; C*H*$(CH_3)_2$), 4.73 (d, J_{gem} 13.9, 1H; NC*H*H'Ph), 4.51 (dd, $J_{7,8}$ 5.1, $J_{6,7}$ 6.6, 1H; H-7), 4.42 (dd, $J_{5,6}$ 4.4, $J_{6,7}$ 6.4, 1H; H-6), 3.94 (dd, $J_{3,4}$ 2.3, $J_{3',4}$ 10.1, 1H; H-4), 3.05 (d, $J_{8,8'}$ 11.1, 1H; H-8'), 2.88 (d, J_{gem} 13.9, 1H; NCH*H*'Ph),

2.60-2.46 (m, 3H; H-2, H-2', and H-5), 2.33-2.14 (m, 2H; H-3 and H-3'), 1.87 (dd, $J_{7,8}$ 5.2, $J_{8,8'}$ 11.2, 1H; H-8), 1.84-1.70 (m, 2H; CH_2CH$_3$), 1.52 (q, J_{Et} 7.5, 2H; CH_2CH$_3$), 1.20 (dd, *J* 3.8, J_{iPr} 6.3, 6H; CH(CH_3)$_2$), 0.98 (t, J_{Et} 7.6, 3H; CH$_2$CH_3), 0.83 (t, J_{Et} 7.5, 3H; CH$_2$CH_3); δ_C (100 MHz, $CDCl_3$): 173.5 (C-1), 143.0, 142.1 (3 × *ipso*-Ar), 128.4, 128.2, 128.1, 127.7, 127.7, 127.2, 127.0, 126.7 (15 × *o/m/p*-C_6H_5), 115.6 (*C*Et$_2$), 82.0, 81.9 (*C*HPh$_2$ and C-6), 78.1 (C-7), 76.2 (C-4), 68.7 (C-5), 67.2 (*C*H(CH$_3$)$_2$), 60.1 (C-8), 59.1 (NCH_2Ph), 32.3 (C-2), 28.8, 28.3 (2 × *C*H$_2$CH$_3$), 27.5 (C-3), 21.8, 21.8 (CH(*C*H$_3$)$_2$), 8.5, 8.2 (2 × CH$_2$*C*H$_3$); LRMS *m/z* (ESI$^+$): 572 (100) $[M + H]^+$; HRMS *m/z* (ESI$^+$) found: 572.3381 $[M+H]^+$; $C_{36}H_{46}NO_5$ requires 572.3371.

iso-Propyl 4-_O_-benzhydryl-_N_-benzyl-6,7-_O_-diethylketal-5,8-imino-2-_C_-methyl-2,3,5,8-tetradeoxy-D-_erythro_-D-_manno_-octoate



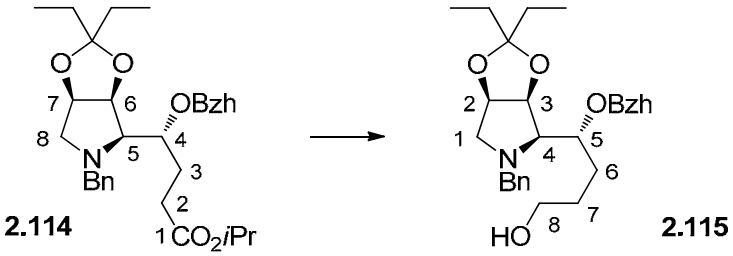
iso-Propyl 4-*O*-benzhydryl-2,3-dideoxy-6,7-*O*-diethylketal-5,8-di-*O*-methanesulfonyl-2-*C*-methyl-D-*erythro*-D-*gluco*-octoate **2.85** (0.580 g, 0.865 mmol, 1 eq) was dissolved in benzylamine (10 mL, 9.81 mmol, 11.3 eq) and the solution heated to reflux (≈155°C). The solution was stirred for 1 h, whereupon t.l.c. (3:1, cyclohexane:EtOAc) indicated complete consumption of starting material (R_f 0.20) and formation of a major product (R_f 0.78). The solution was concentrated *in vacuo* and excess benzylamine removed with azeotroping with *m*-xylene (5 × 5 mL). The crude was purified by flash column chromatography on silica gel (19:1, cyclohexane:EtOAc; re-columned in same to remove benzaldehyde) to afford *iso*-propyl 4-*O*-

benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2-*C*-methyl-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octoate **2.86** (0.376 g, 74%) (80% obtained from 0.19 g) as a colourless syrup in a 1:1 mixture of diastereomers.

$[\alpha]_D^{22}$ -9.9 (*c* 1.946, $CHCl_3$); IR ν_{max} (thin film): 2965, 2932 (Ar-H), 1726 (s, C=O), 1103 (s, C-O); δ_H (400 MHz, $CDCl_3$): 7.45-7.19 (m, 30H; 3 × $C_6H_5^{\mathbf{A}}$ and 3 × $C_6H_5^{\mathbf{B}}$) 5.64 (s, 1H; C*H*$Ph_2^{\mathbf{B}}$), 5.58 (s, 1H; C*H*$Ph_2^{\mathbf{A}}$), 4.97, 4.94 (2 × sept, J_{iPr} 6.3, 2H; C*H*$(CH_3)_2^{\mathbf{A}}$ and C*H*$(CH_3)_2^{\mathbf{B}}$), 4.76 (d, J_{gem} 14.3, 1H; NC*H*H'$Ph^{\mathbf{B}}$), 4.72 (d, J_{gem} 14.0, 1H; NC*H*H'$Ph^{\mathbf{A}}$), 4.52 (dd, $J_{7,8}$ 5.0, $J_{6,7}$ 6.3, 1H; H-$7^{\mathbf{A}}$), 4.50-4.42 (m, 2H; H-$7^{\mathbf{B}}$ and H-$6^{\mathbf{A}}$), 4.28 (dd, $J_{5,6}$ 4.4, $J_{6,7}$ 6.3, 1H; H-$6^{\mathbf{B}}$), 4.14 (dd, $J_{3',4}$ 2.0, $J_{3,4}$ 9.8, 1H; H-$4^{\mathbf{B}}$), 3.99 (dd, $J_{3',4}$ 2.2, $J_{3,4}$ 10.9, 1H; H-$4^{\mathbf{A}}$), 3.05 (d, $J_{8,8'}$ 11.1, 1H; H-$8'^{\mathbf{A}}$), 3.02 (d, $J_{8,8'}$ 10.9, 1H; H-$8'^{\mathbf{B}}$), 2.86 (d, J_{gem} 14.0, 1H; NCH*H*'$Ph^{\mathbf{B}}$), 2.85 (d, J_{gem} 13.8, 1H; NCH*H*'$Ph^{\mathbf{A}}$), 2.75-2.62 (m, 2H; H-$2^{\mathbf{B}}$ and H-$3^{\mathbf{B}}$), 2.61 (d, $J_{5,6}$ 4.3, 1H; H-$5^{\mathbf{A}}$), 2.58-2.43 (m, 2H; H-$2^{\mathbf{A}}$ and H-$3^{\mathbf{A}}$), 2.38 (d, $J_{5,6}$ 4.3, 1H; H-$5^{\mathbf{B}}$), 2.23 (ddd, $J_{3',4}$ 2.2, $J_{2,3'}$ 9.0, $J_{3,3'}$ 14.4, 1H; H-$3'^{\mathbf{A}}$), 1.98 (ddd, $J_{3',4}$ 2.6, $J_{2,3'}$ 10.3, $J_{3,3'}$ 13.0, 1H; H-$3'^{\mathbf{B}}$), 1.88 (dd, $J_{7,8}$ 5.0, $J_{8,8'}$ 11.1, 1H; H-$8^{\mathbf{A}}$), 1.84-1.70 (m, 5H; H-$8^{\mathbf{B}}$, C*H*$_2$$CH_3^{\mathbf{A}}$, and C*H*$_2$$CH_3^{\mathbf{B}}$), 1.53, 1.51 (2 × q, J_{Et} 7.5, 4H; C*H*$_2$$CH_3^{\mathbf{A}}$ and C*H*$_2$$CH_3^{\mathbf{B}}$), 1.21-1.12 (m, 15H; 2-$CH_3^{\mathbf{B}}$, CH(C*H*$_3$)$_2^{\mathbf{A}}$, and CH(C*H*$_3$)$_2^{\mathbf{B}}$), 1.02 (t, J_{Et} 7.4, 3H; CH_2C*H*$_3^{\mathbf{B}}$), 0.98 (t, J_{Et} 7.4, 3H; CH_2C*H*$_3^{\mathbf{A}}$), 0.87-0.81 (m, 9H; 2-$CH_3^{\mathbf{A}}$, CH_2C*H*$_3^{\mathbf{A}}$, and CH_2C*H*$_3^{\mathbf{B}}$); δ_C (100 MHz, $CDCl_3$): 176.9 (C-$1^{\mathbf{A}}$), 176.2 (C-$1^{\mathbf{B}}$), 143.2, 143.1, 142.5, 142.1, 139.8, 139.7 (3 × *ipso*-$Ar^{\mathbf{A}}$ and 3 × *ipso*-$Ar^{\mathbf{B}}$), 129.7, 129.0, 128.5, 128.3, 128.3, 128.2, 128.1, 127.8, 127.6, 127.4, 127.3, 127.2, 127.1, 126.9, 126.6 (15 × *o/m/p*-$C_6H_5^{\mathbf{A}}$ and 15 × *o/m/p*-$C_6H_5^{\mathbf{B}}$), 115.6, 115.5 (*C*$Et_2^{\mathbf{A}}$ and *C*$Et_2^{\mathbf{B}}$), 82.9 (*C*H$Ph_2^{\mathbf{B}}$), 82.0 (C-$7^{\mathbf{B}}$), 81.7 (C-$6^{\mathbf{B}}$), 81.5 (*C*H$Ph_2^{\mathbf{A}}$), 78.1 (C-$7^{\mathbf{A}}$), 78.0 (C-$6^{\mathbf{A}}$), 75.6 (C-$5^{\mathbf{B}}$), 73.8 (C-$5^{\mathbf{A}}$), 69.4 (C-$5^{\mathbf{B}}$), 68.1 (C-$5^{\mathbf{A}}$), 67.0, 67.0 (*C*H$(CH_3)_2^{\mathbf{A}}$ and *C*H$(CH_3)_2^{\mathbf{B}}$), 60.2, 60.0 (C-$8^{\mathbf{A}}$ and C-$8^{\mathbf{B}}$), 59.2, 59.2 (N*C*$H_2$$Ph^{\mathbf{A}}$ and N*C*$H_2$$Ph^{\mathbf{B}}$), 36.8 (C-$2^{\mathbf{A}}$), 36.6 (C-$3^{\mathbf{B}}$), 36.5 (C-$2^{\mathbf{B}}$), 35.1 (C-$3^{\mathbf{A}}$), 28.9 (C*H*$_2$$CH_3^{\mathbf{B}}$), 28.7 (C*H*$_2$$CH_3^{\mathbf{A}}$), 28.3 (C*H*$_2$$CH_3^{\mathbf{B}}$), 28.1 (C*H*$_2$$CH_3^{\mathbf{A}}$), 21.8,

21.7, 21.7, 21.6 ($CH(CH_3)_2^{A}$ and $CH(CH_3)_2^{B}$), 18.6 ($2\text{-}CH_3^{B}$), 16.1 ($2\text{-}CH_3^{A}$), 8.5, 8.5, 8.3, 8.2 ($2 \times CH_2CH_3^{A}$ and $2 \times CH_2CH_3^{B}$); LRMS *m/z* (ESI^+): 586 (100) $[M + H]^+$; HRMS *m/z* (ESI^+) found: 586.3505 $[M + H]^+$; $C_{37}H_{48}NO_5$ requires 586.3527.

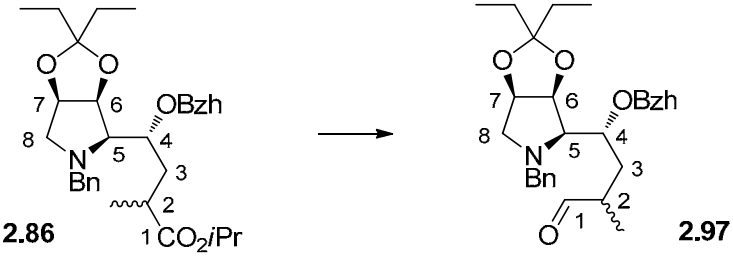
5-*O*-Benzhydryl-*N*-benzyl-2,3-*O*-diethylketal-1,4-imino-1,4,6,7-tetradeoxy-D-*mannono*-octitol



iso-Propyl 4-*O*-benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octoate **2.114** (73 mg, 0.129 mmol, 1 eq) was dissolved in anhydrous THF (5 mL) and the solution cooled to -20°C. Lithium aluminium hydride (2.0 M in THF, 0.13 mL, 0.26 mmol, 2 eq) was added to the stirred solution such that the temperature did not exceed -10°C. The mixture was stirred for 1.5 h, whereupon t.l.c. (3:1, cyclohexane:EtOAc) indicated incomplete consumption of starting material (R_f 0.63) and formation of a major product (R_f 0.33). Further lithium aluminium hydride (0.13 mL, 0.26 mmol, 2 eq) was added, and after a further 1.5 h the reaction had gone to completion. The reaction mixture was quenched with NH_4Cl (aq) (10 mL), stirred for 20 min, and concentrated *in vacuo*. The residue was partitioned between chloroform (25 mL) and water (25 mL) and the aqueous extracted with chloroform (3×25 mL). The organic fractions were combined and concentrated *in vacuo* and the crude purified by flash column chromatography on silica gel (3:1, cyclohexane:EtOAc) to afford 5-*O*-benzhydryl-*N*-benzyl-2,3-*O*-diethylketal-1,4-imino-1,4,6,7-tetradeoxy-D-*mannono*-octitol **2.115** (46 mg, 70 %) as a colourless syrup.

$[\alpha]_D^{25}$ +3.4 (*c* 1.00, $CHCl_3$); IR ν_{max} (thin film): 3450 (br, O-H), 3061, 3029 (w, Ar-H), 2974, 2937 (m, Ar-H), 2796 (m, N-CH_2); δ_H (400 MHz, $CDCl_3$): 7.43-7.18 (m, 15H; 3 × C_6H_5), 5.56 (s, 1H; C*H*Ph_2), 4.69 (d, J_{gem} 14.0, 1H; NC*H*H'Ph), 4.52 (dd, $J_{1,2}$ 5.3, $J_{2,3}$ 6.3, 1H; H-2), 4.45 (dd, $J_{3,4}$ 4.4, $J_{2,3}$ 6.4, 1H; H-3), 3.97 (a-br d, $J_{5,6}$ 9.2, 1H; H-5), 3.51-3.35 (m, 2H; H-8 and H-8'), 3.05 (d, $J_{1,1'}$ 11.3, 1H; H-1'), 2.90 (d, J_{gem} 14.0, 1H; NCH*H*'Ph), 2.59 (d, $J_{3,4}$ 4.3, 1H; H-4), 2.35 (a-ddt, $J_{6,7}$ 1.2, $J_{5,6} \approx J_{6,7'}$ 8.2, $J_{6,6'}$ 14.9, 1H; H-6), 1.97-1.84 (m, 1H; H-6'), 1.88 (d, $J_{1,2}$ 5.1, $J_{1,1'}$ 11.3, 1H; H-1), 1.84-1.67 (m, 1H; H-7), 1.76 (q, J_{Et} 7.4, 2H; C$H_2$$CH_3$), 1.53 (q, J_{Et} 7.5, 2H; C$H_2$$CH_3$), 1.57-1.41 (m, 1H; H-7'), 0.98 (t, J_{Et} 7.5, 3H; CH_2CH_3), 0.83 (t, J_{Et} 7.4, 3H; CH_2CH_3); δ_C (100 MHz, $CDCl_3$): 143.0, 142.2, 139.6 (3 × *ipso*-Ar), 128.4, 128.2, 128.2, 128.0, 127.9, 127.7, 127.2, 126.9, 126.7 (15 × *o/m/p*-C_6H_5), 115.6 (*C*Et_2), 82.1, 82.1 (*C*HPh_2 and C-3), 78.1 (C-2), 75.9 (C-5), 68.8 (C-4), 62.2 (C-8), 60.1 (C-1), 59.1 (N*C*H_2Ph), 29.2, 28.6, 28.1 (2 × *C*$H_2$$CH_3$ and C-7), 27.6 (C-6), 8.5, 8.2 (2 × CH_2*C*H_3); LRMS *m/z* (ESI^+): 516 (100) $[M + H]^+$; HRMS *m/z* (ESI^+) found: 538.2941 $[M + Na]^+$; $C_{33}H_{41}NNaO_4$ requires 538.2933.

4-*O*-Benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2-*C*-methyl-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octose



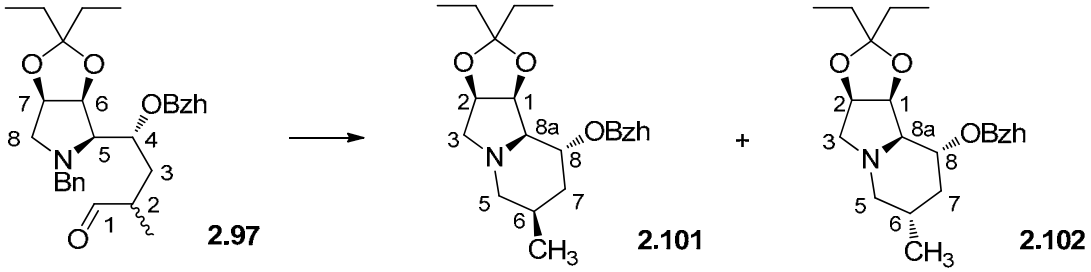
iso-Propyl 4-*O*-benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2-*C*-methyl-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octoate **2.86** (0.129 g, 0.220 mmol, 1 eq) was dissolved in anhydrous dichloromethane (10 mL). The stirred solution was cooled to -78°C and diisobutyl

aluminium hydride (1.5 M in toluene, 0.17 mL, 0.26 mmol, 1.1 eq) added dropwise such that the temperature did not exceed -70°C. After 2 h, mass spectroscopy detected product (528: $[M + H]^+$) and some starting material (586: $[M + H]^+$). The solution was quenched with potassium sodium tartrate solution and stirred vigorously for 2 h. The aqueous fraction was separated, extracted with dichloromethane (3 × 10 mL), and the organic fractions combined and concentrated *in vacuo*. The crude purified by flash column chromatography on silica gel (19:1, cyclohexane:EtOAc) to afford 4-*O*-benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2-*C*-methyl-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octose **2.97** (79 mg, 68%) (80% based on recovered starting material) as a colourless oil in a 1:1 mixture of diastereomers.

$[\alpha]_D^{25}$ +4.8 (*c* 0.79, $CHCl_3$); IR ν_{max} (thin film): 3087, 3064 (w, Ar-H), 3030 (m, Ar-H), 2971, 2937 (s, Ar-H), 2798 (m, N-CH_2), 1723 (s, C=O), 1101 (s, C-O); δ_H (400 MHz, $CDCl_3$): 9.61 (d, $J_{7,8}$ 1.7, 1H; H-1$^{\mathbf{A}}$), 9.44 (d, $J_{7,8}$ 2.1, 1H; H-1$^{\mathbf{B}}$), 7.41-7.21 (m, 30H; 3 × $C_6H_5^{\mathbf{A}}$ and 3 × $C_6H_5^{\mathbf{B}}$) 5.53 (a-br s, 2H; C*H*$Ph_2^{\mathbf{A}}$ and C*H*$Ph_2^{\mathbf{B}}$), 4.71, 4.68 (2 × d, J_{gem} 13.7, 2H; NC*H*H'Ph$^{\mathbf{A}}$ and NC*H*H'Ph$^{\mathbf{B}}$), 4.51 (dd, $J_{5,6}$ 5.0, $J_{6,7}$ 6.4, 1H; H-6$^{\mathbf{A}}$), 4.46 (ddd, $J_{7,8}$ 4.9, $J_{6,7}$ 6.4, $J_{7,8'}$ 8.7, 2H; H-7$^{\mathbf{A}}$ and H-7$^{\mathbf{B}}$), 4.35 (dd, $J_{5,6}$ 4.5, $J_{6,7}$ 6.4, 1H; H-6$^{\mathbf{B}}$), 4.03 (a-dt, $J_{3,4} \approx J_{4,5}$ 2.0, $J_{3',4}$ 10.5, 2H; H-4$^{\mathbf{A}}$ and H-4$^{\mathbf{B}}$), 3.05 (dd, $J_{7,8}$ 4.1, $J_{8,8'}$ 11.3, 2H; H-8$^{\mathbf{A}}$ and H-8$^{\mathbf{B}}$), 2.88 (d, J_{gem} 14.0, 2H; NCH*H*'Ph$^{\mathbf{A}}$ and NCH*H*'Ph$^{\mathbf{B}}$), 2.64 (ddd, $J_{3,4}$ 2.1, $J_{2,3}$ 9.4, $J_{3,3'}$ 14.9, 1H; H-3$^{\mathbf{A}}$), 2.60 (a-d, $J_{5,6}$ 4.3, 1H; H-5$^{\mathbf{A}}$), 2.50 (a-d, $J_{5,6}$ 4.4, 1H; H-5$^{\mathbf{B}}$), 2.53-2.44 (m, 1H; H-2$^{\mathbf{B}}$), 2.44-2.33 (m, 2H; H-3$^{\mathbf{B}}$ and H-2$^{\mathbf{A}}$), 2.13 (ddd, $J_{2,3'}$ 2.0, $J_{3',4}$ 10.0, $J_{3,3'}$ 16.0, 1H; H-3'$^{\mathbf{B}}$), 2.06 (ddd, $J_{2,3'}$ 3.8, $J_{3',4}$ 10.6, $J_{3,3'}$ 15.0, 1H; H-3'$^{\mathbf{A}}$), 1.86 (dd, $J_{7,8'}$ 5.0, $J_{8,8'}$ 11.2, H-8'$^{\mathbf{B}}$), 1.84 (dd, $J_{7,8'}$ 5.1, $J_{8,8'}$ 11.2, H-8'$^{\mathbf{A}}$), 1.81-1.63 (m, 4H; CH_2CH$_3^{\mathbf{A}}$ and CH_2CH$_3^{\mathbf{B}}$), 1.51 (q, J_{Et} 7.6, 2H; CH_2CH$_3^{\mathbf{B}}$), 1.49 (q, J_{Et} 7.5, 2H; CH_2CH$_3^{\mathbf{A}}$), 1.03 (d, $J_{2,Me}$ 7.0, 3H; 2-CH$_3^{\mathbf{B}}$), 0.96 (t, J_{Et} 7.5, 3H; CH$_2$C$H_3^{\mathbf{B}}$), 0.92 (t, J_{Et} 7.5, 3H; CH$_2$C$H_3^{\mathbf{A}}$), 0.82 (t, J_{Et} 7.4, 3H; CH$_2$C$H_3^{\mathbf{A}}$), 0.81 (t, J_{Et} 7.5, 3H; CH$_2$C$H_3^{\mathbf{B}}$), 0.78 (d, $J_{2,Me}$ 6.7, 3H; 2-CH$_3^{\mathbf{A}}$); δ_C (125 MHz,

$CDCl_3$): 205.6 (C-1^{A} and C-1^{B}), 142.7, 142.7, 141.8, 139.5, 139.4 (3 × *ipso*-Ar^{A} and 3 × *ipso*-Ar^{B}), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.3, 127.2, 127.1, 126.8 (15 × *o/m/p*-$C_6H_5^{A}$ and 15 × *o/m/p*-$C_6H_5^{B}$), 115.6, 115.5 (CEt_2^{A} and CEt_2^{B}), 82.3 ($CHPh_2^{A}$ and $CHPh_2^{B}$), 81.9 (C-7^{A} or C-7^{B}), 81.6 (C-6^{B}), 78.0 (C-6^{A}), 77.9 (C-7^{A} or C-7^{B}), 74.5, 74.2 (C-4^{A} and C-4^{B}), 68.5 (C-5^{B}), 68.0 (C-5^{A}), 59.9 (C-8^{A} and C-8^{B}), 59.2, 59.1 (NCH_2Ph^{A} and NCH_2Ph^{B}), 43.8 (C-2^{A}), 43.4 (C-2^{B}), 34.0 (C-3^{A}), 32.9 (C-3^{B}), 28.6 ($CH_2CH_3^{B}$), 27.8 ($CH_2CH_3^{A}$), 14.3 (2-CH_3^{B}), 12.9 (2-CH_3^{A}), 8.5, 8.5, 8.3 (2 × $CH_2CH_3^{A}$ and 2 × $CH_2CH_3^{B}$); LRMS *m/z* (ESI^+): 530 (100) $[M + H]^+$; HRMS *m/z* (ESI^+) found: 530.3099 $[M + H]^+$; $C_{34}H_{42}NO_4$ requires 530.3108.

8-*O*-Benzhydryl-1,2-*O*-diethylketal-(6*R*)-*C*-methyl-D-swainsonine and 8-*O*-benzhydryl-1,2-*O*-diethylketal-(6*S*)-*C*-methyl-D-swainsonine



4-*O*-Benzhydryl-*N*-benzyl-6,7-*O*-diethylketal-5,8-imino-2-*C*-methyl-2,3,5,8-tetradeoxy-D-*erythro*-D-*manno*-octose **2.97** (0.157 g, 0.297 mmol, 1 eq) was dissolved in methanol (15 mL) under an atmosphere of argon. 10% Pd/C (10% by mass, 14 mg) was added to the stirred solution. The reaction vessel was degassed and the reaction mixture placed under an atmosphere of hydrogen. After 22 h, mass spectroscopy detected product (422: $[M + H]^+$) and no starting material. t.l.c. (3:1, cyclohexane:EtOAc) indicated formation of one product (R_f 0.73) but the other product co-spotted with the starting material (R_f 0.68). The reaction mixture was filtered

through Celite®, the filtrate concentrated *in vacuo*, and the crude purified by flash column chromatography on silica gel (99:1 → 19:1, cyclohexane:EtOAc) to afford 8-*O*-benzhydryl-1,2-*O*-diethylketal-(6*R*)-*C*-methyl-D-swainsonine **2.101** (56 mg, 45%) and 8-*O*-benzhydryl-1,2-*O*-diethylketal-(6*S*)-*C*-methyl-D-swainsonine **2.102** (50 mg, 40%) both as colourless syrups.

Data for 8-*O*-benzyhydryl-1,2-*O*-diethylketal-(6*R*)-*C*-methyl-D-swainsonine **2.101**

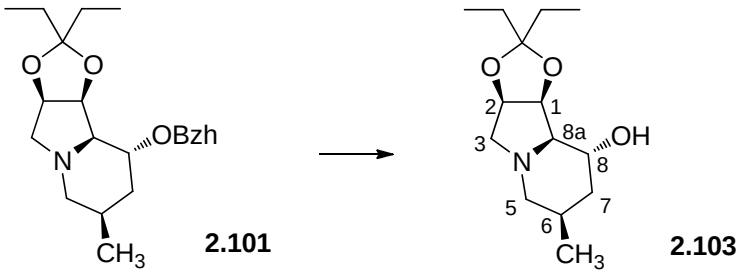
R_f (3:1, cyclohexane:EtOAc) 0.73; $[\alpha]_D^{25}$ -114.6 (*c* 0.78, $CHCl_3$); IR ν_{max} (thin film): 3088, 3063, 3029 (w, Ar-H), 2970, 2934 (s, Ar-H), 2782 (m, N-CH_2); δ_H (400 MHz, $CDCl_3$): 7.42-7.18 (m, 10H; 2 × C_6H_5), 5.80 (s, 1H; C*H*Ph_2), 4.71 (dd, $J_{1,8a}$ 3.9, $J_{1,2}$ 6.3, 1H; H-1), 4.59 (dd, $J_{2,3}$ 4.5, $J_{1,2}$ 6.3, 1H; H-2), 3.92 (ddd, $J_{7',8}$ 4.6, $J_{8,8a}$ 9.0, $J_{7,8}$ 11.4, 1H; H-8), 3.03 (d, $J_{3,3'}$ 10.4, 1H; H-3'), 2.65 (br d, $J_{5,5'}$ 10.5, 1H; H-5'), 2.04 (dd, $J_{2,3}$ 4.6, $J_{3,3'}$ 10.4, 1H; H-3), 1.99 (dd, $J_{5,6}$ 3.2, $J_{5,5'}$ 10.5, 1H; H-5), 1.97-1.89 (m, 1H; H-6), 1.84-1.77 (m, 1H; H-7), 1.73 (q, J_{Et} 7.5, 2H; C$H_2$$CH_3$), 1.70 (dd, $J_{1,8a}$ 4.0, $J_{8,8a}$ 9.1, 1H; H-8a), 1.62 (q, J_{Et} 7.5, 2H; C$H_2$$CH_3$), 1.42 (a-dt, $J_{7',8}$ 4.9, $J_{7,7'} \approx J_{7,8}$ 11.9, 1H; H-7'), 0.94 (t, J_{Et} 7.4, 3H; CH_2CH_3), 0.90 (d, $J_{6,Me}$ 7.2, 3H; 6-CH_3), 0.87 (t, J_{Et} 7.5, 3H; CH_2CH_3); δ_C (125 MHz, $CDCl_3$): 143.2, 142.9 (2 × *ipso*-Ar), 128.1, 128.0, 127.4, 127.2, 127.0 (10 × *o/m/p*-C_6H_5), 115.2 (*C*Et_2), 81.6 3 (*C*HPh_2), 80.1 (C-1), 78.4 (C-2), 73.4 (C-8a), 69.9 (C-8), 61.0 (C-3), 57.0 (C-5), 37.2 (C-7), 29.4, 29.2 (2 × *C*$H_2$$CH_3$), 29.1 (C-6), 18.8 (6-$CH_3$), 8.5, 7.9 (2 × CH_2*C*H_3); LRMS *m/z* (ESI^+): 422 (100) $[M + H]^+$; HRMS *m/z* (ESI^+) found: 422.2692 $[M + H]^+$; $C_{27}H_{35}NO_3$ requires 422.2690.

Data for 8-*O*-benzyhydryl-1,2-*O*-diethylketal-(6*S*)-*C*-methyl-D-swainsonine **2.102**

R_f (3:1, cyclohexane:EtOAc) 0.68; $[\alpha]_D^{25}$ -28.3 (*c* 0.90, $CHCl_3$); IR ν_{max} (thin film): 2968, 2933 (s, Ar-H), 2882 (m, Ar-H), 2784 (m, N-CH_2); δ_H (400 MHz, $CDCl_3$): 7.44-7.18 (m, 10H; 2 × C_6H_5), 5.84 (s, 1H; C*H*Ph_2), 4.72 (dd, $J_{1,8a}$ 4.0, $J_{1,2}$ 6.2, 1H; H-1), 4.59 (dd, $J_{2,3}$ 4.4, $J_{1,2}$ 6.3, 1H; H-2), 3.74 (ddd, $J_{7,8}$ 4.6, $J_{8,8a}$ 8.9, $J_{7',8}$ 10.9, 1H; H-8), 3.08 (d, $J_{3,3'}$ 10.4, 1H; H-3'), 2.86 (br dd,

$J_{5,6}$ 3.6, $J_{5,5'}$ 10.3, 1H; H-5), 2.05 (dd, $J_{2,3}$ 4.5, $J_{3,3'}$ 10.5, 1H; H-3), 1.98 (br a-dt, $J_{7,8} \approx J_{6,7}$ 4.2, $J_{7,7'}$ 12.4, 1H; H-7), 1.75-1.68 (m, 3H; H-8a and CH_2CH$_3$), 1.62 (q, J_{Et} 7.5, 2H; CH_2CH$_3$), 1.62-1.58 (m, 1H; H-6), 1.49 (d, $J_{5,5'}$ 10.5, 1H; H-5'), 1.04-0.97 (m, 1H; H-7'), 0.94 (t, J_{Et} 7.5, 3H; CH$_2$CH_3), 0.86 (t, J_{Et} 7.4, 3H; CH$_2$CH_3), 0.84 (d, $J_{6,Me}$ 6.7, 6-CH$_3$); δ_C (125 MHz, CDCl$_3$): 143.4, 142.9 (2 × *ipso*-Ar), 128.2, 128.1, 127.4, 127.2, 127.1, 127.0 (10 × *o/m/p*-C$_6$H$_5$), 115.2 (*C*Et$_2$), 81.4 (*C*HPh$_2$), 79.5 (C-1), 78.6 (C-2), 72.6 (C-8a), 72.4 (C-8), 60.5 (C-3), 59.2 (C-5), 40.0 (C-7), 30.1 (C-6) 29.1, 28.9 (2 × *C*H$_2$CH$_3$), 18.8 (7-CH$_3$), 8.6, 8.1 (2 × CH$_2$*C*H$_3$); LRMS *m/z* (ESI$^+$): 422 (100) [M + H]$^+$; HRMS *m/z* (ESI$^+$) found: 422.2697 [M + H]$^+$; $C_{27}H_{35}NO_3$ requires 422.2690.

1,2-*O*-Diethylketal-(6*R*)-*C*-methyl-D-swainsonine

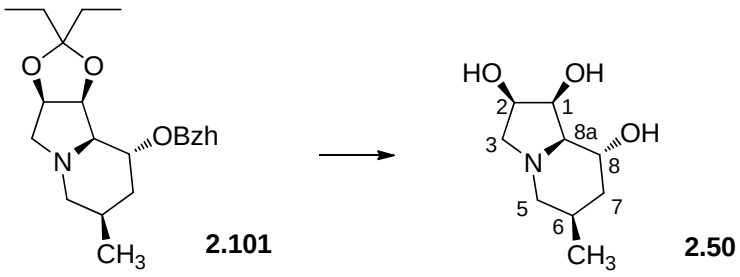


8-*O*-Benzhydryl-1,2-*O*-diethylketal-(6*R*)-*C*-methyl-D-swainsonine **2.101** (38 mg, 0.090 mmol) was dissolved in trifluoroacetic acid (33% *v/v* in water) (1.5 mL). The stirred solution was heated to 35°C and left for 18 h, whereupon t.l.c. indicated consumption of starting material (R_f 0.74) and formation of a single product (R_f 0.55). The solution was concentrated *in vacuo*, redissolved in EtOAc (20 mL), and washed with 1 M NaOH (aq) (15 mL). The phases were separated, the aqueous extracted with EtOAc (2 × 15 mL), and the organic fractions combined and concentrated *in vacuo*. The crude was purified by flash column chromatography on silica gel (19:1 → 9:1,

cyclohexane:EtOAc) to afford 1,2-*O*-diethylketal-(6*R*)-*C*-methyl-D-swainsonine **2.103** (23 mg, 95%) as a colourless syrup.

$[\alpha]_D^{25}$ -80.1 (*c* 1.15, $CHCl_3$); IR ν_{max} (thin film): 3384 (br, O-H); δ_H (400 MHz, $CDCl_3$): 4.62 (dd, $J_{1,8a}$ 4.1, $J_{1,2}$ 5.8, 1H; H-1), 4.58 (dd, $J_{2,3}$ 3.6, $J_{1,2}$ 6.0, 1H; H-2), 4.02 (ddd, $J_{7',8}$ 4.9, $J_{8,8a}$ 9.0, $J_{7,8}$ 11.9, 1H; H-8), 3.11 (br d, $J_{3,3'}$ 10.4, 1H; H-3'), 2.64 (br d, $J_{5,5'}$ 10.2, 1H; H-5'), 2.04 (dd, $J_{2,3}$ 3.8, $J_{3,3'}$ 10.4, 1H; H-3), 1.99 (dd, $J_{5,6}$ 3.2, $J_{5,5'}$ 10.2, 1H; H-5), 1.96-1.88 (m, 1H; H-6), 1.83-1.60 (m, 4H; H-7, H-8a, and CH_2CH_3), 1.69 (q, J_{Et} 7.3, 2H; CH_2CH_3), 1.42 (a-dt, $J_{7',8}$ 4.9, $J_{7,7'} \approx J_{7,8}$ 12.0, 1H; H-7'), 0.96 (t, J_{Et} 7.5, 3H; CH_2CH_3), 0.90 (d, $J_{6,Me}$ 6.9, 3H; 6-CH_3), 0.85 (t, J_{Et} 7.4, 3H; CH_2CH_3); δ_C (100 MHz, $CDCl_3$): 114.6 (CEt_2), 81.0 (C-1), 79.9 (C-2), 72.1 (C-8a), 67.5 (C-8), 61.0 (C-3), 56.9 (C-5), 40.1 (C-7), 29.3, 29.2, 29.0 (C-6 and 2 × CH_2CH_3), 18.8 (6-CH_3), 8.6, 7.8 (2 × CH_2CH_3); LRMS *m/z* (ESI^+): 256 (100) $[M + H]^+$; HRMS *m/z* (ESI^+) found: 278.1733 $[M + Na]^+$; $C_{14}H_{25}NNaO_3$ requires 278.1732.

(6*R*)-*C*-methyl-D-swainsonine



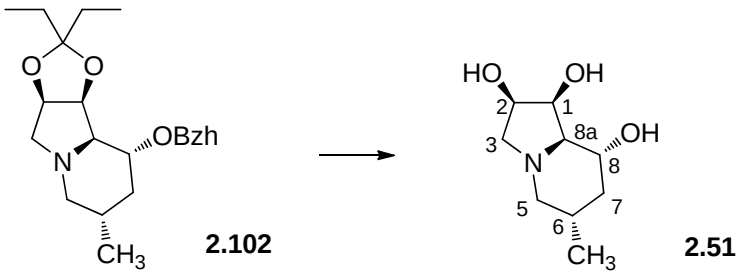
2.101 **2.50**

8-*O*-Benzhydryl-1,2-*O*-diethylketal-(6*R*)-*C*-methyl-D-swainsonine **2.101** (54 mg, 0.128 mmol) was dissolved in trifluoroacetic acid (90% *v/v* in water) (2 mL). The stirred solution was heated to reflux (≈100°C) and left for 5.5 h. The solution was concentrated *in vacuo* and the residue purified using ion exchange resin Dowex 50W X8-200 (H^+ form) and aqueous 2 M ammonium

hydroxide solution as an eluent to afford (6*R*)-*C*-methyl-D-swainsonine **2.50** (22 mg, 92%) as a white solid.

m.p. 148-150°C [Lit. for enantiomer:[166] m.p. 148-150°C]; $[\alpha]_D^{25}$ -39.4 (*c* 1.10, H_2O) [Lit. for enantiomer:[166] $[\alpha]_D^{21}$ +43.7 (*c* 1.72, H_2O)]; IR ν_{max} (thin film): 3342 (br, O-H); δ_H (400 MHz, D_2O, pH 9): 4.33 (ddd, $J_{2,3}$ 2.6, $J_{1,2}$ 5.8, $J_{2,3'}$ 8.2, 1H; H-2), 4.23 (dd, $J_{1,8a}$ 3.4, $J_{1,2}$ 5.8, 1H; H-1), 4.02 (ddd, $J_{7,8}$ 4.3, $J_{8,8a}$ 9.3, $J_{7',8}$ 10.5, 1H; H-8), 2.88 (br dd, $J_{2,3}$ 2.7, $J_{3,3'}$ 11.4, 1H; H-3), 2.78 (br dd, $J_{5,6}$ 1.0, $J_{5,5'}$ 11.3, 1H; H-5), 2.65 (br dd, $J_{2,3'}$ 8.4, $J_{3,3'}$ 11.0, 1H; H-3'), 2.28 (br dd, $J_{5',6}$ 3.6, $J_{5,5'}$ 11.4, 1H; H-5'), 2.11-2.00 (m, 2H; H-6 and H-8a), 1.84 (a-dt, $J_{6,7} \approx J_{7,8}$ 3.3, $J_{7,7'}$ 12.6, 1H; H-7), 1.43 (ddd, $J_{6,7'}$ 5.2, $J_{7',8}$ 11.0, $J_{7,7'}$ 12.5, 1H; H-7'), 1.00 (d, $J_{6,Me}$ 7.3, 3H; 6-CH_3); δ_C (100 MHz, D_2O, pH 9): 73.3 (C-8a), 69.9 (C-1), 69.3 (C-2), 63.4 (C-8), 61.3 (C-3), 58.0 (C-5), 38.0 (C-7), 28.3 (C-6), 18.9 (6-CH_3); LRMS *m/z* (ESI^+): 188 (100) $[M + H]^+$; LRMS *m/z* (ESI^-): 186 (100) $[M - H]^-$; HRMS *m/z* (ESI^+) found: 188.1285 $[M + H]^+$; $C_9H_{18}NO_3$ requires 188.1281.

(6*S*)-*C*-methyl-D-swainsonine



2.102 **2.51**

8-*O*-Benzhydryl-1,2-*O*-diethylketal-(6*S*)-*C*-methyl-D-swainsonine **2.102** (19 mg, 0.0453 mmol) was dissolved in trifluoroacetic acid (90% *v/v* in water) (1 mL). The stirred solution was heated to reflux (≈100°C) and left for 5.5 h. The solution was concentrated *in vacuo* and the residue purified using ion exchange resin Dowex 50W X8-200 (H^+ form) and aqueous 2 M ammonium

hydroxide solution as an eluent to afford (6*S*)-*C*-methyl-D-swainsonine **2.51** (8.4 mg, 98%) as a white crystalline solid.

m.p. 164°C (decomp.) [Lit. for enantiomer:[79] 166-167°C]; $[\alpha]_D^{25}$ -71.2 (*c* 1.25, H_2O) [Lit. for enantiomer:[79] $[\alpha]_D^{22}$ +77.5 (*c* 2.57, H_2O); IR ν_{max} (thin film): 3378 (br, O-H), 1202, 1134 (s, C-N); δ_H (400 MHz, D_2O, pH 9): 4.35 (ddd, $J_{2,3}$ 2.5, $J_{1,2}$ 6.1, $J_{2,3'}$ 8.1, 1H; H-2), 4.23 (dd, $J_{1,8a}$ 3.7, $J_{1,2}$ 6.1, 1H; H 1), 3.82 (ddd, $J_{7,8}$ 4.6, $J_{8,8a}$ 9.5, $J_{7',8}$ 11.1, 1H; H-8), 2.89-2.82 (m, 2H; H-3 and H-5), 2.53 (dd $J_{2,3'}$ 7.9, $J_{3,3'}$ 10.9, 1H; H-3'), 2.05 (ddd, $J_{6,7}$ 4.1, $J_{7,8}$ 4.2, $J_{7,7'}$ 12.3, 1H; H-7), 1.88 (dd, $J_{1,8a}$ 3.8, $J_{8,8a}$ 9.6, 1H; H-8a), 1.76-1.67 (m, 1H; H-6), 1.64 (a-t, $J_{5',6} \approx J_{5,5'}$ 10.8, 1H; H-5'), 0.96 (a-q, $J_{7',8} \approx J_{6,7'} \approx J_{7,7'}$ 11.8, 1H; H-7'), 0.89 (d, $J_{6,Me}$ 6.3, 3H; 6-CH_3); δ_C (125 MHz, D_2O, pH 9): 72.2 (C-8a), 69.2 (C-1), 69.0 (C-2), 65.7 (C-8), 60.1 (C-3), 58.7 (C-5), 41.0 (C-7), 29.1 (C-6), 18.6 (6-CH_3); LRMS *m/z* (ESI^+): 188 (100) $[M + H]^+$; LRMS *m/z* (ESI^-): 186 (100) $[M - H]^-$; HRMS *m/z* (ESI^+) found: 188.1286 $[M + H]^+$; $C_9H_{18}NO_3$ requires 188.1281.

Chapter 3

2-C-Methyl Fucose

3.1 Overview

This chapter describes the biological significance of the naturally occurring sugar L-fucose, and the use of a key lactone intermediate in the synthesis of the novel compound 2-*C*-methyl-L-fucose is described.

3.2 Introduction to fucose

L-(-)-Fucose **3.1-3.3** is a naturally-occurring hexose, equivalent to 6-deoxy-L-galactose.

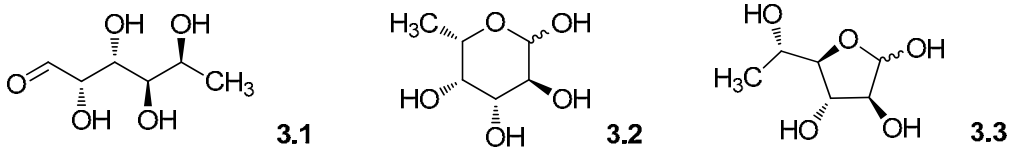


Figure 3.1 L-Fucose in its open chain, pyranose, and furanose forms.

Fucose is named after *Fucus vesiculus*, the seaweed from which the sugar was isolated. However, the sugar is also found in *N*-linked glycans on the cells of mammalian, insect and plants as well in marine algae.[168] Unusually for a mammalian sugar, the L-enantiomer of fucose is the naturally-occurring form. The vast majority of fucose is synthesised *de novo* (in its nucleotide-activated form, GDP-fucose) from GDP-mannose.[169-172]

3.2.1 Biological Role

This section will focus on the biological role of fucose in mammals, particularly the fucosylation of glycans. Fucosylation is usually a terminal modification to mammalian cell surface glycans,[173] which can alter the function of the oligosaccharides. The extent of glycan fucosylation is regulated during cellular differentiation and development of the organism (ontogeny),[174, 175] and alterations in the expression of these glycans is linked with pathological processes such an inflammation, atherosclerosis, and cancer (see also **Chapter 1, Section 1.2.2.1**).

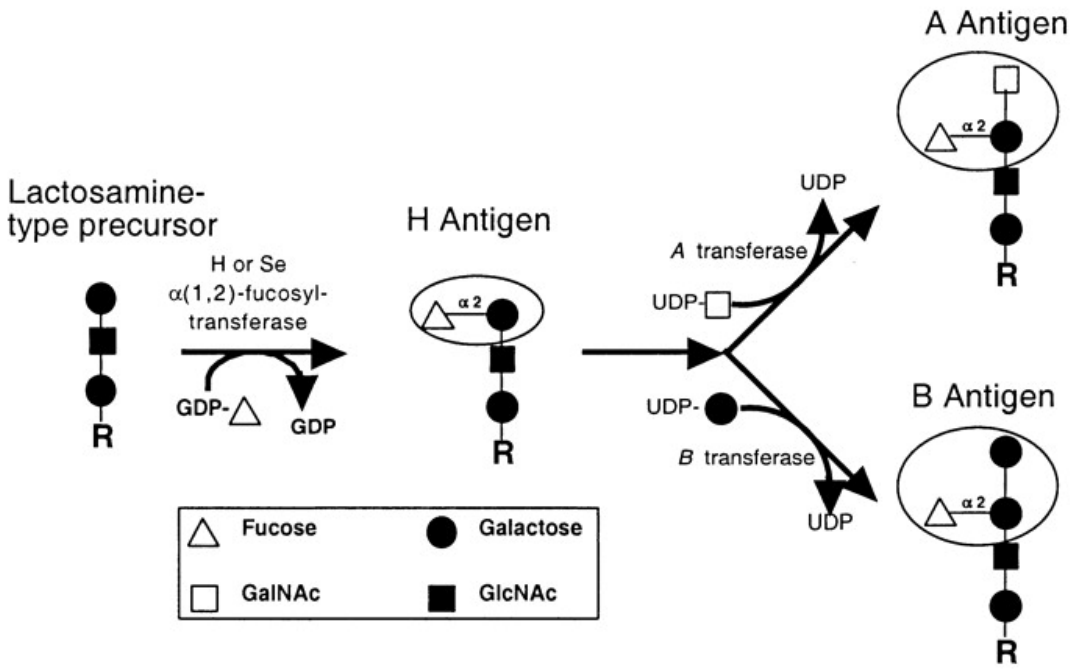


Figure 3.2 Biosynthesis of A and B blood group antigens from the fucosylated precursor H antigen. D. J. Becker and J. B. Lowe, Fucose: biosynthesis and biological function in mammals, *Glycobiology*, 2003, *13*, 41R-53R. Adapted with permission from Oxford University Press.

The A and B blood group antigens are the best-known examples of fucosylated glycans; generated from the H antigen precursor (**Figure 3.2**), they are strongly immunogenic, preventing successful blood transfusions between incompatible individuals.[176] They are also affect other

processes including blood clotting[177, 178] and host-microbe interactions; for example, individuals with type O blood have a higher risk of peptic ulcers, even though type A and B individuals carry the causative bacteria *Helicobacter pylori* just as frequently.[179] Binding of *H. pylori* to the gastric epithelium is mediated by the ABO blood group glycans, and those associated with the O blood group are accessible to all strains of the bacterium.[180, 181]

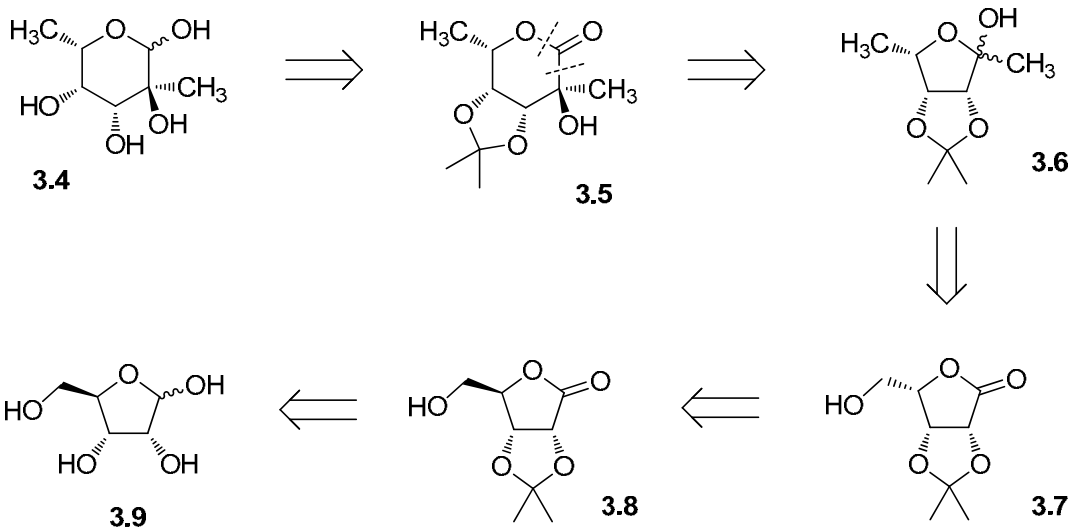
Fucose is also an essential component of the carbohydrate ligands for the selectins, a family of cell adhesion receptors.[182, 183] The interaction between selectins and their ligands is a vital step in the pathway allowing leukocytes to reach the site of infection (extravasion).[184]

3.3 Synthetic target

There is synthetic interest in 2-*C*-methyl branched sugars. Their density of stereocentres means they can be chirons for the enantiospecific synthesis of complex targets.[185-188] This class of compounds may also have chemotherapeutic potential,[189, 190] for example in the treatment of Type 2 diabetes; another application is in the field of novel foodstuffs including low-calorie sweeteners.[191] 2-*C*-Methyl branched sugars are also synthetic precursors to 2'-*C*-methyl nucleosides, a several of which have demonstrated activity against Hepatitis C.[192-194] A retrosynthesis of the 2-*C*-methyl derivative of fucose was devised (**Scheme 3.1**).

3.3.1 Retrosynthesis

2-*C*-Methyl-L-fucose **3.4** could be derived from the protected lactone **3.5**. This 6-carbon lactone could be made from the pentose **3.6** *via* a Kiliani extension. The pentose could be made from the protected L-lyxonolactone **3.7**, which can in turn be made from D-ribose **3.9** *via* a known procedure.[195]

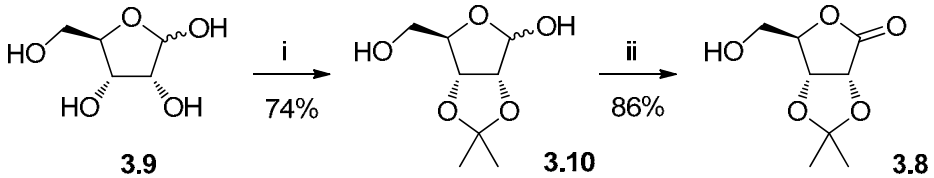


Scheme 3.1 A retrosynthesis of 2-*C*-methyl-L-fucose from D-ribose.

The lactone **3.5** from the Kiliani extension is also a potential intermediate in the synthesis of some branched iminosugars, with the nitrogen being introduced by azide displacement of a triflate leaving group.

3.4 Synthetic route to 2-C-methyl fucose

3.4.1 Protection and oxidation of ribose



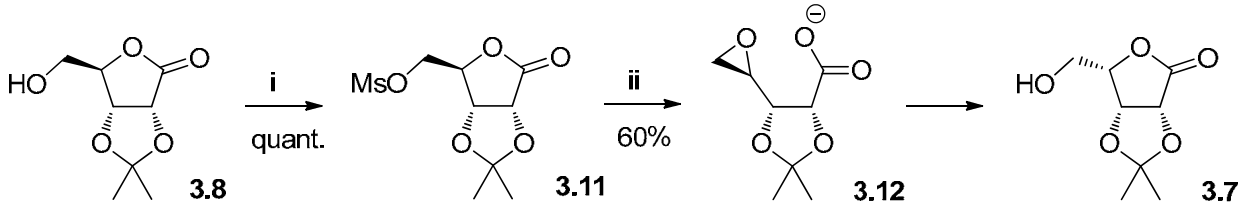
Reagents and conditions: (i) acetone, $CuSO_4$; (ii) Br_2, $BaCO_3$, H_2O, 0°C.

Scheme 3.2 Synthesis of isopropylidene ribonolactone.

Isopropylidene protection of D-ribose **3.9** (**Scheme 3.2**) gave the 2,3-protected product **3.10** in 74% yield, along with small amounts of differently-protected products. The isopropylidene

ribose **3.10** was oxidised to the lactone **3.8** using bromine, for which yield of 86% was obtained. A high infrared absorption value (1775 cm^{-1}) indicated that the product was the 1,4- rather than the 1,5-lactone.

3.4.2 Inversion at C-4



Reagents and conditions: (i) MsCl, Et_3N, DCM, 0°C; (ii) 2 M NaOH (aq), 1,4-dioxane; then 2 M HCl.

Scheme 3.3 Base-catalysed inversion of configuration.

Conversion of the isopropylidene D-ribonolactone **3.8** to the corresponding L-*lyxo* compound **3.7** (**Scheme 3.3**) is a known procedure that can be performed on a large scale.[195] The ribonolactone was mesylated in quantitative yield, and the mesylate **3.11** treated with alkali. This led to formation of an epoxide intermediate **3.12**, which was not isolated. An acidic workup resulted in closure of the lactone with inversion at C-4. The modest yield of 60% was similar to results in the literature, and was not improved by varying the reaction conditions (*e.g.* concentration, reaction time, type of acid used in the workup). In this case, no other side products were observed that would account for the lost material; however, deprotection of the acetonide group has previously been observed as a side reaction.[196]

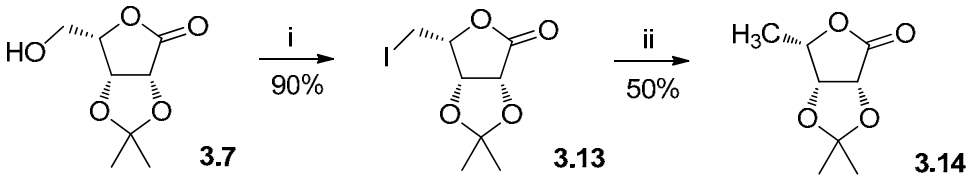
3.4.3 Synthesis of Kiliani precursor

The next step was to convert the hydroxymethyl group to a methyl group. Common strategies for this transformation include: (a) conversion to a halide followed by radical chemistry, usually involving tributyl tin hydride;[197-199] (b) activation of the alcohol (*e.g.* as a mesylate or tosylate)

and subsequent reduction using a metal hydride;[200, 201] and (c) conversion to a halide *via* an oxophosphonium intermediate, followed by hydrogenation.[202-204]

In this case, the proton at the 2-position would be relatively acidic, so the use of metal hydrides such as lithium aluminium hydride was not the preferred approach. Although tributyl tin hydride is an effective reducing agent, it is highly toxic and there are concerns about its effect on the environment.[205] Additionally, products of such reactions can be contaminated with trace organo-tin compounds that are very difficult to remove. It was therefore decided to proceed *via* the halide hydrogenation route.

The alcohol **3.7** was converted to the iodide **3.13** in an Appel reaction (**Scheme 3.4**), and the iodide was then hydrogenated to give the deoxy sugar **3.14**.



Reagents and conditions: (i) PhP_3, imid., I_2, toluene, 85°C; (ii) Pd/C, H_2, NaOAc, EtOH.

Scheme 3.4 Removal of the hydroxyl group *via* iodination and hydrogenation.

The iodination was successful, but only low yields of around 50% were obtained for the hydrogenation step. The reaction was repeated under different conditions (**Table 3.1**, overleaf), but little improvement was seen.

The main reason for the low yield is thought to be the acidity of H-2. The reaction mixture included a base to quench the hydrogen iodide byproduct: without a base (Entry 6), degradation occurred and the resulting complex mixture of products could not be purified to obtain any of the target compound. Unfortunately, as well as neutralising hydrogen iodide, the base could lead to

side reactions *via* removal H-2 or opening of the lactone, leading to decomposition of the molecule.

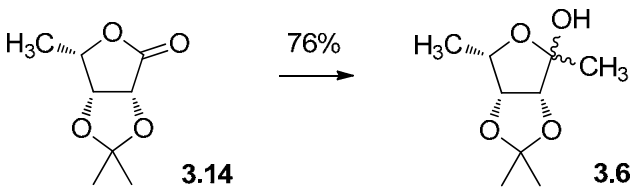
Entry	Base	Catalyst	Workup	Yield
1	Et_3N (1.2 eq)	Pd/C (10% by mass)	$Na_2S_2O_3$ (aq)	45%
2	Et_3N (1.2 eq)	Pd/C (10% by mass)	None	42%
3	Et_3N (1.2 eq)	$Pd(OH)_2$/C (10% by mass)	$Na_2S_2O_3$ (aq)	44%
4	NaOAc (1.2 eq)	Pd/C (10% by mass)	$Na_2S_2O_3$ (aq)	50%
5	NaOAc (1.2 eq)	Pd/C (10% by mass)	None	44%
6	None	Pd/C (10% by mass)	$Na_2S_2O_3$ (aq)	None isolated

Table 3.1 Yields obtained under different reaction conditions.

A review of the literature shows certain patterns in the yields for this type of hydrogenation. Better results are obtained for lactones that do not have an acidic proton.[206, 207] Reactions on substrates that resemble **3.13** except with the iodomethyl branch at C-2 rather than C-4 (*i.e.* they have no H-2) proceeded in very good yields.[188, 208, 209] Reactions on sugar substrates that are lactols rather than lactones also tend to give better yields.[210, 211] Another factor contributing to the disappointing yield in this case could be the volatility of the methyl product.

Enough material was obtained *via* the hydrogenation of the iodomethyl compound **3.13**, but if larger amounts were required, then radical chemistry (perhaps using a silyl radical rather than tin) could be employed.

Finally, the carbon chain of the lactone **3.14** was extended by treatment with methyl magnesium bromide (**Scheme 3.6**). The lactol **3.6** was obtained in 76% yield as a mixture of anomers.

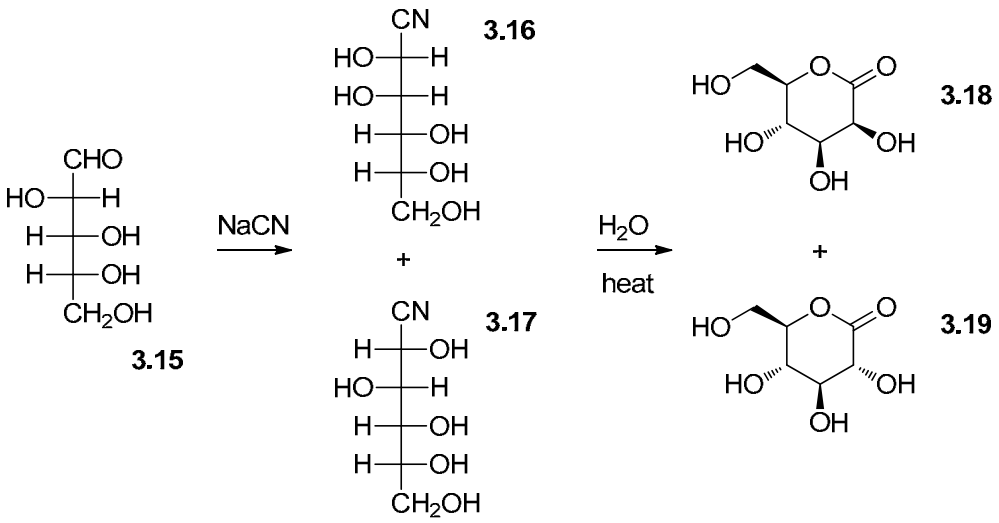


Reagents and conditions: MeMgBr, THF, -78°C.

Scheme 3.5 Conversion of lactone to methyl hemiketal.

3.4.4 Kiliani ascension

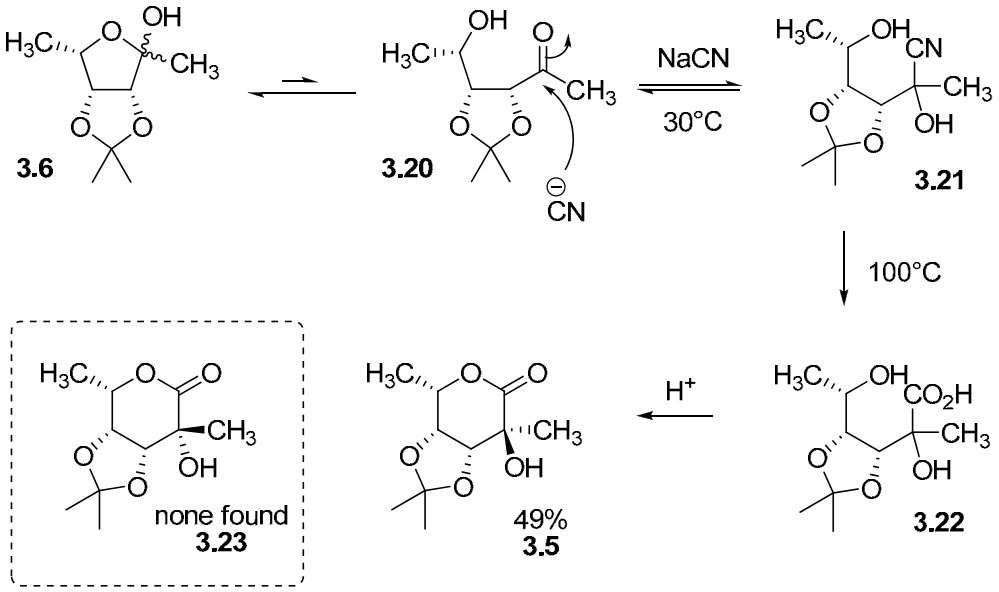
The Kiliani reaction[212-214] is a useful method for extending aldoses and ketoses to make higher-carbon sugar lactones. As an example (**Scheme 3.6**), when D-arabinose **3.15** is treated with sodium cyanide, a mixture of cyanohydrins is formed. Upon heating, the cyanohydrins are hydrolysed to give a mixture of D-mannonolactone **3.18** and D-gluconolactone **3.19**.[215]



Scheme 3.6 Example of the Kiliani reaction: D-arabinose to D-gluconolactone and D-mannonolactone.

This reaction requires the use of a toxic reagent and often gives low yields for protected sugars; however, it is extremely useful in allowing access to novel saccharide structures that may not be possible to make in other ways.

The lactol **3.6** was stirred with sodium cyanide at room temperature to give the cyanohydrin **3.21**; heating the solution to reflux, followed by an acidic workup and column chromatography, yielded the crystalline lactone **3.5** (**Scheme 3.7**).



Reagents and conditions: NaCN, H_2O, 30°C, 41 h; then 100°C, 48 h; then AcCl, MeOH, 5 Å molecular sieves.

Scheme 3.7 Kiliani reaction.

The cyanohydrin **3.21** and open-chain carboxylic acid **3.22** intermediates were detected by mass spectroscopy; however, studies have detected other intermediates (*e.g.* cyclic imidates; open chain amides; amidines) in the Kiliani reaction on D-arabinose.[215, 216] A workup of acetyl chloride in anhydrous methanol had been found to give a cleaner crude reaction mixture compared to the use of Amberlite resin;[217] this was found to be the case with the acetyl chloride workup giving only one product by t.l.c. prior to purification. The 2-*C*-methyl lactone **3.5** was obtained in 49% yield.

3.4.4.1 Structure of Kiliani product

The structure of the Kiliani product was confirmed by X-ray crystallographic analysis (**Figure 3.3**).[218] The molecule adopts a boat conformation in which the 2-hydroxyl group, rather than the methyl group, is in the flagpole position; this is consistent with results for other 3,4-*O*-isopropylidene-1,5-lactones.[219-221]

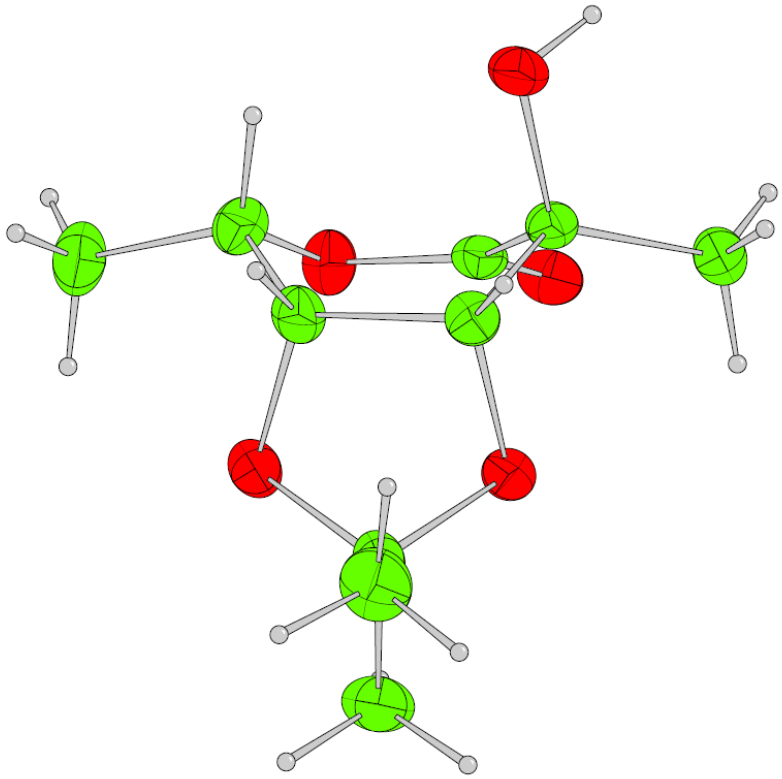


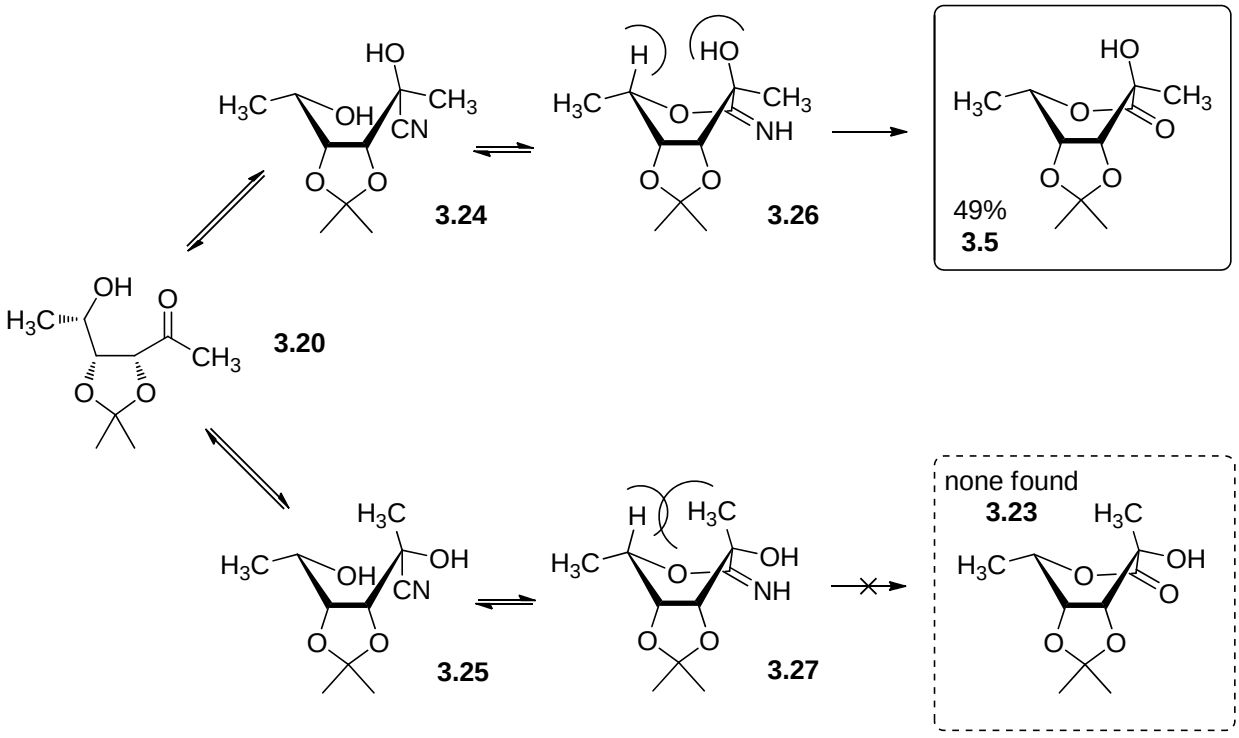
Figure 3.3 Structure of intermediate, obtained by X-ray diffraction. Displacement ellipsoids are drawn at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

3.4.4.2 Rationalisation of stereoselectivity

In general Kiliani reactions give a mixture of two products, as cyanide can attack either face of the carbonyl; however in this case only a single compound was obtained. This diastereoselectivity is opposite to that predicted by the Felkin-Anh model,[222] and can be

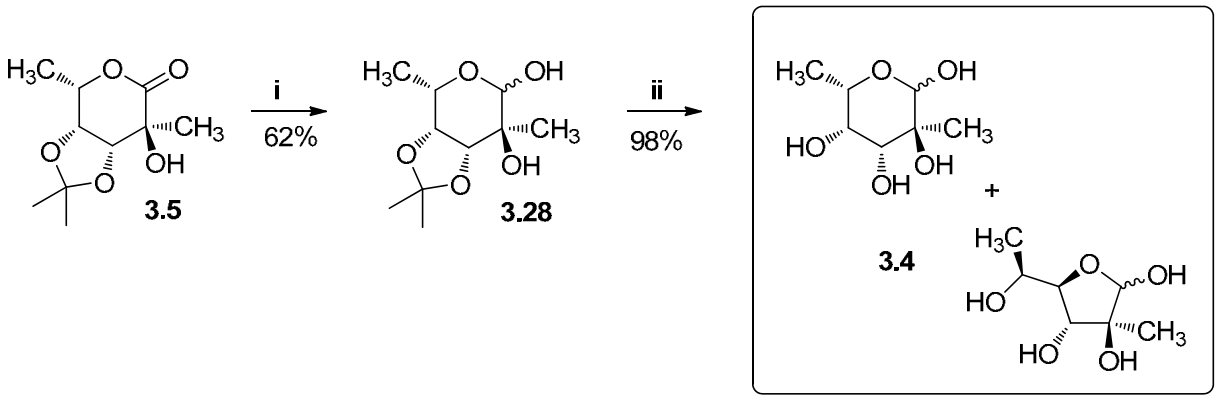
explained in various ways. One explanation is that the product that is formed has less steric congestion, with the smaller hydroxyl group rather than the methyl group in the flagpole position, compared to its C-2 epimer.[223-226]

Another possible explanation is that the formation of an imidate intermediate is the rate-determining step (**Scheme 3.8**), and the more stable intermediate is that with less steric repulsion between the flagpole substituents (**3.26** rather than **3.27**). This contrasts with the Felkin-Anh model, in which the initial nucleophilic attack on the carbonyl group is rate-determining.



Scheme 3.8 Possible mechanism for diastereoselectivity: selective imidate formation.

3.4.5 Reduction and deprotection of the lactone



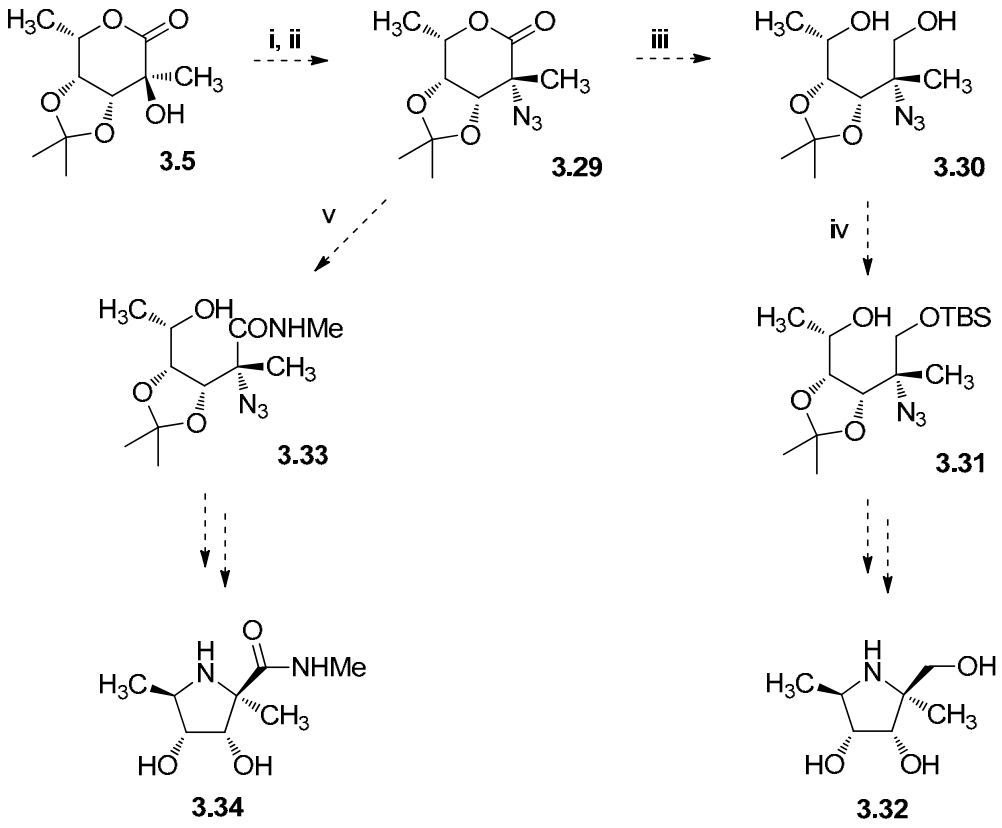
Reagents and conditions: (i) DIBAL-H, DCM, -78°C; (ii) Dowex® (50W X8 H^+ form), 1:1 H_2O:1,4-dioxane.

Scheme 3.9 Reduction and deprotection of the lactone to give 2-*C*-methyl-L-fucose.

The lactone **3.5** formed by the Kiliani reaction was reduced to the lactol **3.28** in 62% yield using DIBAL-H, and the deprotection gave 2-*C*-methyl-L-fucose **3.4** in 98% yield (**Scheme 3.9**). The product was obtained as a mixture of pyranose and furanose rings. The overall yield from D-ribose **3.9** was only 4% over ten steps; certain low-yielding steps such as hydrogenation of the iodide **3.13** and the Kiliani reaction contributed to the low efficiency of this route.

3.5 Other reactions of lactone

The lactone product of the Kiliani reaction was identified as a potential precursor for the synthesis of di-methyl-branched pyrrolidine iminosugars (**Scheme 3.10**).

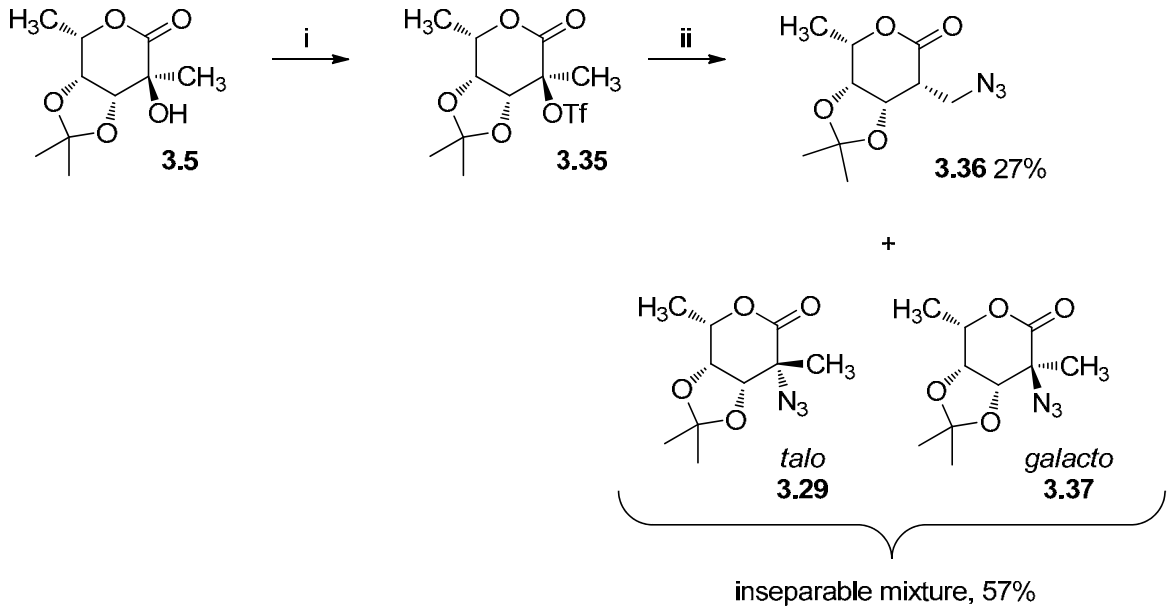


Selected reagents and conditions: (i) Tf_2O, py., DCM, -15°C; (ii) NaN_3, DMF; (iii) $NaBH_4$, MeOH, 0°C; (iv) TBSCl, imid., DMF; (v) $MeNH_2$, 1,4-dioxane.

Scheme 3.10 A route to methyl-branched iminosugars was envisaged.

It was decided to introduce the azide group to the lactone **3.5** *via* displacement of triflate at C-2. Previous work on a similar system[227, 80] suggested that this would occur with inversion of configuration to give the L-*talo* compound **3.29** shown. The resulting azide could then be treated in different ways: reduction of the lactone followed by selective silylation would give compound **3.31**; treatment of the lactone with methylamine would give the open-chain compound **3.33**. For both of these intermediates, mesylation, hydrogenation of the azide and deprotection would give a methyl-branched pyrrolidine iminosugar (compounds **3.32** and **3.34**).

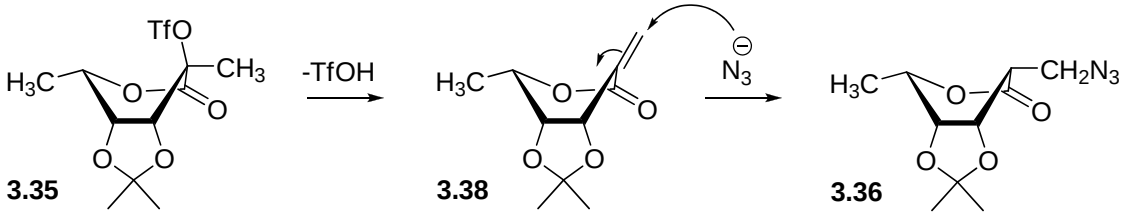
3.5.1 Triflate-azide displacement



Reagents and conditions: (i) Tf_2O, py., DCM, -15°C→RT; (ii) NaN_3, DMF.

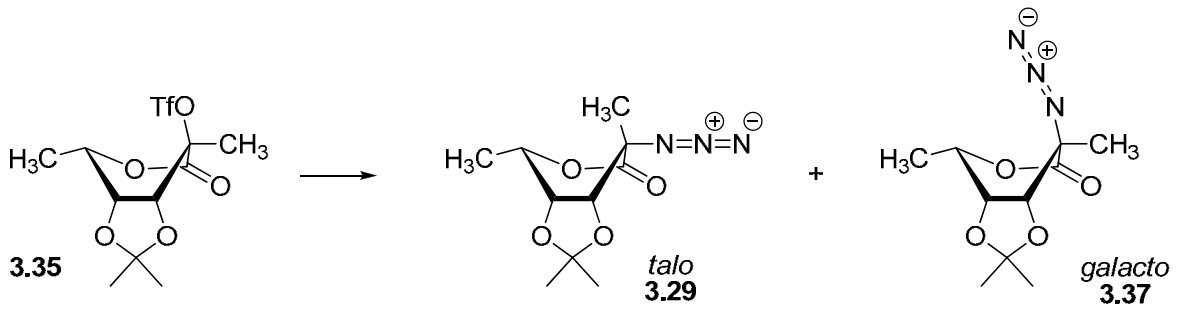
Scheme 3.11 Triflation and treatment with azide leading to three products.

The triflate **3.35** was made and reacted without prior purification (**Scheme 3.11**). After treatment with sodium azide, three products were formed. The unwanted primary azide **3.36** arose from elimination of triflic acid followed by Michael addition to the resulting unsaturated lactone **3.38** (**Scheme 3.12**). The initial elimination is catalysed by azide or by the DMF solvent.



Scheme 3.12 Formation of azidomethyl compound by elimination-addition.

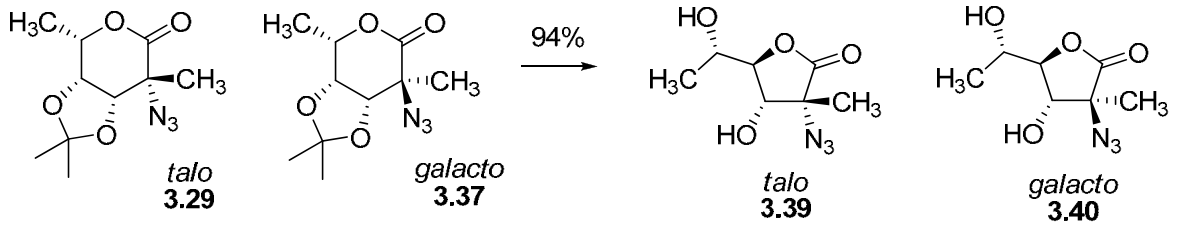
Two inseparable tertiary azides **3.29** and **3.37** were formed. The L-*talo* azide **3.29** resulted from S_N2 displacement of triflate with inversion. The L-*galacto* epimer was also obtained (**Scheme 3.13**).



Scheme 3.13 Formation of two tertiary azides.

The azides were obtained in a 2:1 ratio. It was not possible to definitively assign their stereochemistries by nOe, because the methyl groups at the 2-position and in the acetonide protecting group could not all be differentiated. However, the L-*talo* azide **3.29**, arising from inversion of configuration, is thought to be the major product.

This lack of selectivity was disappointing. The mixture of azides was deprotected in the hope that the products, the 1,4-lactones, could be separated and reacted further (**Scheme 3.14**). Unfortunately the diols **3.40** and **3.41** also proved to be inseparable. Due to the difficulties foreseen in making enough material and finding an opportunity to separate the epimers, this synthetic route was not pursued further.

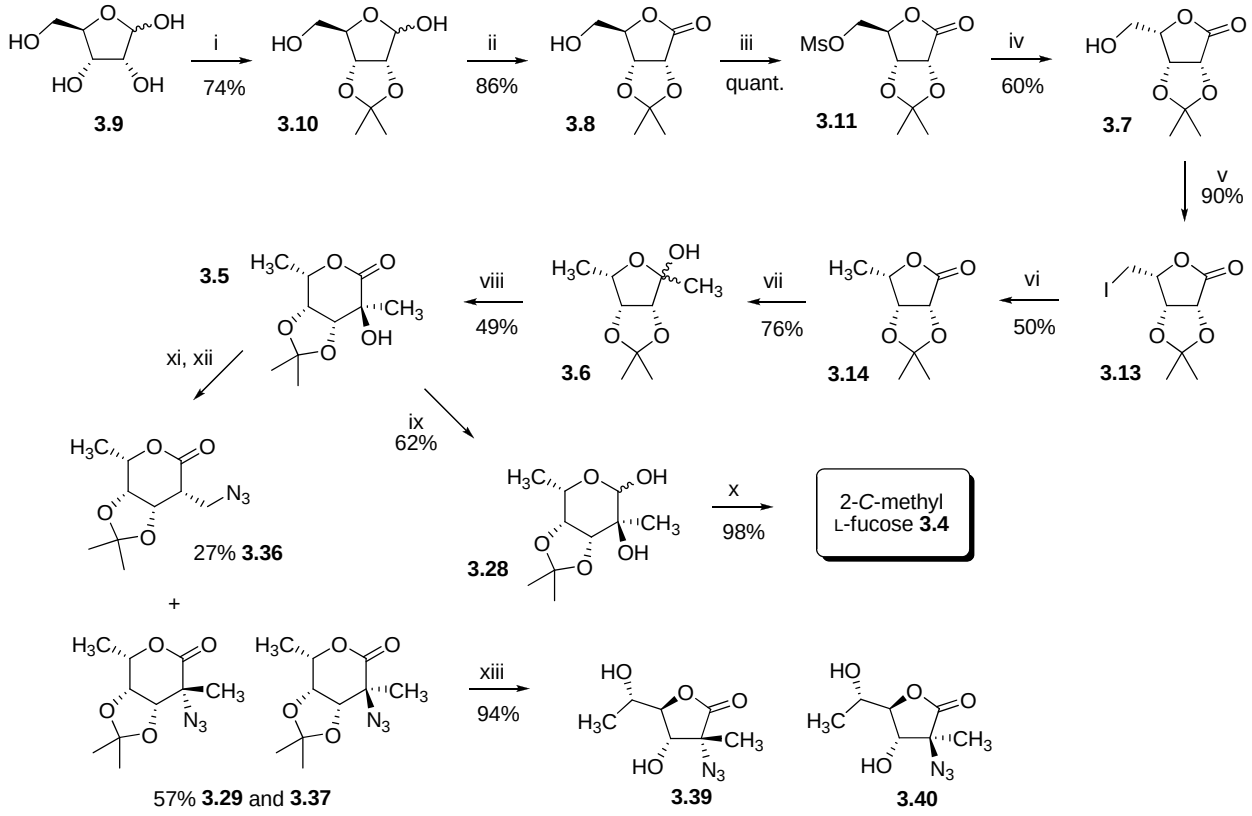


Reagents and conditions: H_2O:1,4-dioxane:TFA, 1:1:4.

Scheme 3.14 Isopropylidene deprotection of azide mixture.

3.6 Conclusion and Future Work

This chapter has described the synthesis of the novel branched sugar 2-*C*-methyl-L-fucose in ten steps, utilising a Kiliani reaction to create a new quaternary stereocentre. An attempt to access di-methyl-branched iminosugars *via* this intermediate is also described; however, due to the low stereoselectivity of the triflate-azide displacement, these iminosugars must be synthesised *via* alternative routes.



Reagents and conditions: (i) acetone, $CuSO_4$; (ii) Br_2, $BaCO_3$, H_2O, 0°C; (iii) MsCl, Et_3N, DCM, 0°C; (iv) 2 M NaOH (aq), 1,4-dioxane; then 2 M HCl; (v) PhP_3, imid., I_2, toluene, 85°C; (vi) Pd/C, H_2, NaOAc, EtOH; (vii) MeMgBr, THF, -78°C; (viii) NaCN, H_2O, 30°C, 41 h; then 100°C, 48 h; then AcCl, MeOH, 5 Å molecular sieves; (ix) DIBAL-H, DCM, -78°C; (ii) Dowex®, 1:1 H_2O:1,4-dioxane; (xi) Tf_2O, py., DCM, -15°C→RT; (xii) NaN_3, DMF; (xiii) H_2O:1,4-dioxane:TFA, 1:1:4.

Scheme 3.15 Synthetic route to 2-*C*-methyl-L-fucose and azides.

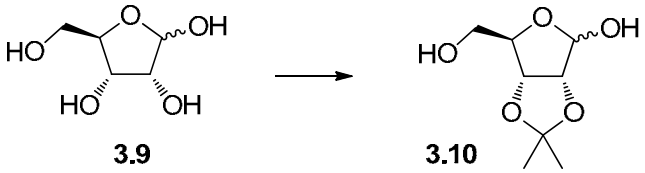
3.7 Experimental

3.7.1 General Experimental

For details of the general experimental procedures used, see **Chapter 2, pages 55-57**.

3.7.2 Experimental

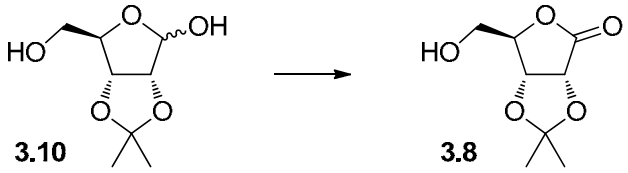
2,3-*O*-Isopropylidene-D-ribose



This compound was prepared according to a known procedure.[228] Anhydrous copper (II) sulfate (52.7 g, 0.329 mol, 1.7 eq) was added to a suspension of D-ribose **3.9** (29.1 g, 0.194 mol, 1 eq) in acetone (1 L). The suspension was stirred at 45°C for 21 h, whereupon t.l.c. (EtOAc) indicated absence of starting material (R_f 0.0) and formation of a major product (R_f 0.64) and two minor products (R_f 0.82, 0.28). The reaction mixture was filtered through Celite® and concentrated *in vacuo*. The crude was purified by flash column chromatography on silica gel (3:2 → 1:1, cyclohexane:EtOAc) to give 2,3-*O*-isopropylidene-D-ribose **3.10** (27.1 g, 74%) as a colourless syrup in a 7:1 mixture of anomers.

$[\alpha]_D^{25}{}_{eqm}$ -24.7 (*c* 0.98, $CHCl_3$) [Lit.:[228] $[\alpha]_D^{25}$ -27.5 (*c* 1.0, $CHCl_3$)]; IR ν_{max} (thin film): 3384 (s, O-H); δ_H (400 MHz, $CDCl_3$), major anomer only: 5.42 (a-s, 1H; anomeric H-1), 4.84 (br-d, $J_{2,3}$ 6.0, 1H; H-3), 4.59 (d, $J_{2,3}$ 6.0, 1H; H-2), 4.41 (a-br t, $J_{4,5} \approx J_{4,5'}$ 2.4, 1H; H-4), 3.76 (dd, $J_{4,5}$ 2.2, $J_{5,5'}$ 11.9; H-5), 3.71 (dd, $J_{4,5'}$ 3.1, $J_{5,5'}$ 12.0; H-5'), 1.49, 1.32 (2 × s, 2 × 3H; $C(CH_3)_2$); LRMS *m/z* (ESI^-): 189 (100) $[M - H]^-$, 379 (82) $[2M - H]^-$.

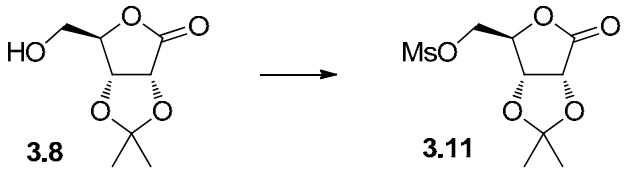
2,3-*O*-Isopropylidene-D-ribono-1,4-lactone



2,3-*O*-Isopropylidene-D-ribose **3.10** (12.0 g, 63.1 mmol, 1 eq) was dissolved in water (300 mL) and cooled to 0°C. The flask was wrapped in foil and barium carbonate (18.7 g, 94.6 mmol, 1.5 eq) added to the stirred solution. Bromine (4.85 mL, 94.6 mmol, 1.5 eq) was added dropwise with an addition funnel over 20 min, such that the temperature did not exceed 5°C. After 2.5 h the solution was allowed to warm to room temperature, and after a further 1 h, t.l.c. (1:2, cyclohexane:EtOAc) indicated trace starting material (R_f 0.42) and the formation of a single product (R_f 0.60). The reaction mixture was quenched with $Na_2S_2O_3$ (aq) (100 mL) and shaken with EtOAc (300mL). The layers were separated, the aqueous extracted with EtOAc (3 × 150 mL), and the organic fractions combined and concentrated *in vacuo*. The crude yellow solid was purified by flash column chromatography on silica gel (2:3, cyclohexane:EtOAc) to yield 2,3-*O*-isopropylidene-D-ribono-1,4-lactone **3.8** (10.2 g, 86%) as a white crystalline solid.

m.p. 132-133°C [Lit.:[229] 138-139°C]; $[\alpha]_D^{25}$ -64.4 (*c* 1.39, acetone) [Lit.:[229] $[\alpha]_D^{20}$ -65.2 (*c* 5.0, acetone)]; IR ν_{max} (thin film): 3468 (s, O-H), 1775 (s, C=O); δ_H (400 MHz, $CDCl_3$): 4.85 (d, $J_{2,3}$ 5.6, 1H; H-2), 4.78 (d, $J_{2,3}$ 5.6, 1H; H-3), 4.64 (a-t, $J_{4,5} \approx J_{4,5'}$ 1.9, 1H; H-4), 4.01 (ddd, $J_{4,5}$ 2.3, $J_{5,OH}$ 5.1, $J_{5,5'}$ 12.0, 1H; H-5), 3.84 (ddd, $J_{4,5'}$ 1.7, $J_{5',OH}$ 5.3, $J_{5,5'}$ 12.1, 1H; H-5'), 1.84 (br d, $J_{5,OH}$ 4.8, 1H; 5-OH), 1.50, 1.40 (2 × s, 2 × 3H; $C(CH_3)_2$); LRMS *m/z* (ESI^+): 211 (100) $[M + Na]^+$.

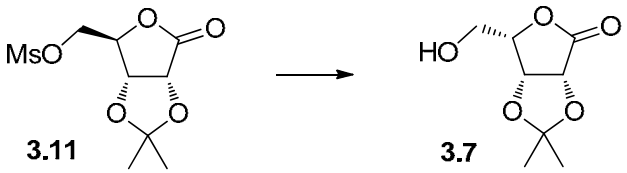
2,3-*O*-Isopropylidene-5-*O*-methanesulfonyl-D-ribono-1,4-lactone



2,3-*O*-Isopropylidene-D-ribono-1,4-lactone **3.8** (17.8 g, 94.4 mmol, 1 eq) was stirred in anhydrous dichloromethane (300 mL) under an atmosphere of argon. Triethylamine (32.9 mL, 0.236 mol, 2.5 eq) was added and the solution cooled to 0°C. Methanesulfonyl chloride (18.3 mL, 0.236 mol, 2.5 eq) was added dropwise over 60 min, such that the temperature did not exceed 5°C. After 2 h, t.l.c. (1:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.21) and formation of a major product (R_f 0.32), and the solution had changed from colourless to orange. The reaction mixture was diluted with additional dichloromethane (200 mL), and water (300 mL) was added. The layers were separated, the aqueous extracted with dichloromethane (3 × 150 mL), and the organic fractions combined and concentrated *in vacuo*. The crude orange syrup was purified by flash column chromatography on silica gel (2:1 → 3:2 → 1:1, cyclohexane:EtOAc) to yield 2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-D-ribono-1,4-lactone **3.11** (25.1 g, 100%) as a white crystalline solid.

m.p. 68-69°C [Lit.:[230] 66-67°C]; $[\alpha]_D^{25}$ -53.0 (*c* 1.1, $CHCl_3$) [Lit.:[230] $[\alpha]_D^{20}$ -49.9 (*c* 1.0, $CHCl_3$)]; IR ν_{max} (thin film): 1795 (s, C=O), 1338, 1172 (O-SO_2); δ_H ($CDCl_3$, 400 MHz): 4.83 (d, $J_{2,3}$ 5.6, 1H; H-2), 4.82-4.78 (m, 2H; H-3 and H-4), 4.49 (dd, $J_{4,5}$ 2.1, $J_{5,5'}$ 11.4, 1H; H-5), 4.45 (dd, $J_{4,5'}$ 2.5, $J_{5,5'}$ 11.5, 1H; H-5'), 3.06 (s, 3H; SO_2CH_3), 1.50, 1.41 (2 × s, 2 × 3H; $C(CH_3)_2$); LRMS *m/z* (ESI^+): 555 (100) $[2M + Na]^+$.

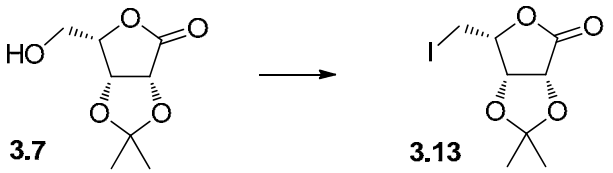
2,3-*O*-Isopropylidene-L-lyxono-1,4-lactone



This compound was prepared according to a known procedure.[195] 2,3-*O*-Isopropylidene-5-*O*-methanesulfonyl-D-ribono-1,4-lactone **3.11** (7.06 g, 26.5 mmol, 1 eq) was dissolved in 1,4-dioxane (90 mL). 2 M NaOH (aq) (39.8 mL, 79.5 mmol, 3 eq) was added and the mixture stirred for 2 h. The solution was acidified to pH 2.5-3.0 using 2 M HCl (aq) and stirred for 30 min. The reaction mixture was extracted with dichloromethane (5 × 150 mL), and the organic fractions combined and concentrated *in vacuo* to give a single product (R_f 0.15) by t.l.c. (1:1, cyclohexane:EtOAc). The crude off-white solid was purified by flash column chromatography on silica gel (1:1 → 1:2, cyclohexane:EtOAc → EtOAc) to yield 2,3-*O*-isopropylidene-L-lyxono-1,4-lactone **3.7** (2.61 g, 52%) (60% obtained from 3.50 g) as a white crystalline solid.

m.p. 96-97°C [Lit.:[195] 98-99°C]; $[\alpha]_D^{25}$ -88.1 (*c* 1.1, acetone) [Lit.:[195] $[\alpha]_D^{25}$ -89 (*c* 1.0, acetone)]; IR ν_{max} (thin film): 3216 (br, O-H), 1778 (s, C=O); δ_H (400 MHz, $CDCl_3$): 4.90 (dd, $J_{3,4}$ 3.7, $J_{2,3}$ 5.5, 1H; H-3), 4.87 (d, $J_{2,3}$ 5.6, 1H; H-2), 4.61 (ddd, $J_{3,4}$ 3.6, $J_{4,5'}$ 5.5, $J_{4,5}$ 6.5, 1H; H-4), 4.06 (dd, $J_{4,5}$ 6.5, $J_{5,5'}$ 12.3, 1H; H-5), 3.98 (dd, $J_{4,5'}$ 5.4, $J_{5,5'}$ 12.2, 1H; H-5'), 2.04 (br-s, 1H; 5-OH), 1.49, 1.41 (2 × s, 2 × 3H; $C(CH_3)_2$); LRMS *m/z* (ESI^+): 399 (100) $[2M + Na]^+$; LRMS (ESI^-): *m/z* 187 (45) $[M - H]^-$.

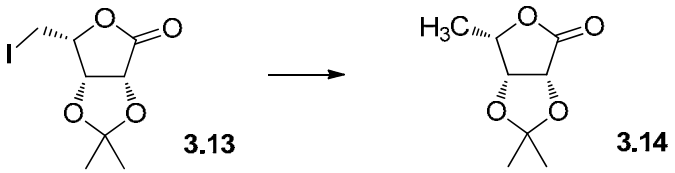
5-Deoxy-5-iodo-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone



2,3-*O*-Isopropylidene-L-lyxono-1,4-lactone **3.7** (2.77 g, 14.7 mmol, 1 eq) was dissolved in toluene (75 mL) and the solution heated to 85°C. Triphenylphosphine (10.0 g, 38.2 mmol, 2.6 eq), imidazole (3.59 g, 52.8 mmol, 3.6 eq), and iodine (9.75 g, 38.4 mmol, 2.6 eq) were added to the stirred solution. After 40 min, t.l.c. (1:1, cyclohexane:EtOAc) indicated consumption of starting material (R_f 0.11) and formation of a major product (R_f 0.78). The reaction mixture was allowed to cool to room temperature, concentrated *in vacuo* and partitioned between dichloromethane (250 mL) and $NaHCO_3$ (aq) (350 mL). The phases were separated, the aqueous fraction extracted with dichloromethane (3 × 150 mL), and the organic layers combined and concentrated *in vacuo*. The crude orange solid was purified by flash column chromatography on silica gel (9:1 → 6:1 → 3:1, cyclohexane:EtOAc) to yield 5-deoxy-5-iodo-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone **3.13** (3.81 g, 87%) (90% obtained from 0.80 g) as a white crystalline solid.

m.p. 96-97°C [Lit. for enantiomer:[231] 89°C]; $[\alpha]_D^{25}$ -19.8 (*c* 1.24, acetone) [Lit. for enantiomer:[231] $[\alpha]_D$ +23.1 (*c* 0.83, acetone)]; IR ν_{max} (thin film): 1787 (s, C=O), 508 (s, C-I); δ_H (400 MHz, $CDCl_3$): 4.93 (dd, $J_{3,4}$ 3.4, $J_{2,3}$ 5.3, 1H; H-3), 4.87 (d, $J_{2,3}$ 5.3, 1H; H-2), 4.67 (ddd, $J_{3,4}$ 3.3, $J_{4,5}$ 5.9, $J_{4,5'}$ 9.1, 1H; H-4), 3.48-3.39 (m, 2H; H-5 and H-5'), 1.49, 1.44 (2 × s, 2 × 3H; $C(CH_3)_2$); δ_C (100 MHz, $CDCl_3$): 173.4 (C=O), 114.4 ($C(CH_3)_2$), 78.8 (C-4), 76.7 (C-2), 75.9 (C-3), 26.8, 26.0 ($C(CH_3)_2$), -2.5 (C-5); HRMS *m/z* (Probe ESI/FI$^+$) found: 297.9705 $[M]^+$; $C_8H_{11}IO_7$ requires 297.9702.

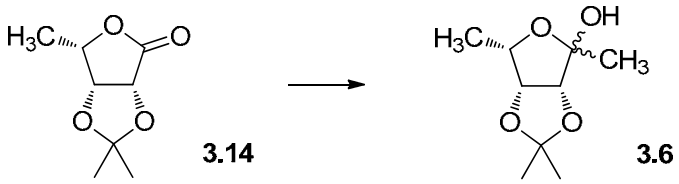
5-Deoxy-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone



5-Deoxy-5-iodo-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone **3.13** (2.51 g, 8.43 mmol, 1 eq) was dissolved in ethanol (75 mL) under an atmosphere of nitrogen. Sodium acetate (0.853 g, 10.4 mmol, 1.2 eq) and 10% Pd/C (10% by mass, 0.262 g) were added to the stirred solution. The reaction vessel was degassed and the reaction mixture placed under an atmosphere of hydrogen. After 66 h, t.l.c. (2:1, cyclohexane:EtOAc) indicated complete consumption of starting material (R_f 0.63, UV active) and formation of a major product (R_f 0.39, UV inactive). The reaction mixture was filtered through Celite® and the yellow filtrate concentrated *in vacuo*. Due to the volatility of the product, the pressure was not reduced below 75 mbar at 25°C. The residue was partitioned between dichloromethane (150 mL) and $Na_2S_2O_3$ (aq) (100 mL), the layers separated, and the aqueous extracted with dichloromethane (2 × 75 mL). The organic fractions were combined and concentrated *in vacuo*. The crude white solid was purified by flash column chromatography on silica gel (3:1, cyclohexane:EtOAc) to yield 5-deoxy-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone **3.14** (0.725 g, 50%) as a white solid that smelt of hazelnuts.

m.p. 71-72°C; $[\alpha]_D^{25}$ -132.2 (*c* 0.86, $CHCl_3$); IR ν_{max} (thin film): 1786 (s, C=O); δ_H (400 MHz, $CDCl_3$): 4.82 (d, $J_{2,3}$ 5.3, 1H; H-2), 4.71 (dd, $J_{3,4}$ 3.6, $J_{2,3}$ 5.5, 1H; H-3), 4.63 (dq, $J_{3,4}$ 3.6, $J_{4,5}$ 6.5, 1H; H-4), 1.50 (d, $J_{4,5}$ 6.6, 3H; 3 × 5-H), 1.48, 1.41 (2 × s, 2 × 3H; $C(CH_3)_2$); δ_H (100 MHz, $CDCl_3$): 174.0 (C-1), 114.0 ($C(CH_3)_2$), 77.5 (C-3), 76.6 (C-2), 76.0 (C-4), 26.8, 26.0 ($C(CH_3)_2$), 14.5 (C-5); LRMS *m/z* (ESI^+): 367 (100) $[2M + Na]^+$; HRMS *m/z* (ESI^+) found: 195.0625 $[M + Na]^+$; $C_8H_{12}NaO_4$ requires 195.0628.

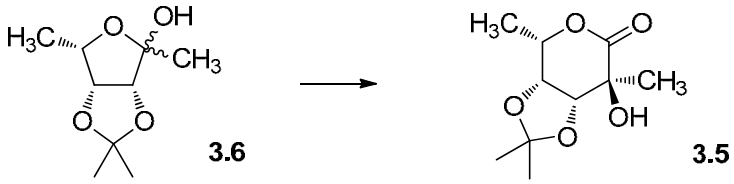
1,6-Dideoxy-3,4-*O*-isopropylidene-L-tagatose



5-Deoxy-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone **3.14** (0.972 g, 5.64 mmol, 1 eq) was dissolved in anhydrous tetrahydrofuran (50 mL) under an atmosphere of argon and cooled to -78°C. Methyl magnesium bromide (1.0 M in Bu_2O, 7.1 mL, 7.1 mmol, 1.25 eq) was added dropwise to the stirred solution. After 1.5 h, IR spectroscopy indicated the presence of O-H and the absence of C=O. The reaction mixture was quenched with excess NH_4Cl (aq) and allowed to warm to room temperature over 1 h. The mixture was partitioned between water (150 mL) and EtOAc (150 mL). The layers were separated, the aqueous extracted with EtOAc (2 × 75 mL), and the organic fractions combined and concentrated *in vacuo*. The crude was purified by flash column chromatography on silica gel (4:1 → 3:1, cyclohexane:EtOAc) to yield 1,6-dideoxy-3,4-*O*-isopropylidene-L-tagatose **3.6** (0.809 g, 76%) as a white solid in a 4:1 mixture of anomers (A:B).

m.p. 54-56°C (sublimes); $[\alpha]_D^{25}$ -74.4 (*c* 0.96, $CHCl_3$); IR ν_{max} (thin film): 3209 (br, s, O-H); δ_H (400 MHz, $CDCl_3$): 4.66 (dd, $J_{4,5}$ 4.0, $J_{3,4}$ 5.6, 1H; H-4$^{\mathbf{A}}$), 4.59 (dd, $J_{4,5}$ 3.8, $J_{3,4}$ 5.8, 0.25H; H-4$^{\mathbf{B}}$), 4.46 (d, $J_{3,4}$ 5.8, 1H; H-3$^{\mathbf{A}}$), 4.31-4.22 (m, 1.25H; H-5$^{\mathbf{A}}$ and H-3$^{\mathbf{B}}$), 4.04 (br s, 0.25H; 2-OH$^{\mathbf{B}}$), 3.75 (dq, $J_{4,5}$ 3.7, $J_{5,6}$ 6.2, 0.25H; H-5$^{\mathbf{B}}$), 1.97 (br s, 1H; 2-OH$^{\mathbf{A}}$), 1.56 (s, 0.75H; $CH_3^{\mathbf{B}}$), 1.52 (s, 3H; $CH_3^{\mathbf{A}}$), 1.49 (s, 3H; $CH_3^{\mathbf{A}}$), 1.39 (s, 0.75H; $CH_3^{\mathbf{B}}$); 1.38 (s, 0.75H; $CH_3^{\mathbf{B}}$), 1.35 (s, 3H; $CH_3^{\mathbf{A}}$), 1.30 (d, $J_{5,6}$ 6.3, 3.75H; 3 × H-6$^{\mathbf{A}}$ and 3 × H-6$^{\mathbf{B}}$); δ_C (100 MHz, $CDCl_3$), major anomer only: 114.0, 112.4 (C-2 and $C(CH_3)_2$), 85.9 (C-3), 81.9 (C-4), 74.8 (C-5), 26.2, 26.2, 25.0, 25.0 (4 × CH_3); LRMS *m/z* (ESI^+): 211 (100) $[M + Na]^+$; LRMS (ESI^-): *m/z* (%) 187 (54) $[M - H]^-$; HRMS *m/z* (ESI^+) found: 211.0941 $[M + Na]^+$; $C_9H_{16}NaO_4$ requires 211.0941.

6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone



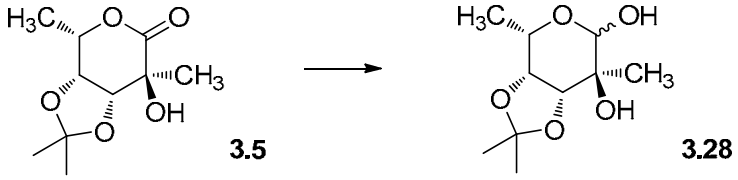
1,6-Dideoxy-3,4-*O*-isopropylidene-L-tagatose **3.6** (0.710 g, 3.77 mmol, 1 eq) was dissolved in water (10 mL) and 1,4-dioxane (0.8 mL). Sodium cyanide (0.370 g, 7.55 mmol, 2 eq) was added and the solution stirred at room temperature for 21 h, followed by a further 20 h at 30°C. At this point mass spectroscopy detected the cyanohydrin intermediate **3.21** (453: $[2M + Na]^+$; 216: $[M + H]^+$; 238: $[M + Na]^+$) as well as starting material (211: $[M + Na]^+$). The solution was heated to 100°C and stirred for a further 48 h, whereupon mass spectroscopy detected the open chain carboxylic acid **3.22** (233: $[M - H]^-$). The solution was concentrated *in vacuo* and redissolved in methanol (15 mL). 5 Å Molecular sieves (10% by mass, 0.355 g) and acetyl chloride (1.7 eq) were added and the solution was stirred for 3 h, whereupon mass spectroscopy detected the lactone (239: $[M + Na]^+$) and none of the open chain carboxylic acid **3.22**. The reaction mixture was preadsorbed on silica and purified by flash column chromatography on silica gel (3:1 → 7:3 → 2:1, cyclohexane:EtOAc) to yield 6-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone **3.5** (0.399 g, 49%) as a white crystalline solid.

m.p. 96-98°C; $[\alpha]_D^{25}$ -84.6 (*c* 1.03, $CHCl_3$); IR ν_{max} (thin film): 3434 (br, s, O-H), 1731 (s, C=O); δ_H (400 MHz, $CDCl_3$): 5.13 (dq, $J_{4,5}$ 1.9, $J_{5,6}$ 6.6, 1H; H-5), 4.38 (dd, $J_{4,5}$ 1.9, $J_{3,4}$ 7.3, 1H; H-4), 4.31 (br d, $J_{3,4}$ 7.3, 1H; H-3), 2.55 (v br s, 1H; 2-OH), 1.58 (d, *J* 1.0, 3H; 2-CH_3), 1.42 (d, $J_{5,6}$ 6.7, 3H; 3 × H-6), 1.41, 1.35 (2 × s, 2 × 3H; $C(CH_3)_2$); δ_C (100 MHz, $CDCl_3$): 171.2 (C-1), 109.8 ($C(CH_3)_2$), 79.0 (C-3), 75.0 (C-4), 73.2 (C-5), 71.9 (C-2), 26.1, 24.2 ($C(CH_3)_2$), 22.5 (2-CH_3), 16.1 (C-6); LRMS *m/z* (ESI^+): 455 (100) $[2M + Na]^+$, 239.08 (86) $[M + Na]^+$; LRMS *m/z* (ESI^-):

431 (100) $[2M - H]^-$, 215 (44) $[M - H]^-$; HRMS *m/z* (ESI^+) found: 239.0891 $[M + Na]^+$; $C_{10}H_{16}NaO_5$ requires 239.0890.

X-ray crystal structure: see **Appendix 1**.

3,4-*O*-Isopropylidene-2-*C*-methyl-L-galactose

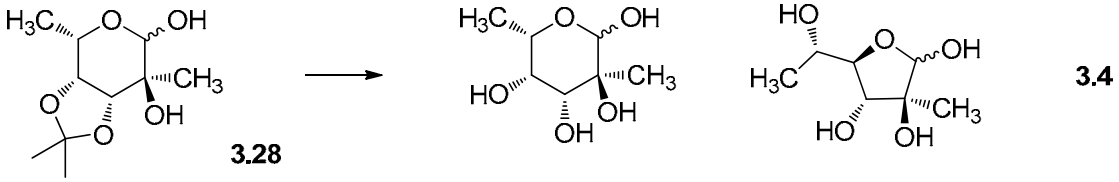


6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone **3.5** (40 mg, 0.185 mmol, 1 eq) was dissolved in anhydrous dichloromethane (5 mL) and cooled to -78°C. Diisobutyl aluminium hydride (25% wt. in toluene, 0.15 mL, 0.22 mmol, 1.2 eq) was added dropwise to the stirred solution. After 2 h, mass spectroscopy detected product (241: $[M + Na]^+$) as well as starting material (239: $[M + Na]^+$). Further diisobutyl aluminium hydride (0.07 mL, 0.10 mmol, 0.6 eq) was added to the reaction mixture, and after a further 45 min, mass spectroscopy detected product and very little starting material. The solution was diluted with dichloromethane (30 mL), quenched with excess sodium potassium tartrate solution (30 mL), and stirred for 30 min. The organic and aqueous layers were separated and the aqueous extracted with dichloromethane (2 × 30 mL). The organic fractions were combined, concentrated *in vacuo* and purified by flash column chromatography on silica gel (3:2 → 1:1, cyclohexane:EtOAc) to yield 6-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactose **3.28** (25 mg, 62%) as a colourless syrup in a 5:1 mixture of anomers (A:B).

$[\alpha]_D^{25}{}_{eqm}$ -6.5 (*c* 0.93, MeOH); IR ν_{max} (thin film): 3373 (br, O-H); δ_H (400 MHz, $CDCl_3$): 5.00 (s, 0.2H; anomeric H-$1^{\mathbf{B}}$), 4.95 (s, 1H; anomeric H-$1^{\mathbf{A}}$), 4.31 (a-br dq, $J_{4,5}$ 1.9, $J_{5,6}$ 6.6, 1.2H; H-$5^{\mathbf{A}}$ and H-$5^{\mathbf{B}}$), 4.28 (d, $J_{3,4}$ 7.5, 1.2H; H-$3^{\mathbf{A}}$ and H-$3^{\mathbf{B}}$), 4.15 (a-br dd, $J_{4,5}$ 1.9, $J_{3,4}$ 7.5, 1.2H;

H-4^{A} and H-4^{B}), 1.46 (s, 3H; CH_3^{A}), 1.45 (s, 0.6H; CH_3^{B}), 1.39 (s, 3H; CH_3^{A}), 1.38 (s, 0.6H; CH_3^{B}), 1.36 (s, 3H; CH_3^{A}), 1.34 (s, 0.6H; CH_3^{B}), 1.30 (d, $J_{5,6}$ 6.7 Hz, 0.6H; 3 × H-6^{B}), 1.21 (d, $J_{5,6}$ 6.7, 3H; 3 × H-6^{A}); δ_C (100 MHz, $CDCl_3$), major anomer only: 109.4 ($C(CH_3)_2$), 93.0 (C-1), 77.6 (C-3), 75.9 (C-4), 69.4 (C-2), 65.4 (C-5), 26.3, 25.0, 24.9 (2-CH_3 and C(CH_3)$_2$), 16.0 (C-6); LRMS *m/z* (ESI$^+$): 201 (100) [M - isopropylidene]$^+$, 241 (87) [M + Na]$^+$; HRMS *m/z* (Probe ESI/FI$^+$) found: 218.1154 [M]$^+$; $C_{10}H_{18}O_5$ requires 218.1154.

6-Deoxy-2-*C*-methyl-L-galactose

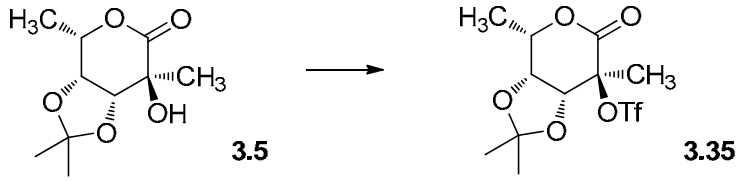


3.28 **3.4**

6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactopyranose **3.28** (24 mg, 0.109 mmol, 1 eq) was dissolved in water/1,4-dioxane 50:50 *v/v* (1 mL). Dowex® (55 mg, 2.3 eq by mass) was added and the solution stirred for 18 h, whereupon t.l.c. (EtOAc) indicated that no starting material (R_f 0.75) remained. The solution was filtered and concentrated *in vacuo* to yield 6-deoxy-2-*C*-methyl-L-galactose **3.4** (19 mg, 98%) as a white solid in a mixture of forms.

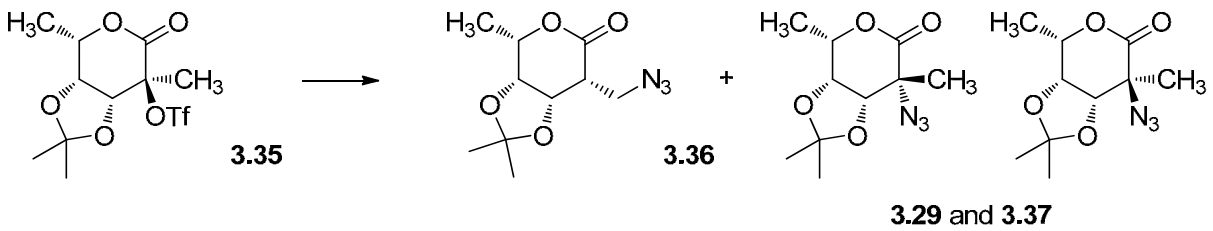
m.p. 121-123°C; $[\alpha]_D^{25}{}_{eqm}$ -35.8 (*c* 0.95, H_2O); IR ν_{max} (thin film): 3364 (br, O-H); δ_H (400 MHz, D_2O), major component only: 4.88 (s, 1H; anomeric H-1), 4.14 (dq, $J_{4,5}$ 1.4, $J_{5,6}$ 6.6, 1H; H-5), 3.76 (dd, $J_{4,5}$ 1.3, $J_{3,4}$ 5.5, 1H; H-4), 3.72 (d, $J_{3,4}$ 5.5, 1H; H-3), 1.17 (s, 3H; 2-CH_3), 1.12 (d, $J_{5,6}$ 6.5, 3H; 3 × H-6); δ_C (100 MHz, D_2O), major component only: 100.1 (C-1), 78.2 (C-3), 76.7 (C-4), 71.8 (C-2), 69.4 (C-5), 17.9 (2-CH_3), 15.6 (C-6); LRMS *m/z* (ESI$^+$): 201 (100) [M + Na]$^+$; LRMS *m/z* (ESI$^-$): 177 (100) [M - H]$^-$, 213 (93), 215 (38) [M + Cl]$^-$; HRMS *m/z* (ESI$^+$) found: 201.0733 [M + Na]$^+$; $C_7H_{14}NaO_5$ requires 201.0733.

6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-2-*O*-trifluoromethanesulfonyl-L-galactono-1,5-lactone



6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone **3.5** (0.265 g, 1.23 mmol, 1 eq) was dissolved in anhydrous dichloromethane (20 mL) under an atmosphere of argon. Pyridine (0.30 mL, 3.68 mmol, 3 eq) was added and the solution cooled to -15°C. Trifluoromethanesulfonic anhydride (0.27 mL, 1.59 mmol, 1.3 eq) was added slowly to the stirred solution, such that the temperature did not exceed 0°C. After 1 h, t.l.c. (1:1, cyclohexane:EtOAc) indicated incomplete consumption of starting material (R_f 0.54) and formation of a single product (R_f 0.78). Additional pyridine (0.30 mL, 3.68 mmol, 3 eq) and trifluoromethanesulfonic anhydride (0.27 mL, 1.59 mmol, 1.3 eq) were added, and the solution was allowed to warm to room temperature. After a further 2 h, no starting material was detected by t.l.c.. The reaction mixture was diluted with dichloromethane (20 mL) and washed with 1 M HCl (aq) (20 mL). The aqueous layer was extracted with dichloromethane (2 × 30 mL) and the organic fractions combined, washed with brine (20 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated *in vacuo* to give the crude 6-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-2-*O*-trifluoromethanesulfonyl-L-galactono-1,5-lactone **3.35** which was reacted on immediately without further purification.

2-Azidomethyl-2,6-dideoxy-3,4-*O*-isopropylidene-L-talono-1,5-lactone, and 2-azido-2,6-dideoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-talono-1,5-lactone/2-azido-2,6-dideoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone



The crude 6-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-2-*O*-trifluoromethanesulfonyl-L-galactono-1,5-lactone **3.35** was dissolved in *N,N*-dimethylformamide (15 mL) under an atmosphere of argon. Sodium azide (0.120 g, 1.84 mmol, 1.5 eq) was added and the solution stirred for 16 h, whereupon t.l.c. (1:1, cyclohexane:EtOAc) indicated complete consumption of staring material (R_f 0.78) and formation of two apparent products (R_f 0.60, R_f 0.47). The reaction mixture was concentrated *in vacuo* and residue redissolved in EtOAc (30 mL). The solution was washed with brine (2 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (5:1 → 4:1, cyclohexane:EtOAc) to yield 2-azidomethyl-2,6-dideoxy-L-talono-1,5-lactone **3.36** (0.081 g, 27%) as a white solid, and an inseparable mixture of 2-azido-2,6-dideoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-talono-1,5-lactone **3.29** and 2-azido-2,6-dideoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone **3.37** (0.167 g, 57%) as a colourless oil in a 2:1 mixture of epimers (A:B).

Data for 2-azidomethyl-2,6-dideoxy-3,4-*O*-isopropylidene-L-talono-1,5-lactone **3.36**

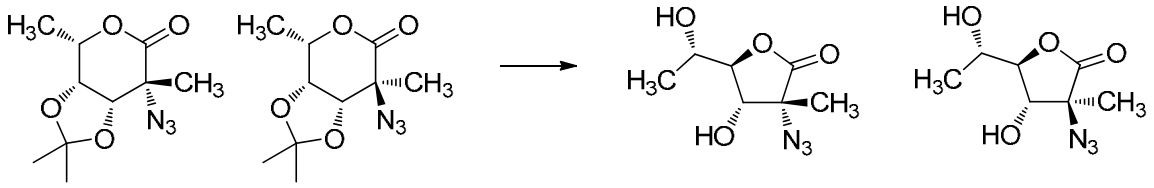
R_f (1:1, cyclohexane:EtOAc) 0.60; m.p. 88-90°C; $[\alpha]_D^{25}$ -124.0 (*c* 1.05, $CHCl_3$); IR ν_{max} (thin film): 2105 (s, N_3), 1752 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 4.73 (dd, $J_{2,3}$ 3.3, $J_{3,4}$ 7.7, 1H; H-3), 4.36 (dd, $J_{4,5}$ 1.7, $J_{3,4}$ 7.7, 1H; H-4), 4.28 (dq, $J_{4,5}$ 1.5, $J_{5,6}$ 6.5, 1H; H-5), 3.94 (dd, $J_{2,2'a}$ 5.6, $J_{2'a,2'b}$ 12.6, 1H; H-2'a), 3.64 (dd, $J_{2,2'b}$ 8.9, $J_{2'a,2'b}$ 12.6, 1H; H-2'b), 2.63 (ddd, $J_{2,3}$ 3.2, $J_{2,2'a}$ 5.6,

$J_{2,2'b}$ 8.9, 1H; H-2), 1.46 (d, $J_{5,6}$ 6.5, 3H; 3 × H-6), 1.42, 1.35 (2 × s, 2 × 3H; $C(CH_3)_2$); δ_C ($CDCl_3$, 100 MHz): 169.9 (C-1), 110.1 ($C(CH_3)_2$), 74.9 (C-4), 73.8 (C-5), 72.2 (C-3), 48.0 (C-2'), 43.1 (C-2), 25.9, 24.3 ($C(CH_3)_2$), 15.7 (C-6); LRMS *m/z* (ESI^+): 264 (97) $[M + Na]^+$, 505 (100) $[2M + Na]^+$; HRMS *m/z* (ESI^+) found: 264.0956 $[M + Na]^+$; $C_{10}H_{15}N_3NaO_4$ requires 264.0955.

Data for mixture of 2-azido-2,6-dideoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-talono-1,5-lactone **3.29** and 2-azido-2,6-dideoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone **3.37**

R_f (1:1, cyclohexane:EtOAc) 0.47; IR ν_{max} (thin film): 2115 (s, N_3), 1742 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 4.71 (dq, $J_{4,5}$ 1.8, $J_{5,6}$ 6.5, 0.5H; H-$5^{\mathbf{B}}$), 4.62 (dq, $J_{4,5}$ 2.3, $J_{5,6}$ 6.4, 1H; H-$5^{\mathbf{A}}$), 4.56 (d, $J_{3,4}$ 7.3, 0.5H; H-$3^{\mathbf{B}}$), 4.49 (d, $J_{3,4}$ 7.5, 1H; H-$3^{\mathbf{A}}$), 4.39 (dd, $J_{4,5}$ 2.2, $J_{3,4}$ 7.3, 1H; H-$4^{\mathbf{A}}$), 4.37 (dd, $J_{4,5}$ 1.9, $J_{3,4}$ 7.3, 0.5H; H-$4^{\mathbf{B}}$), 1.48 (s, 3H; $CH_3^{\mathbf{A}}$), 1.47 (d, $J_{5,6}$ 6.3, 3H; 3 × H-$6^{\mathbf{A}}$), 1.44 (s, 3H; $CH_3^{\mathbf{A}}$), 1.43 (d, $J_{5,6}$ 6.5, 1.5H; 3 × H-$6^{\mathbf{B}}$), 1.42 (s, 3H; 2 × $CH_3^{\mathbf{B}}$), 1.38 (s, 3H; $CH_3^{\mathbf{A}}$), 1.35 (s, 1.5H; $CH_3^{\mathbf{B}}$); δ_C ($CDCl_3$, 100 MHz): 171.1 (C-$1^{\mathbf{A}}$), 169.1 (C-$1^{\mathbf{B}}$), 110.3, 110.3 ($C(CH_3)_2^{\mathbf{B}}$ and $C(CH_3)_2^{\mathbf{A}}$), 80.7 (C-$3^{\mathbf{A}}$), 77.8 (C-$3^{\mathbf{B}}$), 75.0 (C-$4^{\mathbf{B}}$), 74.2 (C-$4^{\mathbf{A}}$), 73.5 (C-$5^{\mathbf{A}}$), 73.3 (C-$5^{\mathbf{B}}$), 63.5, 63.5 (C-$2^{\mathbf{B}}$ and C-$2^{\mathbf{A}}$), 26.0, 24.4 (2-$CH_3^{\mathbf{A}}$ and C-$6^{\mathbf{A}}$), 24.2 (2-$CH_3^{\mathbf{B}}$), 21.0 (C-$6^{\mathbf{B}}$), 19.8 ($C(CH_3)_2^{\mathbf{A}}$), 18.5, 16.4 (2 × $C(CH_3)_2^{\mathbf{B}}$), 16.3 ($C(CH_3)_2^{\mathbf{A}}$); LRMS *m/z* (ESI^+): 264 (100) $[M + Na]^+$; HRMS *m/z* (ESI^+) found: 264.0955 $[M + Na]^+$; $C_{10}H_{15}N_3NaO_4$ requires 264.0955.

2-azido-2,6-dideoxy-2-*C*-methyl-L-talono-1,5-lactone/2-azido-2,6-dideoxy-2-*C*-methyl-L-galactono-1,5-lactone



3.29 and **3.37** **3.39** and **3.40**

The mixture of 2-azido-2,6-dideoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-talono-1,5-lactone **3.29** and 2-azido-2,6-dideoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone **3.37** (73 mg, 0.303 mmol) was dissolved in trifluoroacetic acid (67% *v/v* in 1:1 water:1,4-dioxane) (3 mL) and stirred for 22 h. The solution was concentrated *in vacuo* and the residue purified by flash column chromatography (1:1, cyclohexane:EtOAc) to yield an inseparable mixture of 2-azido-2,6-dideoxy-2-*C*-methyl-L-talono-1,5-lactone **3.39** and 2-azido-2,6-dideoxy-2-*C*-methyl-L-galactono-1,5-lactone **3.40** (57 mg, 94%) as a colourless oil in a 2:1 mixture of epimers (A:B).

Selected data for mixture of 2-azido-2,6-dideoxy-2-*C*-methyl-L-talono-1,5-lactone **3.39** and 2-azido-2,6-dideoxy-2-*C*-methyl-L-galactono-1,5-lactone **3.40**

IR ν_{max} (thin film): 3341 (br, O-H); δ_H ($CDCl_3$, 400 MHz): 4.71 (d, $J_{3,4}$ 8.0, 0.5H; H-3$^{\mathbf{B}}$), 4.10-4.02 (m, 2.5H; H-3$^{\mathbf{A}}$, H-5$^{\mathbf{A}}$, and H-5$^{\mathbf{B}}$), 4.00 (dd, $J_{4,5}$ 2.4, $J_{3,4}$ 6.5, 1H; H-4$^{\mathbf{A}}$), 3.96 (dd, $J_{4,5}$ 3.1, $J_{3,4}$ 8.0, 0.5H; H-4$^{\mathbf{B}}$), 1.64 (s, 3H; 2-$CH_3^{\mathbf{A}}$), 1.56 (s, 1.5H; 2-$CH_3^{\mathbf{B}}$), 1.38 (d, $J_{5,6}$ 6.5, 3 × H-6$^{\mathbf{A}}$), 1.37 (d, $J_{5,6}$ 6.3, 3 × H-6$^{\mathbf{A}}$); LRMS *m/z* (ESI^+): 224 (100) $[M + Na]^+$.

Chapter 4

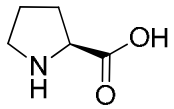
Iminosugar Amides as N-*Acetylhexosaminidase Inhibitors*

4.1 Overview

This chapter concerns polyhydroxylated proline amides, a class of iminosugars with considerable therapeutic potential. Hydroxylated proline analogues, and synthetic approaches to these molecules, are described. The synthesis of methyl (3*S*,4*R*)-dihydroxy-D-proline amide and its L-enantiomer are detailed (**Section 4.5**), along with the results and discussion of biological testing (**Section 4.6**).

4.2 Proline

L-Proline **4.1** is one of the twenty proteinogenic α-amino acids coded for by the universal genetic code. It is the only member of this class in which the amine group is secondary rather than primary.[232, 233]



4.1

Figure 4.1 L-Proline.

The cyclic structure of proline imparts high conformational rigidity, and so it plays a unique role in the conformation of peptides and proteins.[234] For example, it induces β-turns in proteins such as myoglobin,[235] and makes up a large proportion of collagen, the most important structural

protein in mammals; in this case it induces the peptide chains to form tight helices, giving collagen its extremely high tensile strength.[236]

Many modified proline analogues are found in nature. These include hydroxylated, carbon-branched, and nitrogen-substituted proline derivatives.[237]

4.2.1 Proline hydroxylation

Hydroxylation is an important modification to structural proteins such as elastin and collagen. The post-translational modification of proline to (4*R*)-hydroxyproline in collagen alters conformation, fine tuning bond angles to stabilise the triple helical structure.[238, 239] Hydroxylation of proline is also an important biochemical pathway. Hypoxia-Inducible Factor (HIF) is a protein that contains two proline residues. The extent of proline hydroxylation depends indirectly on the levels of oxygen in the cell, and low levels of hydroxylation increase the expression of particular genes. For example, under hypoxic conditions, HIF is not hydroxylated, which leads to the upregulation of genes for glycolysis enzymes (allowing the production of ATP in the absence of oxygen), and vasoendothelial growth factor (promoting angiogenesis and therefore increased oxygen supply).[240, 241]

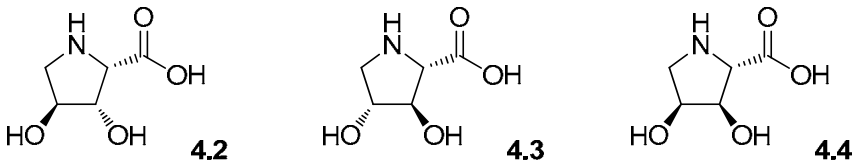
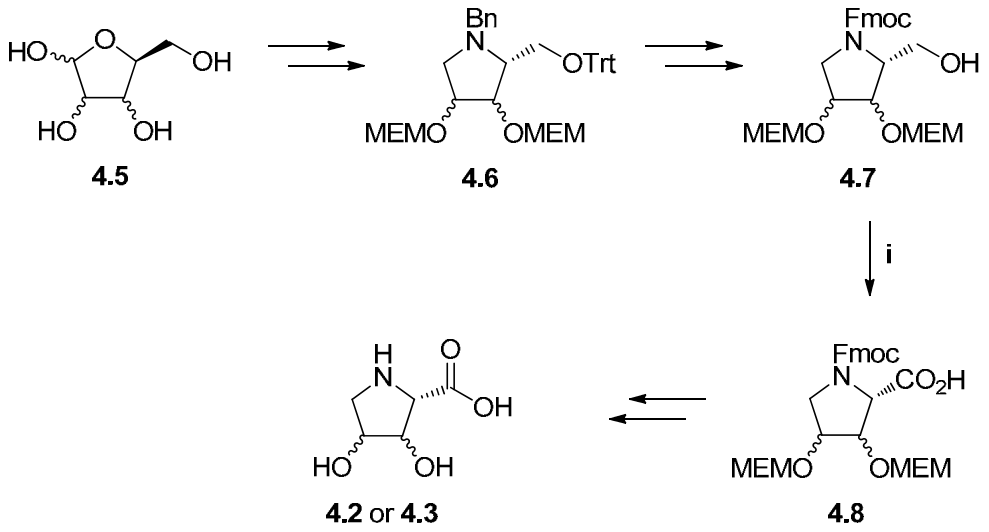


Figure 4.2 Naturally-occurring 3,4-dihydroxy-L-prolines.

Three 3,4-dihydroxy-L-prolines (DHPs) have been discovered from natural sources (**Figure 4.2**). The 2,3-*cis*-3,4-*trans*-DHP **4.2** was first isolated from the freshwater diatom *Navicula pelliculosa*.[242, 243] The 2,3-*trans*-3,4-*trans*-DHP **4.3** was isolated from the toxic peptides of the

Amanita virosa mushroom,[244] and found to be a potent and specific competitive inhibitor of β-D-glucuronidase. Despite being a five-membered ring, the stereochemistry matches that of the six-membered glucuronic acid, which is a component of mucopolysaccharides.[245] The 2,3-*trans*-3,4-*cis*-DHP **4.4** was isolated from the marine mussel *Mytilus edulis*;[246, 247] this amino acid is part of a repeating decapeptide unit found in Mefp1, *M. edulis* foot protein, which helps the organism to adhere to surfaces.

Taylor *et al.* synthesised **4.2** and **4.3** from L-arabinose and L-xylose respectively (**Scheme 4.1**); the stereochemistry of the carbohydrate starting material could in theory be varied to access any of the 8 stereoisomers of 3,4-dihydroxyproline.[248]

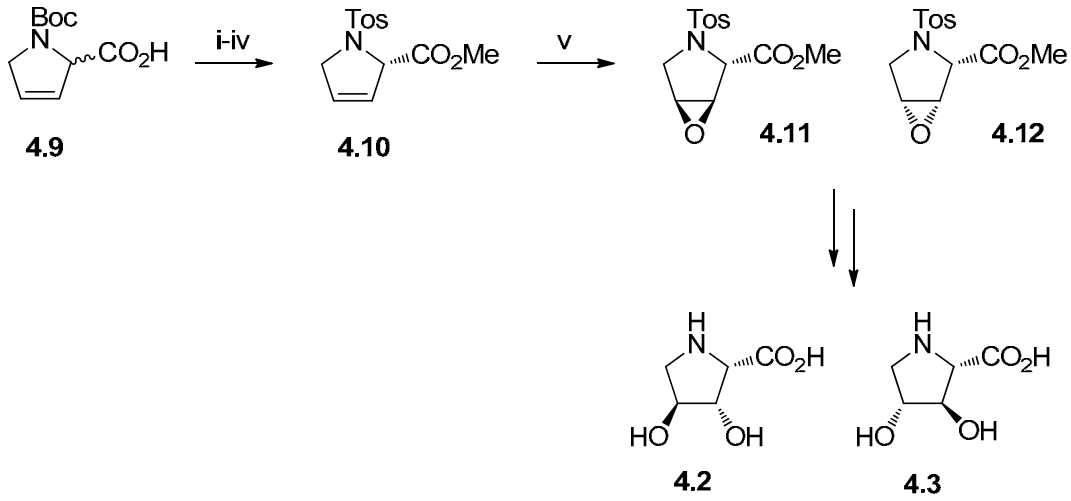


Selected reagents and conditions: (i) TEMPO, $NaClO_2$ bleach, MeCN, NaH_2PO_4 (aq)

Scheme 4.1 Synthesis of DHPs from L-arabinose or L-xylose.

The hexose **4.5** was converted to benzyl pyrrolidine **4.6** and then to the deprotected alcohol **4.7**. The primary alcohol was converted to the carboxylic acid **4.8** *via* a Swern oxidation followed by sodium chlorite oxidation of the aldehyde; deprotection furnished the dihydroxyprolines in an average yield of 27% over ten steps.

4.2 and **4.3** were also made from an achiral starting material by Kahl and Wieland; the racemic carboxylic acid **4.9** was resolved by crystallisation with a chiral amine (**Scheme 4.2**).[249]

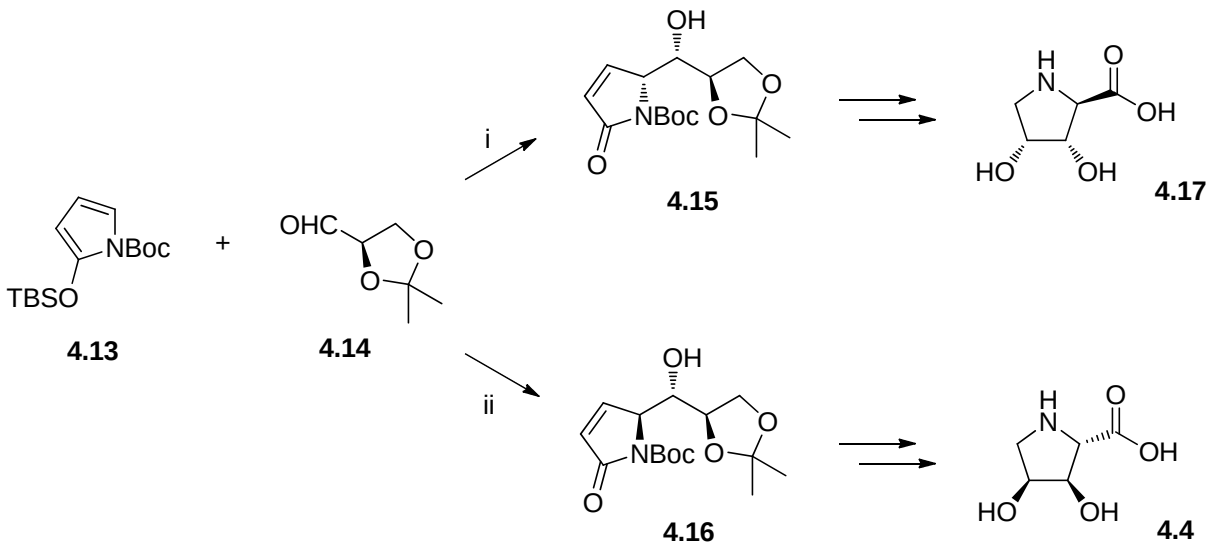


Selected reagents and conditions: (i) Crystallisation with *R*(+)-1-(4-nitrophenyl)ethanamine, then separation; (ii) removal of Boc; (iii) tosylation; (iv) diazomethane; (v) CF_3CO_3H.

Scheme 4.2 Resolution of achiral starting materials leading to enantiopure dihydroxyprolines.

Epoxidation of the double bond, followed by ring opening and deprotection, gave the separate enantiopure DHPs **4.2** and **4.3**.

The third natural product, **4.4**, has also been synthesised.[250, 251] The route published by Zanardi *et al.* (**Scheme 4.3**) allowed either the natural product **4.4** or it its D-enantiomer **4.17** to be made, simply by varying the Lewis acid used in the opening step, the condensation of D-glyceraldehyde **4.14** and protected pyrrole **4.13**.



Selected reagents and conditions: (i) $SnCl_4$, Et_2O, -85°C; (ii) $BF_3{\cdot}OEt_2$, Et_2O, -85°C; parallel syntheses thereafter.

Scheme 4.3 Stereoselective synthesis of both enantiomers of 2,3-*trans*-3,4-*cis*-dihydroxyproline.

4.3 Pyrrolidines as glycosidase inhibitors

There are many examples of natural and artificial pyrrolidine iminosugars acting as glycosidase inhibitors.

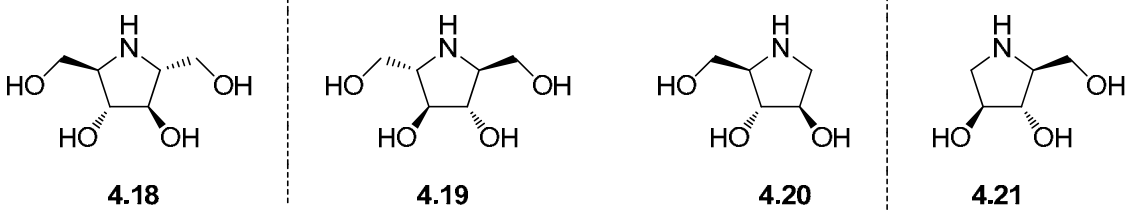


Figure 4.3 DMDP, L-DMDP, DAB, and LAB.

DMDP (2,5-dideoxy-2,5-imino-D-mannitol) **4.18** is a pyrrolidine iminosugar first isolated from the legume *Derris elliptica*[252] and subsequently found in many other plants. DMDP was discovered to be an inhibitor of α- and β-glucosidases.[253, 254] Its enantiomer L-DMDP **4.19** was synthesised and found to be a more potent and specific inhibitor of mammalian α-glucosidases

than the natural product.[255] Likewise, the iminosugar DAB (1,4-dideoxy-1,4-imino-D-arabinitol) **4.20**, first isolated from the fruits of *Angylocalyx boutiqueanus*,[256] is a potent inhibitor of yeast α-glucosidase[257] and a broad range of mammalian α-glycosidases;[258] its synthetic enantiomer LAB **4.21** was found to be a stronger inhibitor of digestive α-glucosidases than DAB.[259] Both L-iminosugars were found to be acting as non-competitive enzyme inhibitors, in contrast to the mode of action for the natural products.

4.3.1 Biological activity of pyrrolidine amides

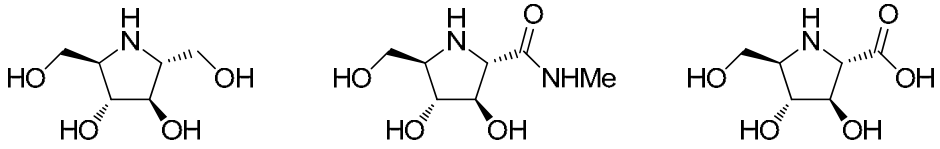


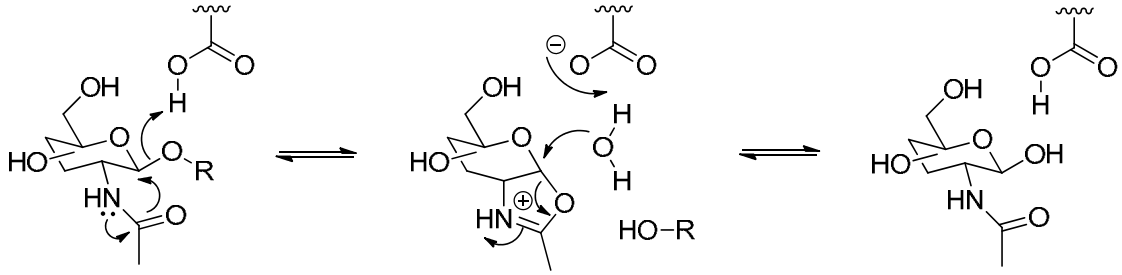
Figure 4.4 DMDP, DMDP methyl amide, and DMDP acid.

Two analogues of DMDP, the methyl amide **4.22** and the carboxylic acid **4.23**, were recently synthesised in the Fleet group.[260] The carboxylic acid did not show any activity except for weak α-glucosidase inhibition. The amide **4.22** was found to be a very good inhibitor of β-*N*-acetylglucosaminidases and -galactosaminidases, and was completely selective for these enzymes over α-*N*-acetylgalactosaminidases (see **Section 4.6, Table 4.1**). In general, this type of iminosugar can be prepared from carbohydrate starting materials, with nitrogen being introduced *via* nucleophilic attack of either a primary amine or an azide anion.[261]

4.3.2 N-*Acetylhexosaminidases as therapeutic targets*

N-Acetylhexosaminidases catalyse the hydrolysis of *N*-acetylhexosamine residues (GlcNAc and GalNAc) from the non-reducing end of oligosaccharides.[262] *N*-Acetylhexosamine residues are common components of mammalian glycans, as well as being found in bacterial cell walls.

β-*N*-Acetylhexosaminidases fall into two main classes, Family 3 and Family 20, which proceed *via* different mechanisms.[263] Family 3 enzymes hydrolyse terminal GlcNAc or GalNAc residues *via* a similar mechanism to the β-retaining glycosidases (**Chapter 1, Section 1.3.2**), whereby the substrate forms a covalently bonded intermediate with the enzyme. The mechanism for Family 20 enzymes involves neighbouring group participation of the acetyl group in the substrate, creating a cyclic oxazolinium ion intermediate (**Scheme 4.4**). This intermediate is subsequently hydrolysed by to give the product with retention of configuration at the anomeric centre.



Scheme 4.4 Mechanism for the action of Family 20 β-*N*-acetylhexosaminidases.

Cancer cells secrete high levels of β-*N*-acetylhexosaminidases, which degrade the extracellular matrix (ECM).[264, 265] The ECM is an array of proteins and glycoconjugates, containing a high proportion of *N*-acetylhexosamine residues. Degradation of the extracellular matrix enables cancer cells to reach the bloodstream and metastasise.[266, 267] Around 90% of cancer deaths are caused by metastases, rather than the primary tumour itself, so prevention of metastasis would slow disease progression and improve the chance of success for other therapies targeting the primary tumour.[268] Therefore the synthesis of *N*-acetylhexosaminidase inhibitors could constitute an approach to the treatment of cancer.

This type of glycosidase inhibitor could also have applications in chaperone-mediated therapy (**Chapter 1, Section 1.5.1.2**) in the treatment of Tay-Sachs disease and Sandhoff disease. These

are both lysosomal storage diseases that arise from defective versions of β-*N*-acetylhexosaminidases.

4.4 Synthetic targets

Work by other members of the Fleet group is in progress to synthesise and evaluate each stereoisomer of the biologically active amide **4.22** (16 stereoisomers, 8 pairs of enantiomers, motif **4.24**). It was also decided to synthesise some derivatives lacking the 5-hydroxymethyl group (*i.e.* analogues of DAB), including variations in stereochemistry (8 stereoisomers, 4 pairs of enantiomers, motif **4.25**).

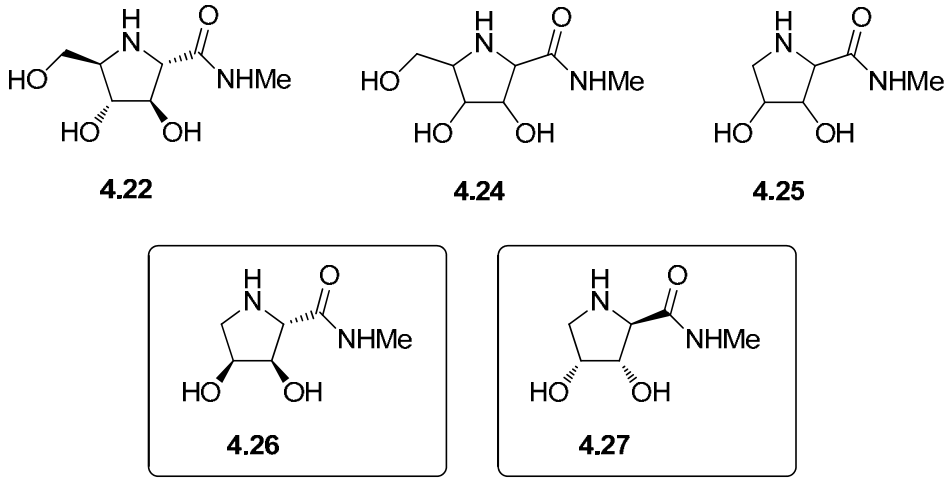


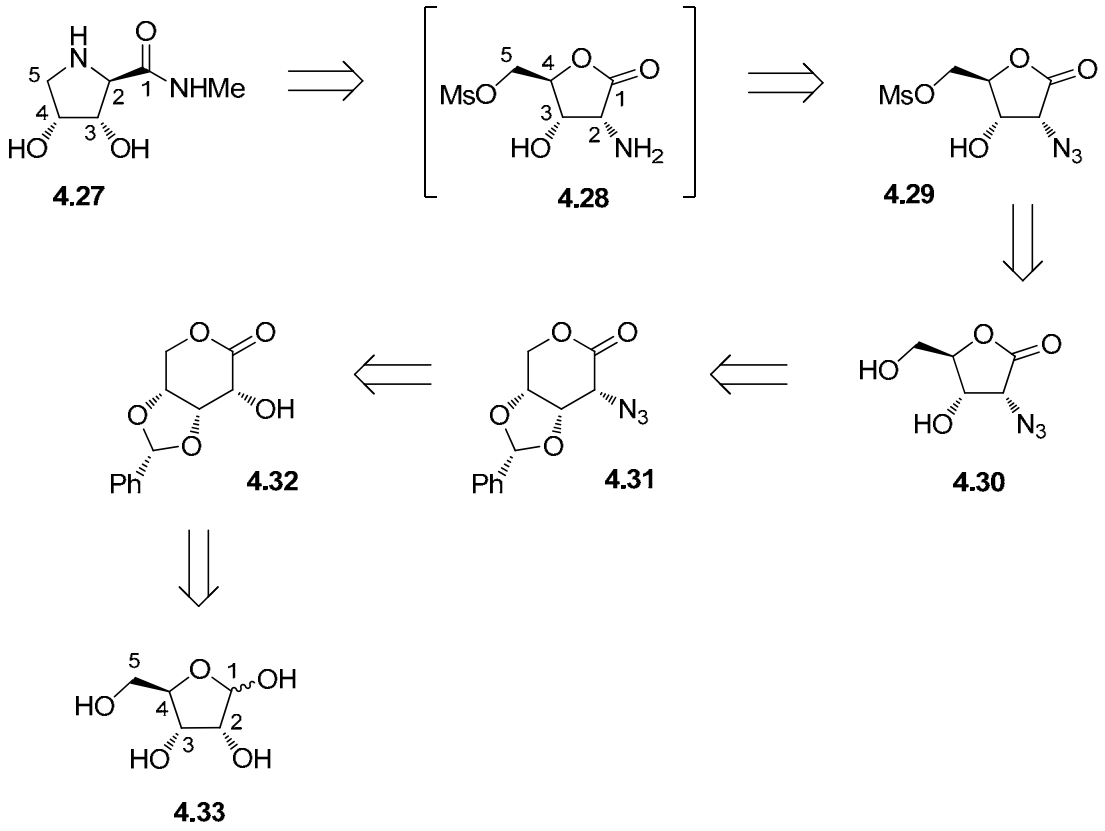
Figure 4.5 Iminosugar amide motifs, and target compounds.

The synthesis of an enantiomeric pair of dihydroxyproline amides is described in this chapter. I synthesised methyl (3*S*,4*R*)-dihydroxy-D-proline amide **4.27** from D-ribose, and the enantiomeric synthesis of **4.26** from L-ribose was completed by Michael Hollas.[269]

The synthesis and biological screening of all the compounds with structures **4.24** and **4.25** will elucidate the characteristics that are most important for successful *N*-acetylhexosaminidase inhibition, and may lead to the discovery of other good inhibitors.

4.4.1 Retrosynthesis

A reasonably short and simple retrosynthesis of the D-proline amide **4.27** from D-ribose **4.33** was envisaged (**Scheme 4.2**). Many of the intermediates are known compounds.[219]



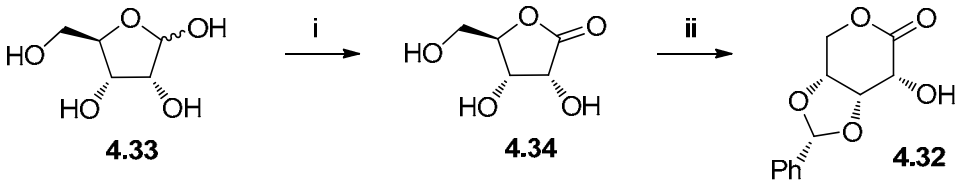
Scheme 4.5 Retrosynthesis of dihydroxyproline amide.

The target compound could be made *via* intramolecular cyclisation of an amine onto a leaving group such as mesylate; the amine **4.28** could be obtained by reduction of the known

azidomesylate **4.29**. This intermediate can be made by the deprotection and selective activation of azidolactone **4.31**, which in turn can be synthesised from D-ribose *via* Zinner's lactone **4.32**.

4.5 Synthesis of proline amide

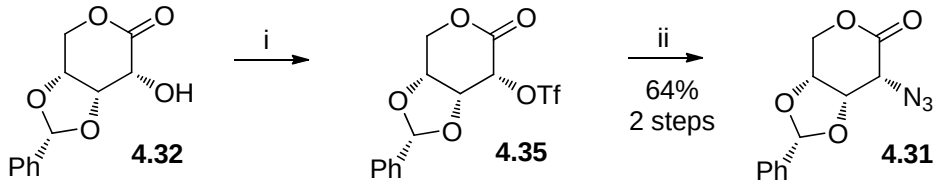
4.5.1 Formation of benzylidene azidolactone



Reagents and conditions: (i) Br_2, Na_2CO_3, H_2O, 0°C; (ii) PhCHO, conc. HCl, H_2O.

Scheme 4.6 Oxidation and protection of ribose.

Bromine oxidation of D-ribose **4.33** gave ribonolactone **4.34**, which was treated with benzaldehyde without prior isolation. This gave the protected 1,5-lactone **4.32**, known as Zinner's lactone, in which only the C-2 position is unprotected.[270, 271] Benzylidene protection typically results in formation of a 6-membered benzylidene with an equatorial phenyl group. In ribonolactone, however, this would result in a highly strained trans acetal, and so the 3,4-protected 1,5-lactone **4.32** is the product.



Reagents and conditions: (i) Tf_2O, py., -15°C; (ii) NaN_3, DMF.

Scheme 4.7 Triflate-azide displacement.

The protected ribonolactone **4.32** was triflated at the free hydroxyl; the crude triflate **4.35** could be purified by recrystallisation. However, better yields were obtained when the crude material was treated with sodium azide without prior purification (64% yield over two steps, compared to 54%). Displacement of the triflate group by azide proceeded with retention of configuration. This was confirmed by an earlier X-ray crystal structure of the azide **4.31** (**Figure 4.6**).[219]

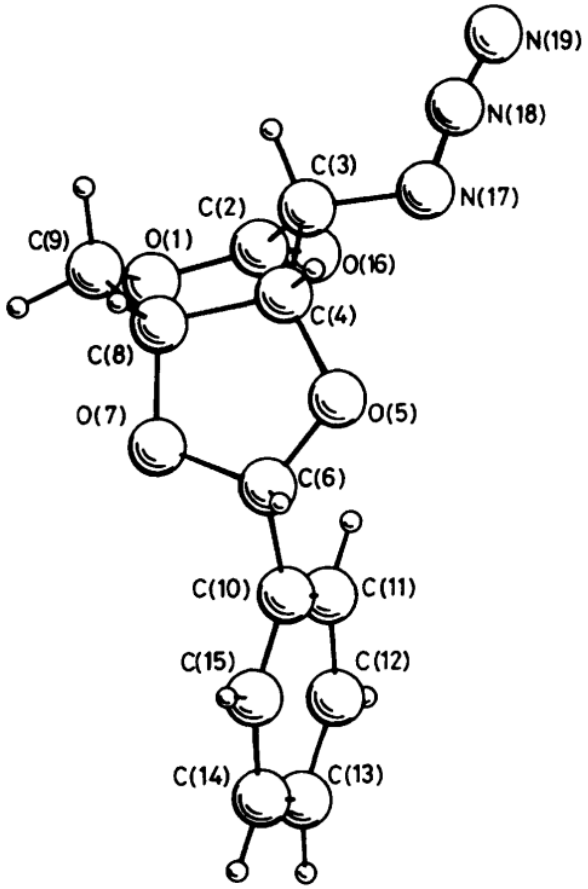
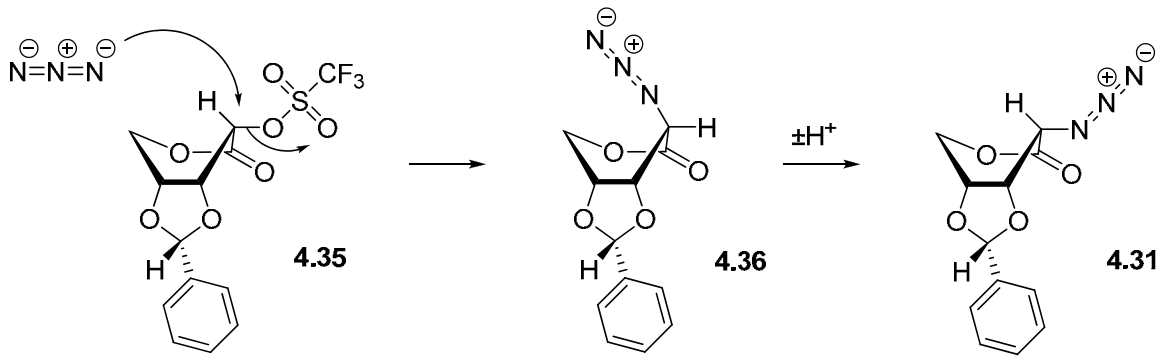


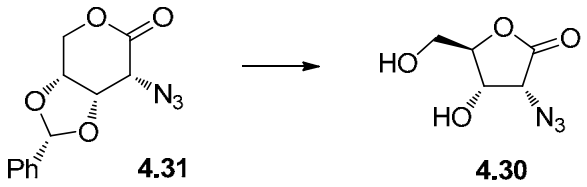
Figure 4.6 X-ray crystal structure of 2-azido-3,4-*O*-(*R*)-benzylidene-D-ribose. Reproduced by permission from RSC Publishing: *Journal of the Chemical Society, Perkin Transactions 1*, P. D. Baird *et al*., 1785-1791, 1987.

The displacement is thought to occur *via* S_N2 displacement of triflate by the azide, giving the inverted *arabino*-azide **4.36** (**Scheme 4.8**). The DMF solvent can act as a base to catalyse epimerisation to the more thermodynamically stable *ribo*-configured azidolactone **4.31**.[272]



Scheme 4.8 S_N2 followed by epimerization gives overall retention of configuration.

4.5.2 Deprotection and selective mesylation



Reagents and conditions: 2:1, TFA:H_2O, 50°C.

Scheme 4.9 Deprotection of benzylidene azide.

Acid-catalysed deprotection of the benzylidene group proceeded in 94% yield. Again, the structure of the product **4.30** was confirmed by an earlier X-ray crystal structure (**Figure 4.7**).[219] Without the steric demands of the benzylidene group, the kinetically favoured 1,4-lactone is obtained, rather than the 1,5-lactone.

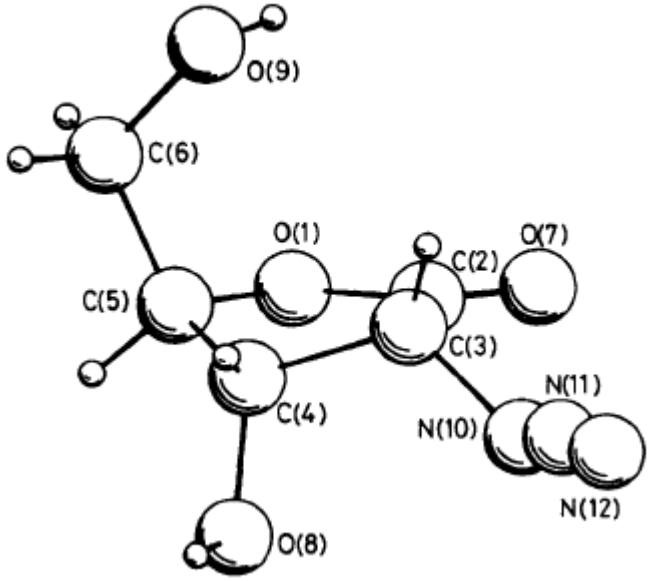
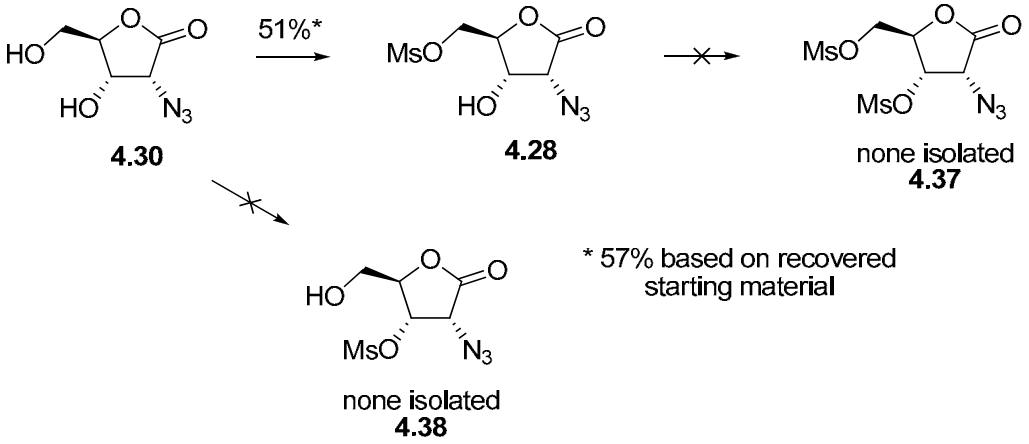


Figure 4.7 X-Ray crystal structure of 2-azido-2-deoxy-D-ribono-1,4-lactone. Reproduced by permission from RSC Publishing: *Journal of the Chemical Society, Perkin Transactions 1*, P. D. Baird *et al*., 1785-1791, 1987.

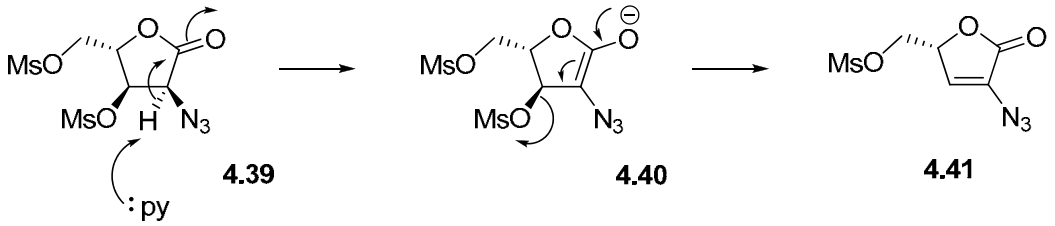
Selective mesylation of the diol **4.30** did not achieve very good yields. In the best case, treatment with 1.1 equivalents of mesyl chloride gave a yield of 51%, along with 10% recovered starting material (**Scheme 4.10**).



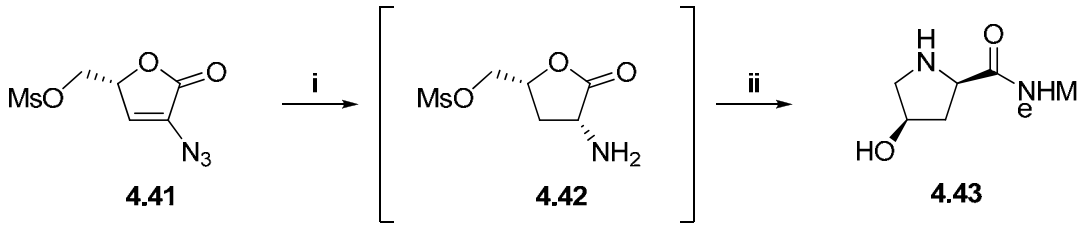
Reagents and conditions: MsCl, py., -10°C.

Scheme 4.10 Selective mesylation of the azidodiol.

The yield of primary mesylate **4.28** was 57% allowing for recovered starting material; none of the dimesylate **4.37** or secondary mesylate **4.38** were detected or isolated under these reaction conditions. The yield for mesylation of the enantiomer was also disappointing, at 31%. Attempts to optimise the reaction were not successful; for example, when the reaction time was extended, the α,β-unsaturated lactone **4.42** was obtained, presumably *via* dimesylation followed by E_1cB elimination. (**Scheme 4.11**). The elimination was catalysed by the pyridine solvent. Due to the insolubility of the diol starting material **4.40** in other solvents, the use of neat pyridine could not be avoided.



Scheme 4.11 Mechanism for the formation of unsaturated lactone.



Reagents and conditions: (i) Pd/C, H_2, 1,4-dioxane; (ii) $MeNH_2$, 1,4-dioxane.

Scheme 4.12 Formation of the (4*R*)-hydroxy-D-proline.

Interestingly, hydrogenation of the unsaturated azidolactone **4.41** followed by treatment with methylamine gave the single (4*R*)-hydroxy-D-proline compound **4.43** in 94% yield over two steps. The absolute stereochemistry of the product was already known from the ribose starting material, and the relative stereochemistry was shown by nOe. From preliminary modelling

(**Figure 4.8**), it appears that the amide group is pseudo-equatorial, *i.e.* the thermodynamically more stable product is obtained.[273]

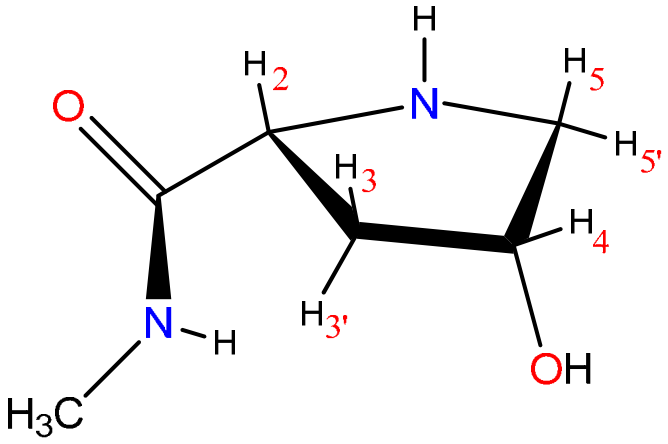
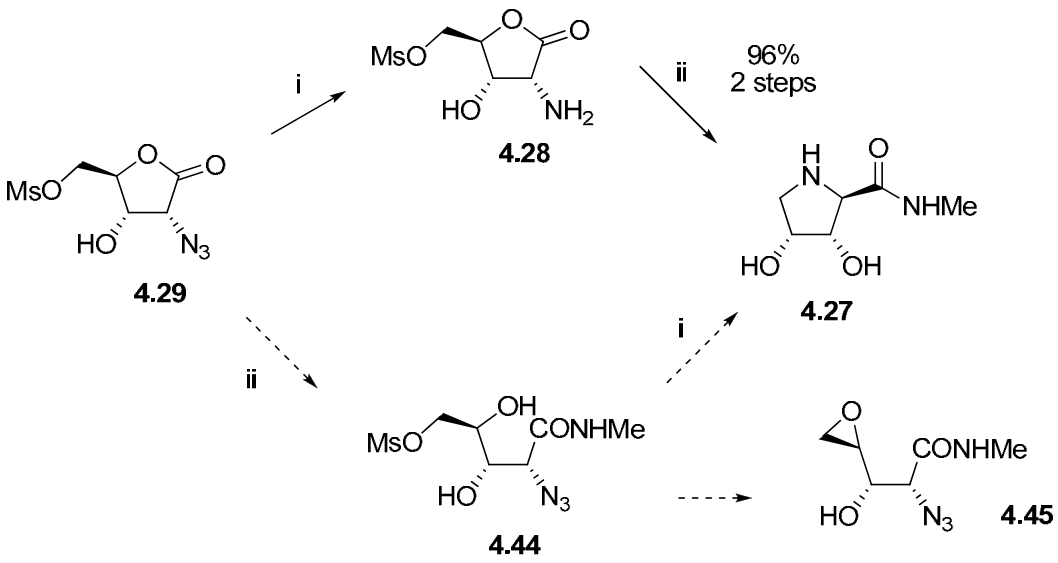


Figure 4.8 Conformation of (4*R*)-hydroxyl D-proline amide according to molecular modelling.

4.5.3 Formation of the dihydroxyproline

This section resumes focus on the synthetic route to dihydroxyproline **4.27** *via* the intended primary mesylate **4.29** (**Scheme 4.13**).



Reagents and conditions: (i) Pd/C, H_2, 1,4-dioxane; (ii) $MeNH_2$, 1,4-dioxane.

Scheme 4.13 Possible routes to the target proline amide.

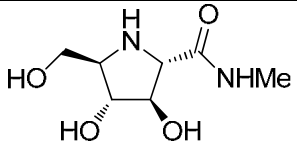
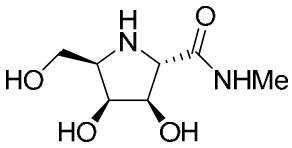
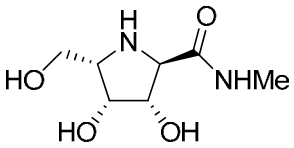
Following hydrogenation of the azide group, the resulting aminomesylate **4.28** was treated with methylamine. This opened the lactone, allowing intramolecular nucleophilic attack of the C-2 amine on the C-5 mesylate to give the target product **4.27**.

Unfortunately, the aminomesylate **4.28** was very unstable: when the filtrate was concentrated *in vacuo* prior to the next step, even without heating, none of the target compound was obtained. Reversal of the last two steps, to avoid this unstable compound, was considered (**Scheme 4.13**). However, this route introduced the possibility that under the basic methylamine conditions, the displacement of mesylate by the adjacent alcohol group to give epoxide **4.45** would compete with the desired attack by the amine. Fortunately, it was found that immediate treatment of the aminomesylate **4.28** with methylamine, without concentration of the filtrate, gave the dihydroxyproline in high yield (96% over two steps). The overall yield for this synthesis was 33% over six steps from the benzylidene ribonolactone **4.32**.

4.6 Results of Biological Testing

The enantiomeric dihydroxyproline amides **4.26** and **4.27** were screened against several *N*-acetyl-hexosaminidases (**Table 4.2**). The enzyme assays were carried out by Prof. Atsushi Kato at the University of Toyama. For comparison purposes, results for the related 5-hydroxymethyl compounds are included first (**Table 4.1**); compound **4.22** was prepared by Sarah Jenkinson, and the enantiomers **4.46** and **4.47** were prepared by Benjamin Ayers.

Concentration of iminosugars giving 50% inhibition of various hexosaminidases

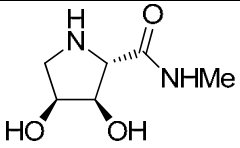
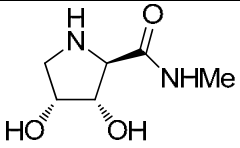
Enzyme	IC_{50} (μM)		
	 **4.22**	 **4.46**	 **4.47**
β-*N*-Acetyl-glucosaminidase			
Human placenta	0.20	0.92	NI (34.6%)
Bovine kidney	0.22	0.69	NI (33.5%)
Jack beans	0.30	0.15	409
HL-60	ND[a]	ND	ND
A. oryzae	0.033	32	NI (11.0%)
α-*N*-Acetyl-galactosaminidase			
Charonia lampas	NI[a]	ND	ND
Chicken liver	NI (0%)[b]	312	NI (2.9%)
β-*N*-Acetyl-galactosaminidase			
HL-60	0.20	ND	ND
A. oryzae	0.35	25	NI (11.0%)

[a] ND: not determined; [b] NI: no inhibition (less than 50% at 1000 μM); [c] ( ): inhibition % at 1000 μM.

Table 1 Results of enzyme assays for iminosugar amides.

Compound **4.22**, the amide derivative of the natural product DMDP, was a highly potent inhibitor of β-GlcNAcase and β-GalNAcase, and was completely selectivity for these enzymes over α-GalNAcase. The C-4 epimer **4.46** retained this potency against β-GlcNAcase, although it was a tenfold weaker inhibitor of β-GalNAcase; it was also less selective that **4.22** for the β-enzymes, showing weak activity against α-GalNAcase. Its enantiomer **4.47** was inactive, apart from weak inhibition of jack bean β-GlcNAcase.

Concentration of iminosugars giving 50% inhibition of various hexosaminidases

Enzyme	IC_{50} (μM)	
	 **4.26**	 **4.27**
β-*N*-Acetylglucosidaminidase		
Human placenta	7.6	NI (0%)
Bovine kidney	3.6	NI (7.3%)
Jack beans	4.6	NI (6.1%)
HL-60	11	NI (11.0%)
A. oryzae	210	NI (0.4%)
α-*N*-Acetylgalactosidaminidase		
Chicken liver	NI[a] (0%)[b]	NI (0%)
β-*N*-Acetylgalactosidaminidase		
HL-60	26	NI (0%)
A. oryzae	170	NI (2.6%)

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

Table 2 Results of enzyme assays for dihydroxyproline amides.

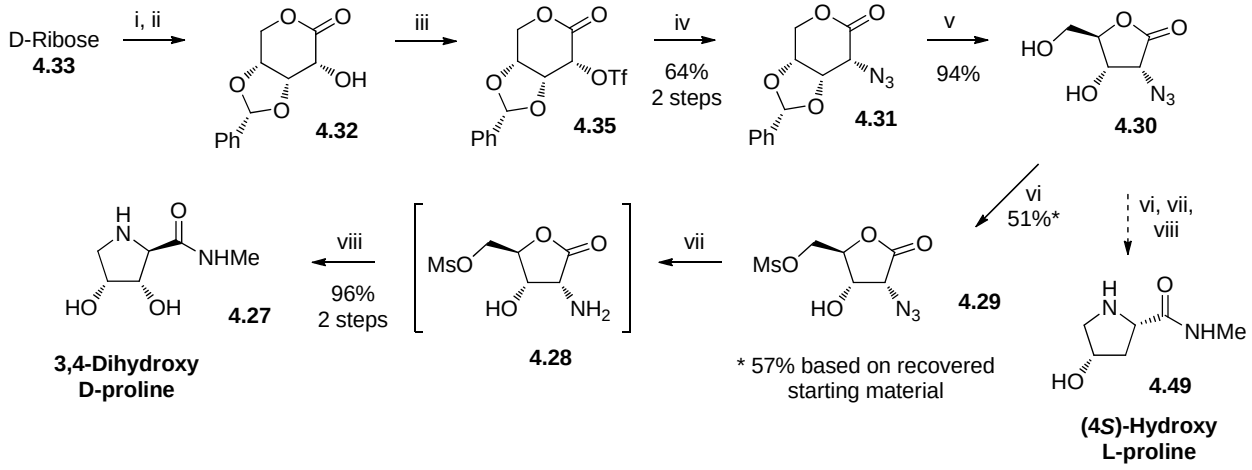
Moving on to the compounds described earlier in the chapter, these also showed a similar pattern of activity. The L-proline amide **4.26** was a good inhibitor of β-GlcNAcase and β-GalNAcase, but the D-proline enantiomer **4.27** was inactive against all the enzymes tested.

The assay results show two trends. First, the hydroxyl group at C-5 is important for inhibition of this class of enzymes: **4.26** is around ten times less active than **4.46**, and **4.27** lacks even the weak activity of **4.47**. Second, the structures based on natural products (D-sugars, L-amino acids)

were biologically active but disappointingly the enantiomeric compounds showed very little activity, in contrast to the pattern shown by DMDP/L-DMDP and DAB/LAB.

4.7 Conclusion and Future Work

This chapter has described the synthesis of the enantiomeric dihydroxyproline amides **4.26** and **4.27**. They were synthesised from D-ribose in eight steps and screened for activity against *N*-acetyl-hexosaminidases. The L-proline derivative **4.26** was a good inhibitor of β-*N*-acetylhexosaminidases, whereas the enantiomer **4.27** showed no inhibition against any of the *N*-acetylhexosaminidases tested. These results contribute to a larger project on pyrrolidine iminosugar amides, helping to elucidate which functional groups and stereochemistry are needed for the successful inhibition of this class of enzymes.



Reagents and conditions: (i) Br_2, Na_2CO_3, H_2O, 0°C; (ii) PhCHO, conc. HCl, H_2O; (iii) Tf_2O, py., -15°C; (iv) NaN_3, DMF; (v) 2:1, TFA:H_2O, 50°C; (vi) MsCl, py., -10°C; (vii) Pd/C, H_2, 1,4-dioxane; (viii) $MeNH_2$, 1,4-dioxane.

Scheme 4.14 The synthesis of methyl (3*S*,4*R*)-dihydroxy-D-proline amide from D-ribose.

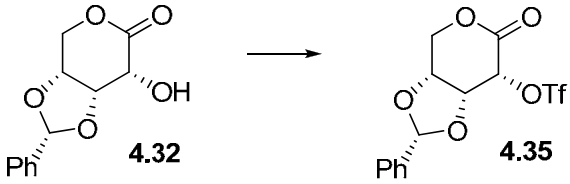
4.8 Experimental

4.8.1 General Experimental

For details of the general experimental procedures used, see **Chapter 2, pages 55-57**.

4.8.2 Experimental

3,4-*O*-(*R*)-Benzylidene-2-*O*-trifluoromethanesulfonyl-D-ribose

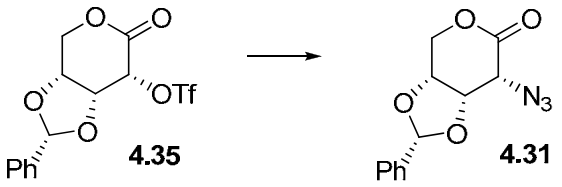


3,4-*O*-Benzylidene-D-ribose **4.32** (1.59 g, 6.74 mmol) was suspended in dry pyridine (20 mL) under an atmosphere of argon, and cooled to -15°C. Trifluoromethanesulfonic anhydride (1.70 mL, 10.1 mmol, 1.5 eq) was added slowly to the stirred solution, such that the temperature did not exceed -10°C. After 2 h, t.l.c. (EtOAc) indicated complete consumption of starting material (R_f 0.20) and formation of a single product (R_f 0.76). The mixture was concentrated *in vacuo* and the residue partitioned between EtOAc (30 mL) and 2 M HCl (aq). The aqueous layer was extracted with EtOAc (2 × 30 mL) and the organic fraction combined, dried ($MgSO_4$) and concentrated *in vacuo* to give the crude 3,4-*O*-(*R*)-benzylidene-2-*O*-trifluoromethanesulfonyl-D-ribose **4.35** as a white solid.

m.p. 164-166°C (decomp.) [Lit.:[219] m.p. 172-173°C (decomp.)]; $[\alpha]_D^{25}$ -121.0 (*c* 0.54, EtOAc) [Lit.:[219] $[\alpha]_D^{23}$ -128.6 (*c* 0.56, EtOAc)]; IR ν_{max} (thin film): 1781 (s, C=O); δ_H (400 MHz, $(CD_3)_2SO$): 7.49-7.38 (m, 5H; C_6H_5), 6.30 (br d, $J_{2,3}$ 3.6, 1H; H-2), 5.87 (s, 1H; C*H*Ph), 5.09 (br dd, $J_{2,3}$ 3.6, $J_{3,4}$ 8.2, 1H; H-3), 4.84 (a-br d, $J_{3,4}$ 8.2, 1H; H-4), 4.75 (br dd, $J_{4,5}$ 1.0, $J_{5,5'}$ 13.3, 1H; H-5), 4.54 (a-br d, $J_{5,5'}$ 13.3, 1H; H-5'); δ_C ($(CD_3)_2SO$, 100 MHz): 164.8 (C-1), 135.2 (*ipso*-Ar),

130.2 (*p*-ArH), 128.5, 128.4, 127.1, 127.1 (2 × *o*-ArH, 2 × *m*-ArH), 103.5 (*C*HPh), 80.9 (C-2), 74.1 (C-3), 73.6 (C-4), 67.8 (C-5); LRMS (ESI^+): *m/z* (%) 391.0 (100) $[M + Na]^+$.

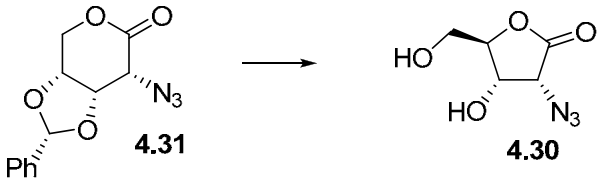
2-Azido-3,4-*O*-(*R*)-benzylidene-2-deoxy-D-ribose



The crude 3,4-*O*-(*R*)-benzylidene-2-*O*-trifluoromethanesulfonyl-D-ribose **4.35** was dissolved in *N,N*-dimethylformamide (15 mL) under an atmosphere of argon. Sodium azide (0.768 g, 11.8 mmol, 1.75 eq) was added and the solution stirred for 2 h. The reaction mixture was concentrated *in vacuo* and the residue partitioned between EtOAc (30 mL) and water (30 mL). The organic fraction was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude was recrystallised (EtOAc/cyclohexane) to yield 2-azido-3,4-*O*-(*R*)-benzylidene-D-ribose **4.31** (1.12 g, 64% over 2 steps) as a white crystalline solid.

m.p. 148-150°C (decomp) [Lit.:[274] m.p. 145°C (decomp)]; $[\alpha]_D^{25}$ -225.9 (*c* 0.54, EtOAc) [Lit.:[274] $[\alpha]_D^{20}$ -245 (*c* 0.42, EtOAc)]; IR ν_{max} (thin film): 2111 (s, N_3), 1756 (s, C=O); δ_H (400 MHz, $CDCl_3$): 7.52-7.38 (m, 5H; C_6H_5), 5.77 (s, 1H; C*H*Ph), 4.90 (dd, $J_{2,3}$ 3.3, $J_{3,4}$ 8.0, 1H; H-3), 4.66 (dd, $J_{4,5'}$ 1.8, $J_{3,4}$ 8.0, 1H; H-4), 4.61 (d, $J_{5,5'}$ 13.4, 1H; H-5), 4.24 (dd, $J_{4,5'}$ 1.7, $J_{5,5'}$ 13.4, 1H; H-5'), 3.94 (d, $J_{2,3}$ 3.3, 1H; H-2); δ_C ($CDCl_3$, 100 MHz): 166.4 (C-1), 134.4 (*ipso*-C_6H_5), 130.3, 130.3, 128.6, 128.5, 127.1 (5 × *o/m/p*-C_6H_5), 104.6 (*C*HPh), 76.3 (C-4), 73.1 (C-3), 67.3 (C-5), 58.9 (C-2); LRMS (ESI^+): *m/z* (%) 284.1 (100) $[M + Na]^+$; HRMS (ESI^+) found 284.0639 $[M + Na]^+$; $C_{12}H_{11}N_3NaO_4$ requires 284.0642.

2-Azido-2-deoxy-D-ribose

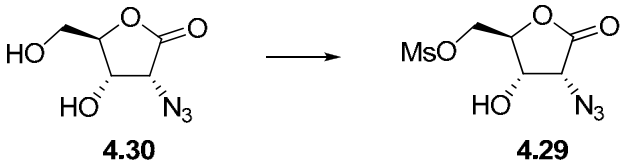


4.31 **4.30**

2-Azido-3,4-*O*-(*R*)-benzylidene-2-deoxy-D-ribose **4.31** (1.11 g, 4.23 mmol) was dissolved in trifluoroacetic acid (67% *v/v* in water) (21 mL) and stirred for 1.5 h at 50°C. The solution was concentrated *in vacuo* and the residue purified by flash column chromatography (4:3 → 2:1 → 1:1, cyclohexane:EtOAc) to yield 2-azido-2-deoxy-D-ribose **4.30** (0.691 g, 94%) as a white crystalline solid.

m.p. 86-88°C [Lit.:[274] m.p. 84-85°C]; $[\alpha]_D^{25}$ +50.2 (*c* 0.38, EtOAc) [Lit.:[274] $[\alpha]_D^{20}$ +54.2 (*c* 0.28, EtOAc)]; IR ν_{max} (thin film): 3396 (br, O-H), 2106 (s, N_3), 1766 (s, C=O); δ_H (400 MHz, $(CD_3)_2CO$): 5.28 (br s, 1H; 3-OH or 5-OH), 4.64 (d, $J_{2,3}$ 5.1, 1H; H-3), 4.51 (dd, $J_{4,5}$ 2.9, $J_{4,5'}$ 3.1, 1H; H-4), 4.47 (d, $J_{2,3}$ 5.1, 1H; H-2), 3.88-3.84 (m, 2H; H-5 and H-5'), 3.24 (v br s, 1H; 3-OH or 5-OH); δ_C ($(CD_3)_2SO$, 100 MHz): 173.6 (C-1), 87.6 (C-4), 71.3 (C-3), 61.4, 61.2 (C-2 and C-5); LRMS (ESI^+): *m/z* (%) 191.1 (100) $[M + NH_4]^+$; HRMS (ESI^+) found 196.0331 $[M + Na]^+$; $C_5H_7N_3NaO_4$ requires 196.0329.

2-Azido-2-deoxy-5-*O*-methanesulfonyl-D-ribose



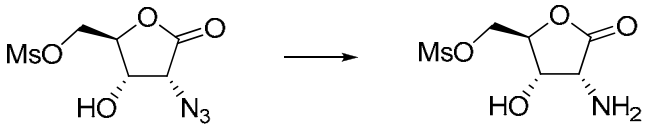
4.30 **4.29**

2-Azido-2-deoxy-D-ribose **4.30** (1.44 g, 8.31 mmol, 1 eq) was dissolved in dry pyridine (40 mL) and cooled to -10°C. Methanesulfonyl chloride (0.71 mL, 9.2 mmol, 1.1 eq) was added slowly to the stirred solution, such that the temperature did not exceed -5°C. After 3 h t.l.c.

(1:2, cyclohexane:EtOAc) indicated a small amount of starting material (R_f 0.35) and formation of a major product (R_f 0.42). The mixture was concentrated *in vacuo*, redissolved in EtOAc (100 mL), and the solution washed successively with 2 M HCl (aq) (50 mL), water (50 mL), and $NaHCO_3$ (aq) (50 mL). The organic fraction was dried (Na_2SO_4) and concentrated *in vacuo*. The crude was purified by flash column chromatography on silica gel (1:1 → 1:2 → 1:3, cyclohexane:EtOAc) to afford 2-azido-2-deoxy-5-*O*-methanesulfonyl-D-ribose **4.29** (1.07 g, 51%) (57% based on recovered starting material) as a white crystalline solid.

m.p. 68-70°C [Lit.:[274] m.p. 68-69°C]; $[\alpha]_D^{25}$ +40.4 (*c* 0.48, EtOAc) [Lit.:[274] $[\alpha]_D^{20}$ +37.3 (*c* 0.48, EtOAc)]; IR ν_{max} (thin film): 3446 (br, O-H), 2110 (s, N_3), 1780 (s, C=O); δ_H (400 MHz, $CDCl_3$): 4.68 (a-dt, $J_{3,4}$ 1.5, $J_{4,5} \approx J_{4,5'}$ 2.6, 1H; H-4), 4.52 (ddd, $J_{3,4}$ 1.4, $J_{3,OH}$ 2.7, $J_{2,3}$ 5.5, 1H; H-3), 4.50-4.45 (m, 3H; H-2, H-5, and H-5'), 3.09 (s, 3H; SO_2CH_3), 2.68 (br d, $J_{3,OH}$ 2.7, 1H; 3-OH); δ_H ($CDCl_3$, 100 MHz): 175.5 (C-1), 86.6 (C-4), 73.3 (C-3), 71.5 (C-5), 64.1 (C-2), 41.3 (SO_2CH_3); LRMS (ESI^+): *m/z* (%) 274.1 (100) $[M + Na]^+$; HRMS (ESI^+) found 252.2089 $[M + H]^+$; $C_6H_{10}N_3O_6S$ requires 252.2090.

2-Amino-2-deoxy-5-*O*-methanesulfonyl-D-ribose

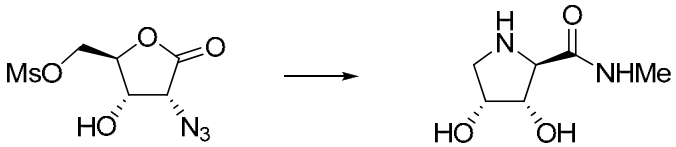


4.29 **4.28**

2-Azido-2-deoxy-5-*O*-methanesulfonyl-D-ribose **4.29** (26 mg, 0.102 mmol, 1 eq) was dissolved in 1,4-dioxane (3 mL) under an atmosphere of argon. 10% Pd/C (20% by mass, 5.6 mg) was added to the stirred solution. The reaction vessel was degassed and the reaction mixture placed under an atmosphere of hydrogen. After 1.5 h, mass spectroscopy detected the product 2-amino-2-deoxy-5-*O*-methanesulfonyl-D-ribose **4.28** (226: $[M + H]^+$) and no starting material. The

reaction mixture was filtered through Celite® and reacted on without being concentrated *in vacuo*.

Methyl (3*S*,4*R*)-dihydroxy-D-proline amide



4.28 **4.27**

The crude solution of 2-amino-2-deoxy-5-*O*-methanesulfonyl-D-ribose **4.28** in 1,4-dioxane was treated with methylamine (33% wt. in EtOH, 0.06 mL, 0.46 mmol, 4 eq). The solution was stirred for 20 h, whereupon mass spectrometry detected product (161: $[M + H]^+$) and no starting material. The solution was concentrated *in vacuo* and the residue purified using ion exchange resin Dowex 50WX8-200 (H^+ form) and aqueous 2 M ammonium hydroxide solution as an eluent to afford methyl (3*S*,4*R*)-dihydroxy-D-proline amide **4.27** (16 mg, 96%) as a white solid.

m.p. 150-152°C; $[\alpha]_D^{25}$ -17.0 (*c* 0.31, H_2O) [enantiomer:[269] $[\alpha]_D^{25}$ +14.0 (*c* 0.27, H_2O)]; IR ν_{max} (thin film): 3272 (br, O-H/N-H), 1673 (s, C=O); δ_H (400 MHz, D_2O, HCl salt): 4.34 (a-dt, $J_{4,5'}$ 2.1, $J_{3,4} \approx J_{4,5}$ 3.9, 1H; H-4), 4.29 (br dd, $J_{3,4}$ 3.9, $J_{2,3}$ 7.9, 1H; H-3), 4.08 (d, $J_{2,3}$ 8.0, 1H; H-2), 3.53 (dd, $J_{4,5}$ 3.8, $J_{5,5'}$ 12.9, 1H; H-5), 3.36 (dd, $J_{4,5'}$ 1.9, $J_{5,5'}$ 12.8, 1H; H-5'), 2.76 (br s, 3H; NHCH_3); δ_C (125 MHz, D_2O, HCl salt, ref. 1,4-dioxane): 168.0 (C-1), 74.7 (C-3), 69.9 (C-4), 61.3 (C-2), 50.2 (C-5), 26.2 ($NHCH_3$); LRMS (ESI^+): *m/z* (%) 161.10 (100) $[M + H]^+$; HRMS (ESI^+) found 161.0928 $[M + H]^+$; $C_6H_{13}N_2O_3$ requires 161.0926.

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Appendix

Publications arising from this thesis

Sarah F. Jenkinson, Loren L. Parry, Francis X. Wilson, George W. J. Fleet, David J. Watkin **6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone** *Acta Crystallographica Section E* **2011**, *67*, o2531-o2532

Papers concerning the work described in Chapters 2 and 4 have been drafted for publication.

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6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone

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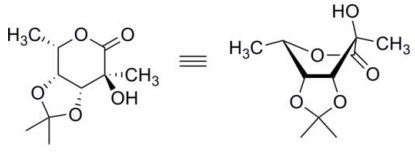
Key indicators: single-crystal X-ray study; T = 150 K; mean σ(C–C) = 0.003 Å; R factor = 0.038; wR factor = 0.090; data-to-parameter ratio = 10.7.

X-ray crystallography unequivocally confirmed the stereochemistry of the 2-*C*-methyl group in the title molecule, $C_{10}H_{16}O_5$, in which the 1,5-lactone ring exists in a boat conformation. The absolute stereochemistry was determined by the use of D-ribose in the synthesis. The crystal exists as O—H···O hydrogen bonded chains of molecules running parallel to the *a* axis with each molecule acting as a donor and acceptor for one hydrogen bond.

Related literature

For branched iminosugars, see: Håkansson *et al.* (2007, 2008); Asano *et al.* (2000); da Cruz *et al.* (2011); Best *et al.* (2010) and for branched sugars, see: Booth *et al.* (2008, 2009); da Cruz *et al.* (2008); Hotchkiss *et al.* (2006, 2007); Jenkinson *et al.* (2007); Jones *et al.* (2007, 2008); Rao *et al.* (2008). For conformations of related 1,5-lactones, see: Baird *et al.* (1987); Booth *et al.* (2007*a*,*b*); Bruce *et al.* (1990); Punzo *et al.* (2005, 2006); Dai *et al.* (2010).



Experimental

Crystal data

$C_{10}H_{16}O_5$
M_r = 216.23
Orthorhombic, $P2_12_12_1$
a = 6.1132 (2) Å
b = 12.2963 (4) Å
c = 14.6367 (5) Å
V = 1100.24 (6) Å^3
Z = 4
Mo $K\alpha$ radiation
μ = 0.11 mm^{-1}
T = 150 K
0.20 × 0.20 × 0.04 mm

Data collection

Nonius KappaCCD diffractometer
Absorption correction: multi-scan (*DENZO*/*SCALEPACK*; Otwinowski & Minor, 1997)
T_{min} = 0.95, T_{max} = 1.00
8628 measured reflections
1454 independent reflections
1131 reflections with $I > 2\sigma(I)$
R_{int} = 0.067

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.038
$wR(F^2)$ = 0.090
S = 0.89
1454 reflections
136 parameters
H-atom parameters constrained
$\Delta\rho_{max}$ = 0.40 e Å^{-3}
$\Delta\rho_{min}$ = −0.29 e Å^{-3}

Table 1
Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
O11—H111···O1^{i}	0.86	1.93	2.793 (3)	172

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

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

Supplementary data and figures for this paper are available from the IUCr electronic archives (Reference: LH5321).

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supplementary materials

Acta Cryst. (2011). E**67**, o2531-o2532 [**doi:10.1107/S1600536811034957**]

6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone

S. F. Jenkinson, L. L. Parry, F. X. Wilson, G. W. J. Fleet and D. J. Watkin

Comment

2-*C*-Methyl branched sugars, as well as being chirons for the enantiospecific synthesis of complex targets (Hotchkiss *et al.*, 2006; Hotchkiss *et al.*, 2007; da Cruz *et al.*, 2008; Booth *et al.*, 2009) including 2'-*C*-methyl nucleosides (Jenkinson *et al.*, 2007), are a class of rare sugars with chemotherapeutic potential (Rao *et al.*, 2008; Jones *et al.*, 2008; Booth *et al.*, 2008). Branched iminosugars have also been shown to exhibit interesting biological activity. For example: 6-*C*-methyl-swainsonine is a more potent inhibitor of *L*-rhamnosidase than *L*-swainsonine (Håkansson *et al.*, 2007; Håkansson *et al.*, 2008; Asano *et al.*, 2000); 4-*C*-methylDAB and 4-*C*-methylLAB are both potent and specific α-glucosidase inhibitors (da Cruz *et al.* 2011) and isoLAB has been shown to partially rescue the defective F508del-CFTR function and therefore may have a role in the study of cyctic fibrosis (Best *et al.*, 2010).

D-ribose **1** was converted by a number of steps to the lactols **2** (Fig. 1). The reaction of **2** with sodium cyanide in water gave a chain extension to afford a single isolated crystalline product **3** (Fig. 2).

3,4-*O*-Isopropylidene-1,5-lactones, such as **3**, invariably crystallize in boat conformations (Baird *et al.*, 1987; Bruce *et al.*, 1990; Punzo *et al.*, 2005). The diastereoselectivity of the reaction may be rationalized by the formation of the lactone **3** with less steric congestion (Punzo *et al.*, 2006; Booth *et al.*, 2007*a*; Booth *et al.*, 2007*b*; Dai *et al.* 2010) with the smaller hydroxy group rather than the methyl group in the flagpole position (Fig. 2). The structure of **3** was confirmed by the X-ray crystallographic analysis. The absolute configuration was assigned from the use of D-ribose as the starting material. The title compound exists as O—H···O hydrogen bonded chains of molecules running parallel to the *a*-axis (Fig. 3). Each molecule acts as a donor and acceptor for 1 hydrogen bond. Only classical hydrogen bonding has been considered.

Experimental

The title compound was recrystallized by diffusion from a mixture of ethyl acetate and cyclohexane: m.p. 369–371 K; $[\alpha]_D^{25}$ -84.6 (*c*, 1.03 in $CHCl_3$).

Refinement

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

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

Figures

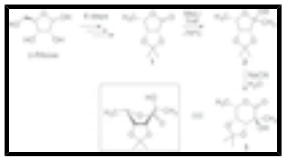

Fig. 1. Synthetic Scheme.

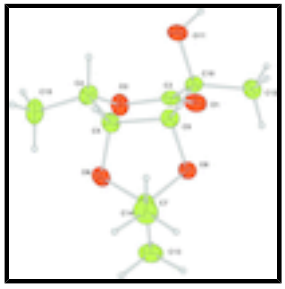

Fig. 2. The title compound with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as spheres of arbitary radius.

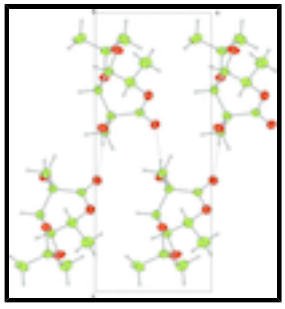

Fig. 3. Packing diagram of the title compound projected along the *b*-axis. Hydrogen bonds are shown by dotted lines.

6-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-galactono-1,5-lactone

Crystal data

$C_{10}H_{16}O_5$	$F(000) = 464$
$M_r = 216.23$	$D_x = 1.305$ Mg m^{-3}
Orthorhombic, $P2_12_12_1$	Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å
Hall symbol: P 2ac 2ab	Cell parameters from 1433 reflections
$a = 6.1132$ (2) Å	$\theta = 5$–27°
$b = 12.2963$ (4) Å	$\mu = 0.11$ mm^{-1}
$c = 14.6367$ (5) Å	$T = 150$ K
$V = 1100.24$ (6) Å^3	Plate, colourless
$Z = 4$	0.20 × 0.20 × 0.04 mm

Data collection

Nonius KappaCCD diffractometer	1131 reflections with $I > 2\sigma(I)$
graphite	$R_{int} = 0.067$
ω scans	$\theta_{max} = 27.5°$, $\theta_{min} = 5.2°$
Absorption correction: multi-scan (*DENZO*/*SCALEPACK*; Otwinowski & Minor, 1997)	$h = -7 \rightarrow 7$
$T_{min} = 0.95$, $T_{max} = 1.00$	$k = -15 \rightarrow 15$

8628 measured reflections
1454 independent reflections

$l = -18 \rightarrow 19$

Refinement

Refinement on F^2

Least-squares matrix: full

$R[F^2 > 2\sigma(F^2)] = 0.038$

$wR(F^2) = 0.090$

$S = 0.89$

1454 reflections

136 parameters

0 restraints

Primary atom site location: structure-invariant direct methods

Hydrogen site location: inferred from neighbouring sites

H-atom parameters constrained

Method = Modified Sheldrick $w = 1/[\sigma^2(F^2) + (0.06P)^2 + 0.14P]$, where $P = [\max(F_o^2,0) + 2F_c^2]/3$

$(\Delta/\sigma)_{max} = 0.000259$

$\Delta\rho_{max} = 0.40$ e Å^{-3}

$\Delta\rho_{min} = -0.29$ e Å^{-3}

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	*y*	*z*	U_{iso}*/U_{eq}
O1	−0.0165 (3)	0.80556 (12)	0.89995 (10)	0.0290
C2	0.1164 (4)	0.75275 (16)	0.85712 (12)	0.0226
O3	0.0426 (3)	0.68163 (11)	0.79561 (10)	0.0287
C4	0.1986 (4)	0.60986 (16)	0.74843 (14)	0.0289
C5	0.3861 (4)	0.67578 (16)	0.71001 (14)	0.0271
O6	0.3108 (3)	0.73556 (12)	0.63264 (9)	0.0355
C7	0.4068 (4)	0.84132 (17)	0.63637 (13)	0.0292
O8	0.4222 (3)	0.86365 (10)	0.73198 (9)	0.0261
C9	0.4744 (4)	0.76299 (15)	0.77626 (13)	0.0240
C10	0.3643 (4)	0.76166 (16)	0.86972 (13)	0.0221
O11	0.4337 (3)	0.66259 (11)	0.91228 (10)	0.0301
C12	0.4222 (4)	0.86099 (16)	0.92581 (13)	0.0277
C13	0.2506 (5)	0.92177 (19)	0.59465 (16)	0.0401
C14	0.6306 (5)	0.8415 (2)	0.59246 (16)	0.0422
C15	0.0664 (5)	0.55020 (19)	0.67809 (16)	0.0402
H41	0.2554	0.5572	0.7948	0.0338*
H51	0.5068	0.6260	0.6926	0.0337*
H91	0.6348	0.7543	0.7832	0.0290*
H121	0.5803	0.8627	0.9363	0.0432*
H122	0.3490	0.8570	0.9864	0.0426*
H123	0.3742	0.9272	0.8933	0.0431*
H131	0.3082	0.9961	0.6014	0.0583*
H132	0.2349	0.9048	0.5289	0.0582*
H133	0.1099	0.9171	0.6262	0.0586*
H141	0.6868	0.9161	0.5934	0.0627*
H142	0.7280	0.7929	0.6268	0.0622*
H143	0.6166	0.8179	0.5275	0.0623*

H153	0.1616	0.4999	0.6465	0.0610*
H152	−0.0508	0.5092	0.7077	0.0613*
H151	0.0062	0.6022	0.6340	0.0612*
H111	0.4361	0.6716	0.9708	0.0460*

Atomic displacement parameters (Å^2)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O1	0.0284 (9)	0.0329 (8)	0.0257 (7)	0.0061 (7)	0.0025 (7)	0.0035 (7)
C2	0.0264 (11)	0.0224 (10)	0.0191 (8)	0.0006 (10)	0.0006 (9)	0.0049 (9)
O3	0.0263 (8)	0.0302 (8)	0.0295 (8)	−0.0029 (7)	0.0011 (6)	−0.0051 (6)
C4	0.0344 (13)	0.0224 (10)	0.0299 (11)	0.0027 (9)	0.0008 (10)	−0.0023 (9)
C5	0.0315 (12)	0.0276 (10)	0.0222 (9)	0.0034 (10)	0.0012 (10)	−0.0035 (9)
O6	0.0500 (11)	0.0332 (8)	0.0234 (7)	−0.0145 (8)	−0.0068 (7)	0.0026 (7)
C7	0.0354 (13)	0.0305 (12)	0.0216 (10)	−0.0092 (11)	−0.0011 (10)	−0.0005 (9)
O8	0.0326 (8)	0.0250 (7)	0.0208 (7)	−0.0002 (7)	0.0006 (6)	0.0007 (6)
C9	0.0220 (11)	0.0253 (10)	0.0248 (9)	0.0032 (9)	0.0000 (9)	−0.0009 (9)
C10	0.0246 (11)	0.0198 (10)	0.0220 (9)	0.0042 (9)	−0.0017 (9)	0.0009 (8)
O11	0.0405 (9)	0.0267 (7)	0.0233 (7)	0.0089 (7)	−0.0047 (7)	0.0023 (6)
C12	0.0300 (12)	0.0288 (11)	0.0243 (10)	0.0005 (10)	−0.0028 (10)	−0.0022 (9)
C13	0.0453 (16)	0.0394 (13)	0.0355 (12)	−0.0026 (13)	−0.0080 (13)	0.0110 (12)
C14	0.0470 (16)	0.0480 (14)	0.0316 (11)	−0.0057 (14)	0.0137 (12)	−0.0004 (12)
C15	0.0509 (17)	0.0330 (12)	0.0368 (12)	−0.0104 (12)	−0.0011 (13)	−0.0088 (10)

Geometric parameters (Å, °)

O1—C2	1.214 (2)	C9—H91	0.992
C2—O3	1.334 (2)	C10—O11	1.433 (2)
C2—C10	1.530 (3)	C10—C12	1.514 (3)
O3—C4	1.472 (3)	O11—H111	0.864
C4—C5	1.512 (3)	C12—H121	0.979
C4—C15	1.501 (3)	C12—H122	0.995
C4—H41	1.000	C12—H123	0.987
C5—O6	1.426 (2)	C13—H131	0.985
C5—C9	1.543 (3)	C13—H132	0.989
C5—H51	0.992	C13—H133	0.978
O6—C7	1.428 (3)	C14—H141	0.979
C7—O8	1.429 (2)	C14—H142	0.982
C7—C13	1.505 (3)	C14—H143	0.997
C7—C14	1.512 (3)	C15—H153	0.967
O8—C9	1.433 (2)	C15—H152	0.977
C9—C10	1.525 (3)	C15—H151	0.980
O1—C2—O3	118.2 (2)	C2—C10—O11	106.55 (17)
O1—C2—C10	124.18 (18)	C9—C10—O11	105.58 (16)
O3—C2—C10	117.60 (18)	C2—C10—C12	110.78 (18)
C2—O3—C4	119.38 (17)	C9—C10—C12	112.01 (17)
O3—C4—C5	110.13 (15)	O11—C10—C12	112.40 (15)
O3—C4—C15	105.41 (19)	C10—O11—H111	109.1

C5—C4—C15	114.54 (18)	C10—C12—H121	109.5
O3—C4—H41	107.1	C10—C12—H122	109.8
C5—C4—H41	109.7	H121—C12—H122	107.8
C15—C4—H41	109.6	C10—C12—H123	109.5
C4—C5—O6	109.09 (18)	H121—C12—H123	110.5
C4—C5—C9	113.83 (17)	H122—C12—H123	109.7
O6—C5—C9	104.69 (15)	C7—C13—H131	110.0
C4—C5—H51	109.2	C7—C13—H132	108.5
O6—C5—H51	110.7	H131—C13—H132	109.2
C9—C5—H51	109.3	C7—C13—H133	109.2
C5—O6—C7	107.85 (16)	H131—C13—H133	108.7
O6—C7—O8	103.85 (15)	H132—C13—H133	111.2
O6—C7—C13	108.81 (18)	C7—C14—H141	108.2
O8—C7—C13	108.23 (18)	C7—C14—H142	109.3
O6—C7—C14	110.92 (18)	H141—C14—H142	110.5
O8—C7—C14	110.89 (19)	C7—C14—H143	109.1
C13—C7—C14	113.65 (18)	H141—C14—H143	108.4
C7—O8—C9	106.95 (14)	H142—C14—H143	111.3
C5—C9—O8	103.77 (15)	C4—C15—H153	108.4
C5—C9—C10	113.70 (17)	C4—C15—H152	110.1
O8—C9—C10	108.47 (15)	H153—C15—H152	108.9
C5—C9—H91	109.6	C4—C15—H151	109.6
O8—C9—H91	111.1	H153—C15—H151	109.1
C10—C9—H91	110.1	H152—C15—H151	110.7
C2—C10—C9	109.25 (16)		

Hydrogen-bond geometry (Å, °)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
C13—H131···O11^{i}	0.99	2.59	3.536 (3)	161
O11—H111···O1^{ii}	0.86	1.93	2.793 (3)	172

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

Fig. 1

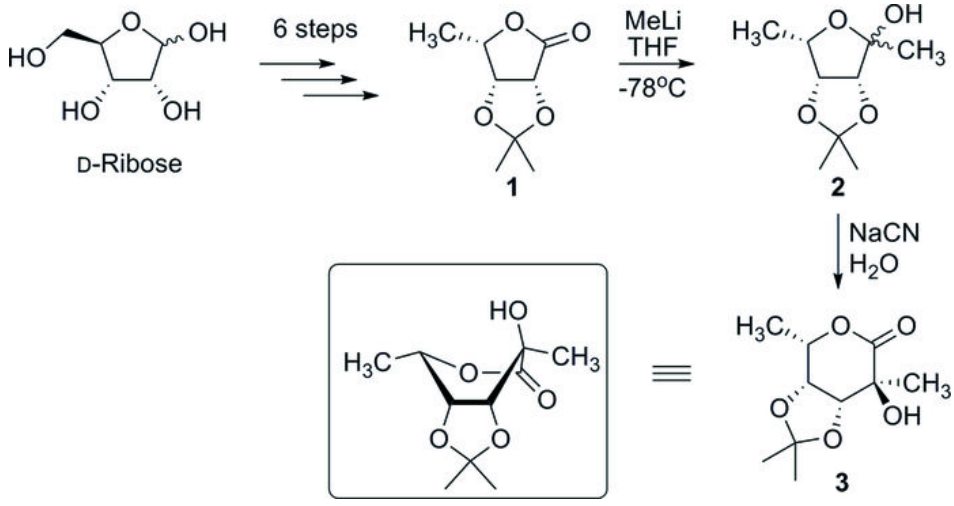
HO
OH
HO
OH
D-Ribose
6 steps
H3C
O
O
O
O
1
MeLi
THF
-78°C
H3C
OH
CH3
O
O
2
NaCN
H2O
HO
H3C
CH3
O
O
O
O
H3C
O
CH3
O
OH
O
O
3

Fig. 2

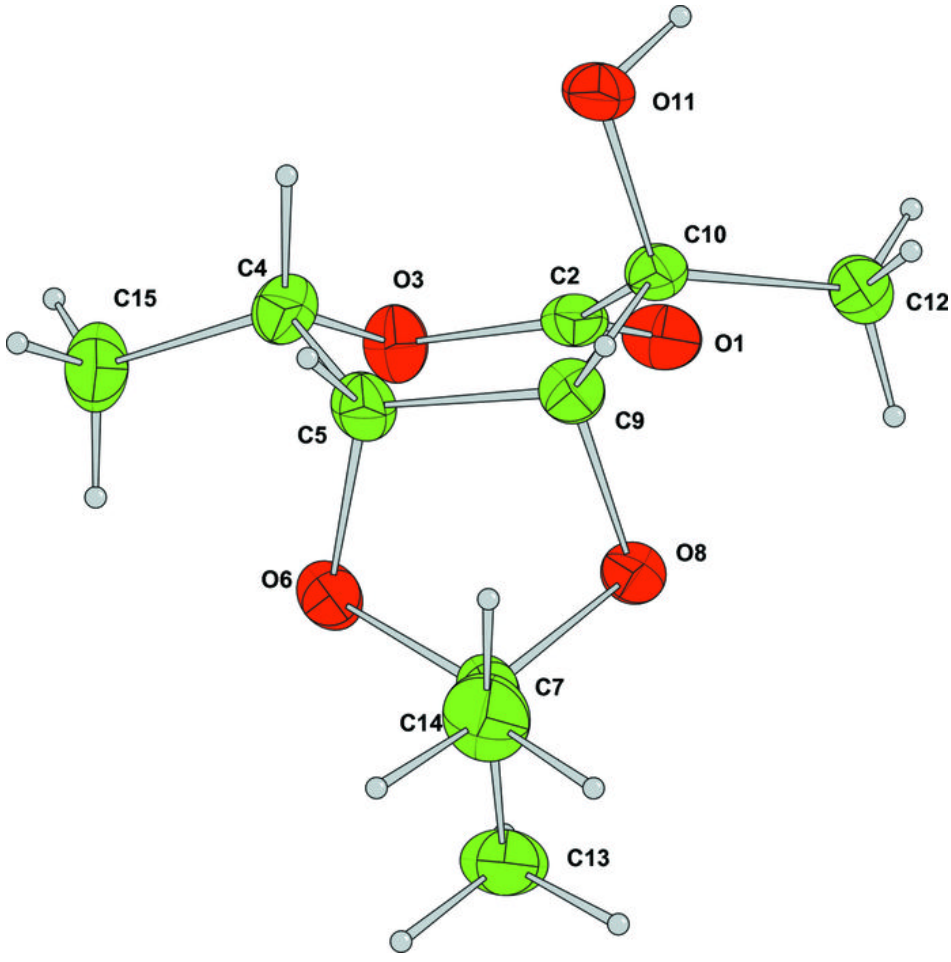

Fig. 3