NOVEL DI-BRANCHED MONOSACCHARIDES AND IMINO SUGARS

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Abstract

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Branched chain sugars display a varied and valuable range of biological activities. This thesis concerns the synthesis of 3,5-di-*C*-methyl-D-glucose, a potential inhibitor of glycogen phosphorylase (GP), and therefore a proposed therapeutic agent for type 2 diabetes. **Chapter 1** looks at the occurrence of branched sugars in the natural world and current therapies for type 2 diabetes. Inhibition of GP is explored, and the molecular modelling studies which led to the design of the project target. **Chapter 1** also looks into the development of new foodstuffs, the chemistry and biochemistry of imino sugars and branched hydroxy proline analogues.

In **Chapter 2**, a range of different approaches to 3,5-di-*C*-methyl-D-glucose are investigated. Most of the initial investigations were carried out on the L-enantiomer, a readily available test system deriving from 2-*C*-methyl-D-ribono lactone. 2-*C*-Methyl-D-ribono lactone is synthesised rapidly from D-glucose in a one-pot reaction; as the key starting material for this work, the scalability of this process was investigated. One of the attempted syntheses of di-*C*-methyl glucose lead to the development of a route towards 3,5-di-*C*-methyl fructose, a novel dibranched ketose sugar. It was envisaged that through an enzymatic transformation, it might be possible to produce 3,5-di-*C*-methyl glucose stereoselectively. Synthesis of both enantiomers of 3,5-di-*C*-methyl glucose and mannose are reported, alongside results of GPb inhibition studies. Analysis of the preferred ring size of a range of di-*C*-methyl branched sugars and sugar lactones generated in this work is also presented.

Chapter 3 explores the chemistry of 2,4-di-*C*-methyl-L-arabinono lactone, a key intermediate in the synthesis of 3,5-di-*C*-methyl-L-glucose. From this lactone a novel deoxy sugar, 2-deoxy-2,4-di-*C*-methyl-L-arabinono lactone, was generated. Routes towards a selection of imino sugars were explored, resulting in the synthesis of a methyl branched isofagomine analogue. A substituted aziridine was synthesised, from which a route to a di-*C*-methyl branched piperidine was proposed, and a pyrrolidine. Also presented is a synthesis of a dihydroxy di-*C*-methyl branched proline analogue.

Detailed NMR analysis of several of the sugars generated in this work was carried out by Dr M. Wormald, of the University of Oxford Biochemistry department. The results of these investigations are presented in the **Appendix**.

Throughout this work, the presence of quaternary centres has posed a problem with the assignment of relative configuration. As a result, this work has been greatly supported by X-ray crystallography, and the structures shown herein were wholly generated by me. Several other crystals were run during the course of this work, not all pertaining to these projects, and are provided in the **CD appendix**.

Acknowledgements

Its hard to believe that the thesis is finally written and 7 years at Oxford are coming to an end. I wouldn't have got to this point without an awful lot of help and I'd like to firstly thank George for his endless patience when 'Any news?' is met with no news at all, and for being thoroughly decent throughout my many illnesses/injuries and allergies in the last three years. Secondly, huge 'thank yous' go to Sarah for the endless proofreading and showing me back in the very beginning how to do a reaction, a work up, a column and then work out what my product actually was. Ed has been fantastic, letting me muscle into his lab when I needed to, and providing much help along the way.

A lot of collaborators have helped with this project and I'd like to thank Mark Wormald for tackling all the NMR's that I couldn't, Rob Nash for cleaning up my imino sugar and Atsushi for the GP inhibition testing. Also, Barbara Odell and the NMR staff for the endless HMBC's and Amber and David in the Crystallography department for providing so much help with all the publications. Without the initial molecular modelling work, there would have been no project, so thanks are due to Jenny Martin and Louise Johnson for that.

Huge thanks are due to the various Fleet group members over the last four years, in particular Dave for being the all-knowing DPhil student when I was a lowly Part II; the fun times had with the part II's and poor Dan, who had the misfortune to try and copy some of my reactions. Thanks also go to my second surrogate group and MC; I really never knew distilling solvents could be quite so entertaining!

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Contents

List of Abbreviations i

Note Concerning Nomenclature iii

Chapter 1 General Introduction

1.1 Introduction to Branched Carbohydrates 1

1.2 Diabetes 6

1.3 New Approaches to the Treatment of Type 2 Diabetes 15

1.4 New Foods from Novel Monosaccharides 27

1.5 Imino Sugars 31

1.6 Proline Analogues 36

1.7 Contents Of This Thesis 38

Chapter 2 Synthesis of 3,5-di-*C*-methyl glucose

2.1 Synthesis of Branched Chain Sugars 44

2.2 Synthesis of 3,5-Di-*C*-methyl-L-glucose 52

2.3 Alternative Approaches to the Synthesis of 3,5-Di-*C*-methyl-L-glucose 83

2.4 From Fructose to Glucose? 97

2.5 Di Branched Lactones: Pyranose or Furanose? 110

2.6 Conclusions and Future Work 115

2.7 Experimental 117

Chapter 3 Synthetic utility of di-*C*-methyl branched sugar lactones

3.1 Synthesis of 2-Deoxy-2,4-di-*C*-methyl-L-arabinose 181

3.2 Introduction of Nitrogen into Arabinono Lactone 184

3.3 Branched Proline Synthesis from Azide **207** 189

3.4 Branched Piperidine Synthesis from Tertiary Azide 197

3.5 Azidomethyl Branched Piperidine 205

3.6 Conclusions and Future Work 208

3.7 Experimental 209

Appendices 238

Crystallographic data is provided on a CD inside the back cover

Abbreviations

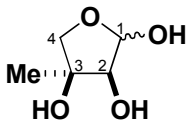
The following abbreviations are used in this thesis:

a	apparent
Ac	acetyl
AD mixβ	$(DHQD)_2PHAL$, potassium carbonate, potassium ferricyanide, potassium osmate dihydrate
AMP	adenosine monophosphate
approx.	approximately
aq.	aqueous
Bn	benzyl
br	broad
c	concentration
cat.	catalytic amount
conc.	Concentrated
COSY	correlation spectroscopy
1D	One Dimensional
2D	Two Dimensional
d	doublet
DAB	1,4-dideoxy-1,4-imino-D-arabinitol
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
dd	double doublet
dddd	double double double doublet
d.e.	diasteromeric excess
DEPT	Distortionless Enhancement by Polarization Transfer
DFJ	deoxyfuconojirimycin
DGJ	1-deoxy-galactonojirimycin
DIBAL	Diisobutylaluminium hydride
DIM	1,4-dideoxy-1,4-imino-D-mannitol
DIPEA	*N*,*N*-Diisopropylethylamine
DMDP	2,5-dideoxy-2,5-imino-D-mannitol
2,2-DMP	2,2-dimethoxypropane
DMAP	*para*-*N*,*N*-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMJ	1-deoxymannojirimycin
DMSO	dimethylsulfoxide
DNA	Deoxyribonucleic acid
DNJ	1-deoxyynojirimycin
DPP IV	Dipeptidyl peptidase IV
dt	double triplet
DTBB	4,4'-*di*-*tert*butylbiphenyl
eq.	equivalents
ESI	electro-spray ionisation
Et	ethyl
EtOH	ethanol
FT	Fourier transform
GIP	Gastric Inhibitory Peptide
G_L	glycogen binding subunit of protein phosphatase-1 (PP1)
GLP-1	Glucagon-Like Peptide-1
GP	Glycogen Phosphorylase
GPa	active form GP
GPb	inactive form GP
GS	Glycogen Synthase
h	hours
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HPLC	high pressure liquid chromatography
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation

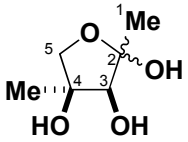
HRMS	high resolution mass spectrometry
IC_{50}	half maximal inhibitory concentration
IR	infra-red
Im	imidazole
J	Scalar coupling constant
KHMDS	potassium hexamethyldisilazane
K_i	binding affinity of the inhibitor
LDA	lithium di*iso*propylamide
Lit.	literature value
LRMS	low resolution mass spectrometry
m	multiplet (NMR), medium (IR)
Me	methyl
MeOH	methanol
min	minute(s)
mol	mole(s)
m.p.	melting point
Ms/ mesylate	methanesulphonyl
Na_2SO_4	anhydrous sodium sulfate powder
NIDDM	Non-Insulin Dependent Diabetes
NIS	*N*-iodosuccinamide
NMO	4-methylmorpholine *N*-oxide
NMM	4-methylmorpholine
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
p	para
Pd/C	Palladium on activated carbon
$Pd(OH)_2$	Palladium hydroxide
Ph	phenyl
PMA	phosphomolybdic acid
PP1	Protein phosphatase-1
PPAR	peroxisome proliferator activated receptor
ppm	parts per million
*p*TSA	*para*-toluenesulfonic acid
Py.	pyridine
q	quartet
R_f	retention factor
RT	room temperature
s	singlet (NMR) , strong (IR)
sat.	saturated
S_N2	Bimolecular nucleophilic substitution
t	triplet
TBAI	*tetra*-butyl ammonium iodide
TBDMS	*tert*-butyldimethylsilyl
TEA	triethylamine
TES	triethylsilyl
Tf/ triflate	trifluoromethanesulphonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
T.l.c.	thin layer chromatography
Ts	*p*-toluenesulphonyl
UV	ultraviolet
w	weak
WHO	World Health Organisation
w/v	weight to volume
% wt.	% by weight

Note Concerning Nomenclature

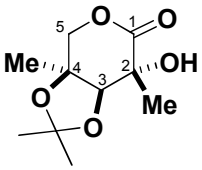
The numbering convention used in this report for assignment of spectroscopic data is derived from the systematic naming of materials according to IUPAC recommendations.[1] Some examples are given below for reference.



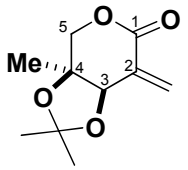
3-*C*-methyl-L-erythrose



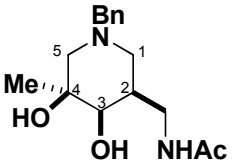
1-deoxy-4-*C*-methyl-L-ribulo furanose



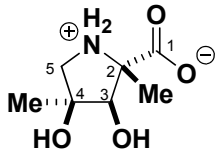
2,4-di-*C*-methyl-3,4-*O*-isopropylidene -L-arabinono-1,5-lactone



2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-2-*C*-methylene-L-erythro-pentono-1,5-lactone



N-Benzyl-2-deoxy-2-*C*-*N*-acetyl-methyl-1,5-imino-4-*C*-methyl-L-ribitol



2,5-Dideoxy-2,5-imino-2,4-di-*C*-methyl-L-ribonic acid [3(*R*),4(*S*)-Hydroxy-2,4-di-*C*-methyl-L-proline]

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

Chapter 1

Introduction

This thesis describes the synthesis of both enantiomers of 3,5-di-*C*-methyl glucose **1** (**Figure 1.1**), of which the D-enantiomer has been predicted to be a potential inhibitor of glycogen phosphorylase.

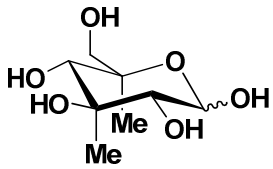


Figure 1.1: 3,5-Di-*C*-methyl-D-glucose, **1**.

Herein are also described routes towards other branched chain sugars and novel imino sugars, which derive from a common intermediate in the synthesis of 3,5-di-*C*-methyl glucose **1**. Also discussed are routes towards branched amino acids, and singly branched sugars.

1.1 Introduction to Branched Carbohydrates

1.1.1 The Carbohydrate Family

Carbohydrates comprise the most abundant group of natural products.[1] The name derives from the French '*hydrate de carbone*', implying a molecule consisting solely of carbon, hydrogen and oxygen; more recently, the term carbohydrate has expanded to include a variety of heteroatoms such as nitrogen, leading to molecules called imino (or aza) sugars, or replacement of the ring oxygen by carbon, giving carbasugars. Oxidation and reduction of the anomeric centre further enhances the variety of monosaccharides, providing aldonolactones and alditols; oxidation at C2, rather than C1 generates the ketose class of carbohydrates. Replacing a hydroxyl group with hydrogen gives deoxy sugars, of biological significance as 2-deoxy-D-ribose forms the sugar backbone of DNA (**Figure 1.2**).

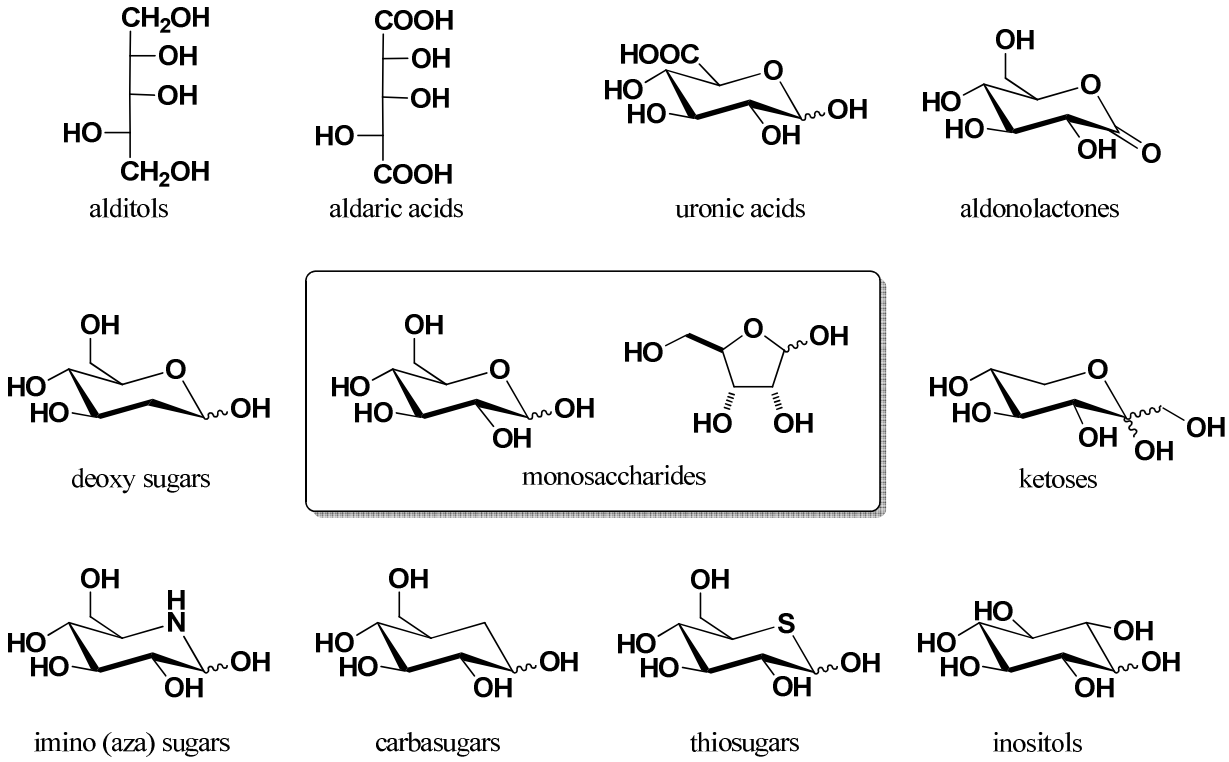


Figure 1.2: Classes of carbohydrates.

Carbohydrates perform a variety of biologically important roles. The most well known is that of an energy source; the word 'carbohydrate' has been adopted by nutritionalists to denote a food with little or no other nutritional value, beyond being a source of starch and therefore energy.[2] Starch is an insoluble glucose oligomer, synthesised by plants as their principal food reserve; animals store glucose as the polymer glycogen. Polysaccharides (carbohydrate oligomers) fulfil a variety of significant roles, including providing structural support within organisms. The polysaccharide chitin forms the exoskeleton of invertebrates, whilst the glucose polymer cellulose constitutes the supportive tissue of plant cell walls. The polysaccharides dextran and pectin possess gelling properties, whilst murein is found adding rigidity to bacterial cell walls.[1]

Cell walls contain other polysaccharides concerned with cell signalling. Lectins, carbohydrate-specific receptors are present on cell surfaces and can interact with sugars on apposing cells. This can result in the adhesion of the two cells and is important in many intercellular activities including infection by bacteria and viruses,[3] and sperm-egg recognition.[4]

1.1.2 Singly Branched Sugars

A further class of carbohydrates exists, namely branched chain sugars. These are carbohydrates possessing one or more additional functional groups along the carbohydrate backbone, for example a methyl or hydroxy methyl group. This class of carbohydrates was first discovered in the early twentieth century, with the discovery of apiose **2** and hamamelose **3** in parsley and witch-hazel (*Hamamelis*) respectively (**Figure 1.3**).[5]

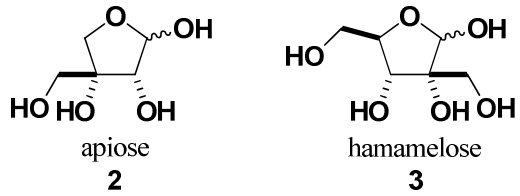


Figure 1.3: The first isolated examples of branched sugars.

Until relatively recently, these compounds were classed as 'rare sugars', and were thought to hold little biological significance.[6] In the last few decades however, increasing numbers of branched sugars have been discovered, frequently in polysaccharides, and in particular as components of antibiotic or bacterial cell walls. For example, 3-*C*-methyl-D-mannose **4** (**Figure 1.4**) has been found to be one of the three sugars in the trisaccharide repeating unit from the outer membrane of the *Helicobacter pylori* bacterium. This pathogen is thought to be the major cause of chronic gastritis, gastric and duodenal ulcers and has been linked to the development of gastric cancer.[7,8] A derivative of 3-*C*-methyl-L-mannose is one of the sugars in the pentasaccharide hapten of *Mycobacterium avium serovar*.[9] Another 3-*C*-methyl branched sugar, L-olivomycose **5**, has been found to be present in the trisaccharide unit of several antitumor antibiotics of the aureolic acid group.[10] Moenuronic acid **6**, a 4-*C*-methyl branched sugar, is found in moenomycin A, a trans-glycosylation inhibitor,[11,12,13] and aceric acid **7** was found in the pectic polysaccharide rhamnogalacturonan-II (**Figure 1.4**).[14] Other examples include 6-deoxy-3-*C*-methyl mannose **8** (evalose), isolated from the O antigen of *Nitrobacter hamburgensis*[15] and 6-deoxy-3-*C*-methyl-gulose (virenose) **9** isolated as the D-enantiomer from the anti-tumor antibiotic virenomycin.[16]

Although full structural confirmation is pending, L-virenose is believed to also have been found in the bacterium *Coxiella burnettii* (**Figure 1.4**).[17]

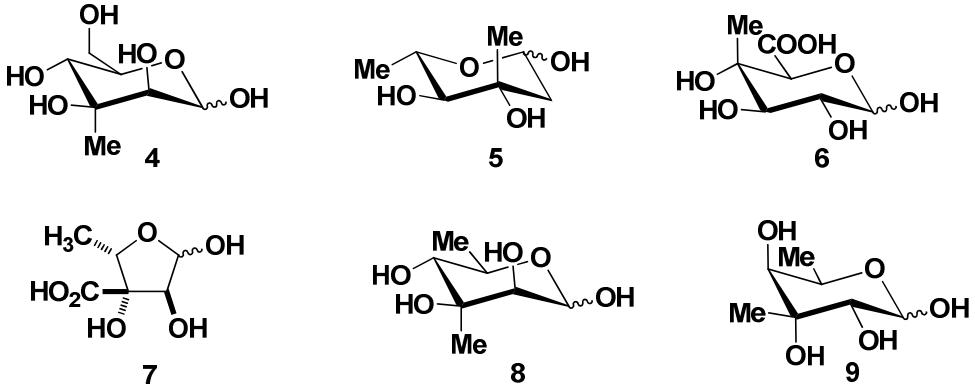


Figure 1.4: Branched sugars from polysaccharides.

1.1.3 Biological Importance of Singly Branched Sugars

Despite the ever increasing number of branched sugars isolated, the role of these types of molecules is far from clear. One of the few examples of a known, clearly defined role of a branched sugar in a biological system is that of potassium 2-*C*-methyl-D-erythronate **10**, which was found to control circadian rhythm in leguminous plants.[18] The closely related structure, 2-*C*-methyl-D-erythritol-4-phosphate **11**, is known to be an intermediate in the non-mevalonate pathway of terpene biosynthesis (**Figure 1.5**).[19,20,21]

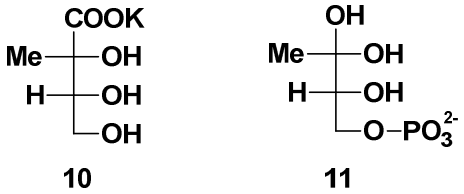


Figure 1.5: Biologically active branched sugar derivatives.

One proposed explanation for the existence of unusual sugars in bacterial cell walls is that the differing surface polysaccharides exhibited by these pathogens may exist as a form of antigenic variation and/or reflect the adaptation of bacterial strains to a particular host population, as has been reported for other pathogenic factors.[22] What is known, however, is that the introduction of a branch point can drastically alter biological activity. For example, branched nucleosides, such as **12** and **13**,

have been shown to inhibit the replication of the hepatitis C virus,[23,24] whilst **14** exhibited anti-HIV activity (**Figure 1.6**).[25]

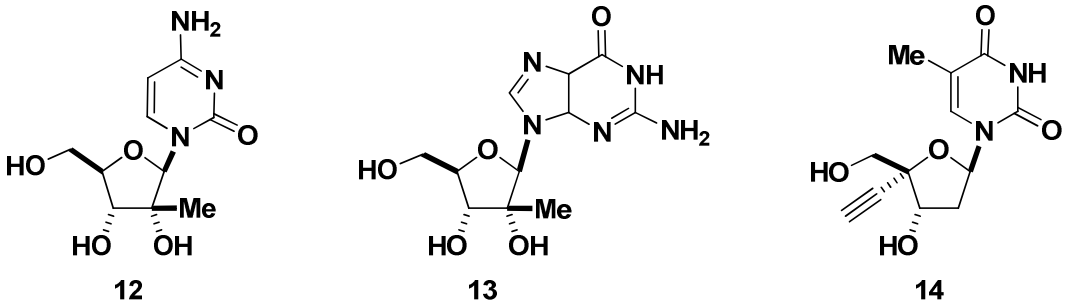


Figure 1.6: Branched nucleoside analogues as therapeutic agents.

The introduction of branch points can negatively impact upon biological activity. Introduction of a 4-*C*-methyl branch into known potent glycosidase inhibitor DFJ (deoxyfuconojirimycin) **15** resulted in the complete removal of biological activity (**Figure 1.7**).[26]

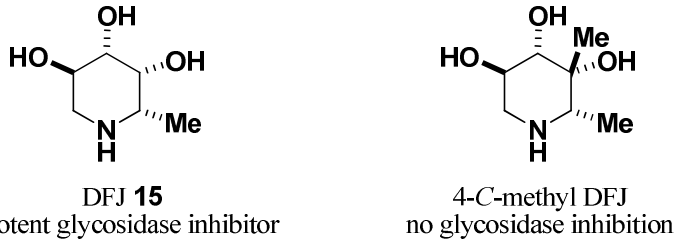


Figure 1.7: An example of methyl branching completely removing biological activity.

1.1.4 Doubly Branched Sugars

Whilst singly branched sugars are now known to be relatively common in the natural world, sugars with two or more branch points are considerably rarer, and to date, few true examples have been found. Often one of the branches can be more correctly identified as a deoxygenated centre, rather than a true branch point, for example in the oligosaccharide segment of apoptolidin **16**.[27,28,29,30] Here, the carbohydrate shown in green is more correctly described as a 6-deoxy species, whilst the apparent '3,5-di-*C*-methyl sugar' shown in blue can be otherwise considered as a 6-deoxy, 3-*C*-methyl sugar, *i.e.* singly branched. The complex oligosaccharide portion of everninomicin 13,384-1 **17** contains an example of a 2,3-di-*C*-methyl sugar, with additional deoxy centres at the 2- and 6-positions (**Figure 1.8**).[31]

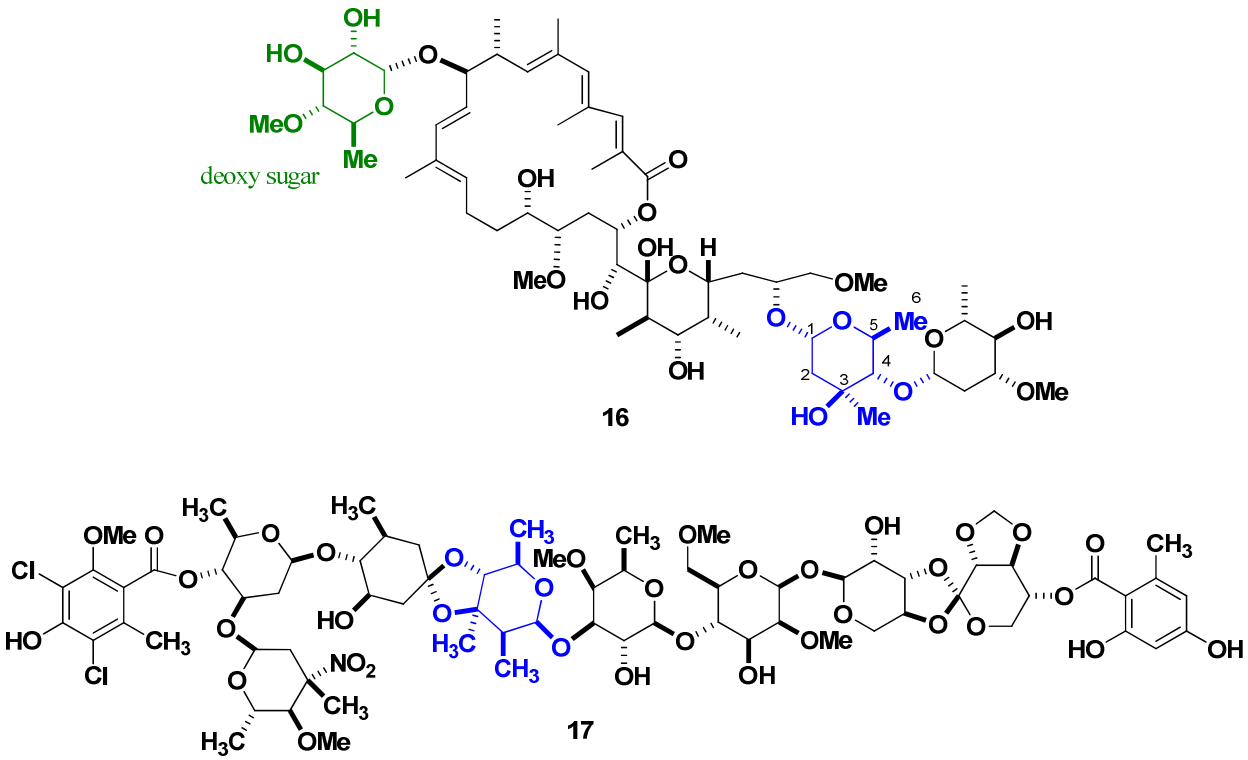


Figure 1.8

As is the case with many of the singly branched sugars, the role of the di-*C*-methyl branched sugars is not clear, but it is known that apoptolidin **16** possesses anti tumor activity.[32,33] Moreover, the carbohydrate portion containing the branched sugar has been shown to be essential for maintaining biological activity.[29] Everninomicin **17** is a potent antibiotic.[34] As with the singly branched case, it has been demonstrated that the recently synthesized 2',4'-di-*C*-methyl nucleosides possess biological activity.[35]

1.2 Diabetes

1.2.1 Diabetes

Diabetes is a condition which results in too much glucose in the blood. Its full name, *Diabetes Mellitus*, reflects the presentation of the disease; 'diabetes' derives from Greek meaning 'siphon', reflecting excessive passing of urine, and 'mellitus' coming from the Latin for honey, reflecting the sweetness that ancient physicians noted in the urine. There are two main forms of the disease, called

type 1 and type 2 diabetes. Type 1 diabetics do not produce insulin themselves and therefore regular injections of insulin must be administered. Type 2 diabetes (non insulin dependent diabetes or NIDDM) is characterised by insulin resistance, whereby the body still produces insulin, but the other enzymes and pathways responsible for bringing down blood sugar levels are unresponsive. The leading cause of this type of diabetes is obesity; insulin resistance is believed to derive from overindulgence in sugary/high fat foods over a prolonged period of time. The aging world population, coupled with the rise in the availability of sugary and/or high fat food stuffs, and the increasingly sedentary nature of the majority of jobs and hobbies has lead to a marked rise in obesity and consequently in the number of cases of type 2 diabetes being diagnosed. The World Health Organization (WHO) estimates that more than 180 million people worldwide currently have diabetes, and that this number is likely to double by 2030.[36]

Much investigation of the link between obesity and diabetes has been carried out, the most striking evidence comes from the Pima Indian population in the USA, where 80% of adult Pima Indians are obese, and 40% have type 2 diabetes. This was contrasted with evidence that a Pima Indian population living a more traditional lifestyle in Mexico has virtually no obesity or type 2 diabetes. Studies suggest that 80-90% of patients with NIDDM are overweight or obese.[37]

Ethnic background and gender could be important, as a recent study showed that non-Hispanic black women exhibited poorer glycaemic control than both non-Hispanic black men and non-Hispanic whites.[38] This may suggest a possible genetic link, however, no gene has yet been found. Body fat distribution may be significant, as it was demonstrated that the waist-to-hip ratio was positively and significantly associated with the risk for diabetes in a study of Swedish men.[39]

A third form of diabetes (Type 3 diabetes) has been recently proposed. This form is characterised by a shortage of insulin in the brain, and has been linked to the development of Alzheimers disease.[40,41] Lastly, gestational diabetes develops during approximately 2-3% of pregnancies. This

leads to an increased risk of miscarriage/stillbirth, and cardiac risk in the mother caused by elevated blood pressure. Although this type of diabetes usually disappears within days of giving birth, around 60% of affected women go on to develop diabetes later in life.[42]

1.2.2 Complications of Diabetes

Untreated diabetes can lead to a variety of complications through failure to adequately control blood glucose levels. Skin diseases and gastrointestinal complaints cause a serious reduction in quality of life for the patient. More serious complications also develop; these include neural degeneration, kidney disease, joint problems, capillary degeneration and increased risk of heart attacks and stroke. Neural degeneration in the eye leads to sight problems and potentially blindness, whilst nerve damage in the extremities leads to a tingling 'pins and needles' type sensation. Capillary degeneration leads to increased risk of stroke, heart attack, reduced ability of the body to heal wounds and erectile dysfunction. Failure to adequately control brain blood sugar levels is believed to cause mood disorders and psychiatric disturbance.[43] The WHO estimates that around 2.9 million deaths per year are currently attributable to diabetes.[44]

1.2.3 Type 2 diabetes treatments

"Non-insulin dependent diabetes has been regarded, traditionally, as a mild condition with simple therapeutic needs" [45]

The above statement from the Royal College of Physicians in 1992 summarises the classical approach to type 2 diabetes. Largely, the patient managed his or her condition through diet, using sugar replacements to avoid hyperglycaemia after meals. However, due to the rising number of cases and increased understanding of the complications related to the disease, this therapeutic area has received much attention.

Diet still remains the first strategy undertaken, as a majority of patients with NIDDM are overweight.[46] Weight loss can lead to increased sensitivity to insulin, and in some cases delay the

progress of the disease. However, weight loss is not an easy task, and diabetics can struggle as the complications of the disease, such as reduced joint mobility, can hamper their ability to exercise. In these cases, medical intervention would be appropriate.

1.2.4 Insulin Secretagogues

The earliest type of drugs developed for the treatment of NIDDM act by stimulating insulin production from pancreatic β-cells. By interrupting the flow of potassium ions through cell walls, the level of calcium ions rises inside cells; the rise in calcium levels leads to increased secretion of insulin. Sulphonylureas are the leading drug type in this category. Tolbutamide **18** and glimepiride **19** are two examples of drugs in this class (**Figure 1.9**).

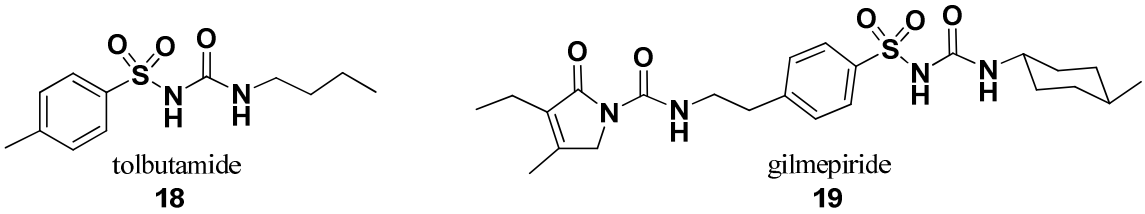


Figure 1.9: Sulphonylurea type anti-diabetic drugs.

Use of sulphonylureas can easily lead to hypoglycaemia.[47] They have been associated with increased mortality due to cardiovascular disease, deriving from their interaction with the potassium pumps in the walls of heart cells.[48] Other side-effects include gastrointestinal upset, weight gain, liver disease, blood disorders, allergic reactions and intolerance to alcohol.[49] Many of the early types of sulfonylureas were shown to be teratogenic in animal studies;[50] later studies have shown no increased risk of foetal mortality, but the stigma remains and sulphonylureas are largely avoided by most medical practitioners during pregnancy.[51]

More recently, a new family of insulin secretagogues has been discovered, the 'glinide' class of compounds (**Figure 1.10**). They act in a similar fashion to the sulphonylureas and may preserve β cell function over time better than hypoglycaemic sulfonylureas.[52]

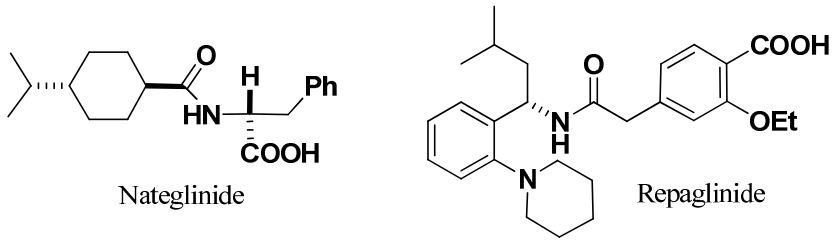


Figure 1.10: Two currently available drugs in the glinide class.

The glinides have similar side effects as the sulfonylureas, but hypoglycemia may be less likely, due to their shorter duration of action.[53]

1.2.5 Insulin sensitizers

Thiazolidinediones (glitazones) act as insulin sensitizers; they increase the sensitivity of muscle, fat, and liver to insulin.[54] Glitazones bind to receptors known as PPAR's (peroxisome proliferator activated receptors). After binding to the glitazone, the PPAR joins with another receptor protein and the resulting complex binds to the DNA within the cell nucleus to activate a number of genes, resulting in greater insulin sensitivity.

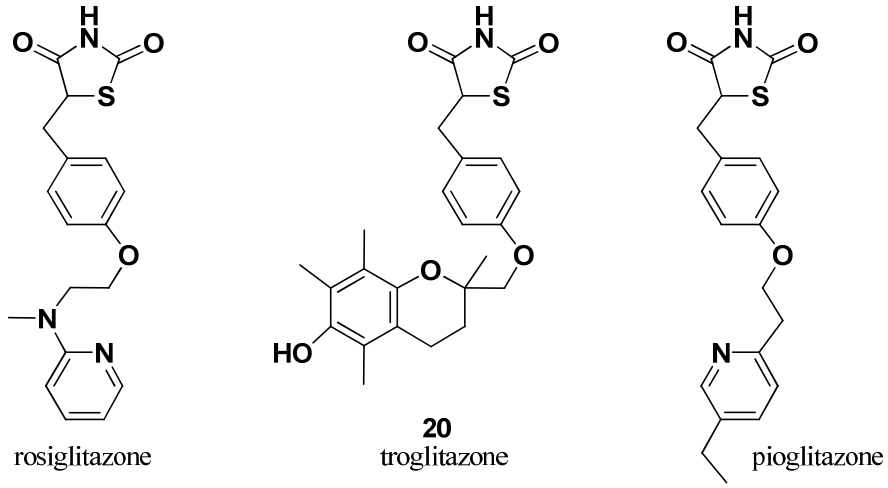


Figure 1.11: Three examples of drugs in the thiazolidinedione (glitazone) class.

The main drawback of this class of drug is the risk of fluid retention, potentially resulting in heart failure.[55] Troglitazone **20**, the first thiazolidinedione approved for treatment of type 2 diabetes, was found to cause serious liver damage,[56,57] and was subsequently withdrawn from the market (**Figure 1.11**).[58,59]

1.2.6 Insulin

Insulin is the most effective of diabetes medications in lowering glycaemia however, relatively large doses may be necessary to overcome the insulin resistant nature of type 2 diabetes.[45] Because it is so effective in lowering blood sugar levels, the risk of hypoglycaemia is high. Other problems relate to its being a drug administered *via* injection; the site of injection alters the absorption rate, whilst repeated injections into the same site have an adverse effect on the consistency of insulin absorption. Wrongly injecting into the muscle leads to too rapid absorption and a hypoglycaemic state.[45] Much effort has been placed into finding an alternative form of insulin delivery, such as nasal sprays,[60,61] but so far the same level of efficacy as the injected form has not been achieved.[62]

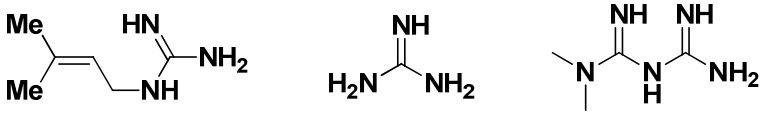
1.2.7 Biguanides

In medieval times, a prescription of *Galega officinalis* (Goat's rue or French Lilac) was said to relieve the intense urination accompanying the disease we now recognise as diabetes. The active ingredient was found to be galegine **21** or isoamylene guanidine. While guanidine **22** itself and most derivatives are too toxic for drug use, the biguanides (two linked guanidine moieties) have proved useful.[63] Metformin **23** is the leading example of a drug in this class (**Figure 1.12**).[64] Its mode of action is much more complicated than that of the sulphonylureas. It is not an insulin secretagogue, but works in three ways:[65]

(1) Improves glucose uptake by skeletal muscles

(2) Promotes the formation of glycogen

(3) Decreases absorption of glucose



galegine **21** guanidine **22** metformin **23**

Figure 1.12: Biguanide **23**, a derivative of guanidine **22**.

Metformin is not metabolised, it is removed from the body *via* the kidneys and there is therefore a risk of accumulation in susceptible individuals, which can prove fatal.[66] The most common adverse effect of metformin is gastrointestinal upset,[67] and there is some evidence of a link to vitamin B12 deficiency.[68,69]

1.2.8 α-Glucosidase Inhibitors

Complex carbohydrates eaten as part of a meal must be broken down into their constituent monosaccharides if they are to be absorbed by the small intestine. The enzymes responsible for this process are a class of glycosidases; α-glucosidase inhibitors therefore prevent digestion of complex carbohydrates and consequently slow the rate of absorption of glucose into the blood stream. Accordingly, drugs in this class could help to prevent the common complications of diabetes caused by alternating hyper- and hypoglycaemia.[70] The leading drugs in this class are the polysaccharide, acarbose **24**, the imino sugar miglitol **25**, and carbasugar voglibose **26** (**Figure 1.13**).

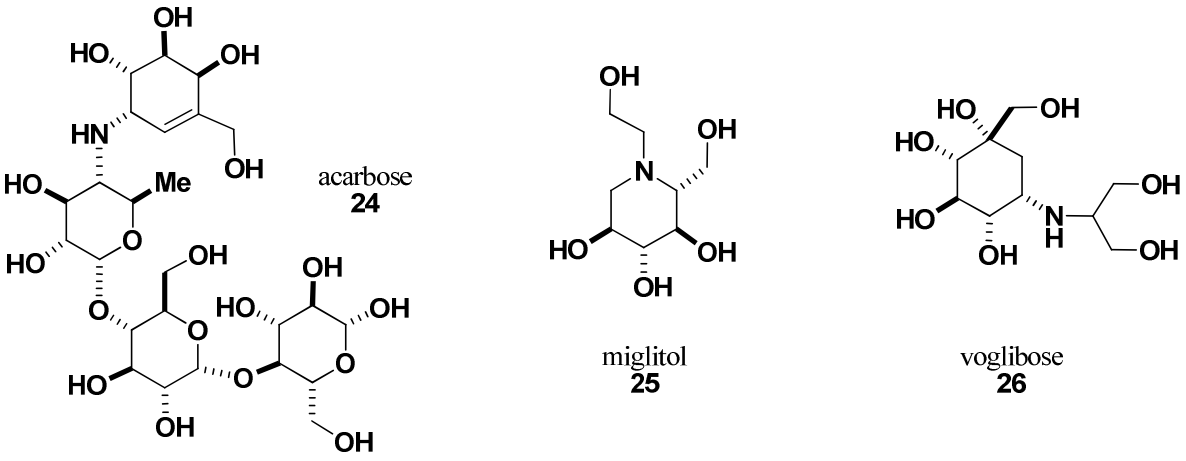
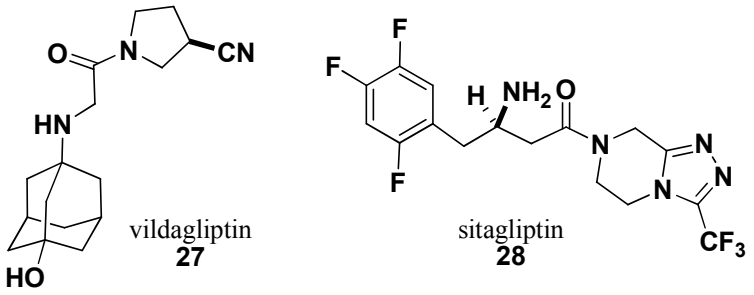


Figure 1.13: α-Glucosidase inhibitors for the treatment of NIDDM.

One advantage of this drug class is that a patient can quickly be brought round from a hypoglycaemic state by administration of glucose; they are, however, associated with gastrointestinal upset,[71] which is usually mild and decreases over time. As yet, no serious side effects have been reported. [72]

1.2.9 DPP IV inhibitors

The latest class of drugs for the treatment of NIDDM act by inhibiting the enzyme dipeptidyl peptidase IV (DPP IV), which is responsible for the breakdown of a hormone class known as incretins. Incretins cause an increase in the amount of insulin released from pancreatic β-cells. They also slow the rate of absorption of nutrients into the blood stream by reducing gastric emptying, this may directly reduce food intake, by promoting a feeling of satiety. They also inhibit glucagon release from the pancreatic α-cells. There are two main types of incretins, glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP); both are degraded by DPP IV.[73] Inhibition of DDP IV as a therapeutic strategy is a fairly new approach, and as such there are currently few drugs in this class; vildagliptin **27** and sitagliptin **28**, are two examples (**Figure 1.14**).



vildagliptin **27** sitagliptin **28**

Figure 1.14: DPP IV inhibitors

The risk of hypoglycaemia is low with this mode of action. Adverse effects observed so far have been mild (upper respiratory tract infection, sinus problems, sore throat and headache)[74] but the role of DPP IV in lymphocytes has raised theoretical concerns about effects on immune function.[75]

1.2.10 GLP-1 and GIP analogues and agonists

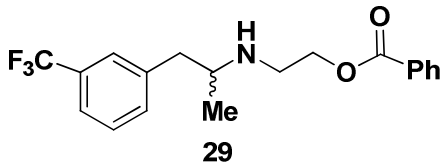
Expanding on the above idea, it should be possible to control blood sugar levels by boosting the levels of GLP-1 or GIP. Proof of concept was achieved with the release of complex polypeptide exenatide, a GLP-1 agonist, in the US.[76] Unfortunately this drug requires twice daily injections;[75] Novo Nordisk hopes to compete with their GLP-1 analogue liraglutide.[77] Several alternatives are currently under investigation which would require only one dosage *via* injection per week.[78] At the time of writing, no GIP analogues or agonists are on the market.

1.2.11 Natural therapies

Natural products have long been used as treatments for diabetes. There is evidence to suggest that spices such as cinnamon, cloves, bay leaves and turmeric display insulin-enhancing activity *in vitro*.[79] Vegetables such as karela, prickly pear cactus and Korean ginseng have shown hypoglycaemic effects in trials, possibly due to their high soluble fibre content.[80] The key advantage of this form of therapy is the lower cost compared to pharmaceutical products, an important factor in controlling diabetes in the developing world.

1.2.12 Combination therapies

Type 2 diabetes is a progressive disease, so any treatment that is undertaken is likely to become ineffective over time, and may need to be combined with another drug. Pramlintide is an analogue of amylin, a hormone released into the bloodstream by the pancreas. Like insulin, amylin is deficient in individuals with diabetes. Amylin, and therefore pramlintide, slows gastric emptying, producing the sensation of satiety, and allows glucose to be absorbed at a more steady rate.[81] It has been linked to weight loss in clinical trials, but is associated with nausea as a temporary side effect.[82] Benfluorex **29** has been shown to improve insulin sensitivity, decrease hepatic glucose production, and improve glucose uptake by skeletal muscles (**Figure 1.15**). It is used in conjunction with sulphonylureas, in patients for whom metformin is unsuitable.[83,84]



29

Figure 1.15: Benfluorex.

1.3 New approaches to the Treatment of Type 2 Diabetes

It is clear that treatment of type 2 diabetes has received much attention in recent years, and despite the improvement in the number of treatments available, there remains much room for development. The major drawbacks of existing therapies include:

- The increased risk of hypoglycaemia.
- The need for regular dosing (*e.g.* before each meal) raises the risk of missed treatments.
- The need to be administered *via* injection in some instances.
- Risk of organ damage, particularly the liver and kidneys.

As many of these problems relate to the nature of the drug and its mode of action, alternative therapeutic areas are being sought. One of these is inhibition of Glycogen Phosphorylase (GP), a key enzyme in the control of blood sugar levels. This work focuses on the synthesis of 3,5-di-*C*-methyl-D-glucose **1** (**Figure 1.16**), a proposed competitive GP inhibitor.

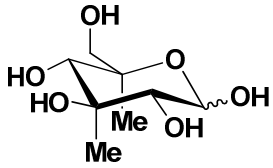


Figure 1.16: 3,5-Di-*C*-methyl-D-glucose, **1**.

1.3.1 Glycogen

Animals will often take in more glucose than is required for immediate use and the valuable excess is stored as a glucose homopolymer called glycogen, which can be rapidly degraded into glucose when energy requirements demand. It is therefore found in organs with a high energy usage – mainly the liver and muscles – of mammals, fish and insects. Glycogen is an extremely large polymer, consisting of α-(1,4) linked glucose units forming a semi rigid backbone. Every 10-12 residues along this chain there is an α-(1,6) linked glucose moiety, creating a branch point (**Figure 1.17**). This branching results in a large number of terminal residues that can be cleaved easily, alongside improving the solubility of the polymer.

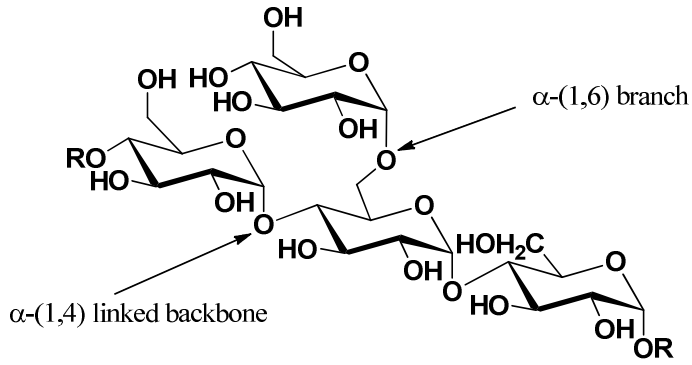


Figure 1.17: Glycogen.

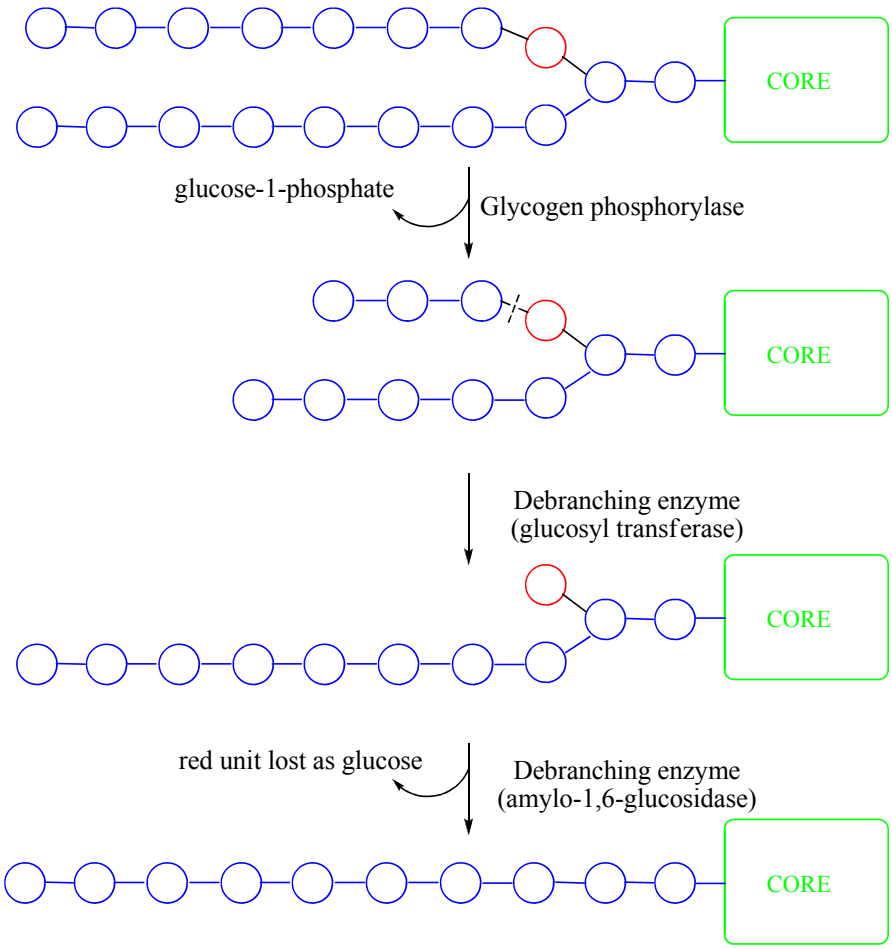
1.3.2 Glycogen Degradation

Glycogen phosphorylase catalyses the phosphorylation of terminal glucose units on the glycogen molecule into α-D-glucose-1-phosphate (**Scheme 1.1**).

$$\text{Glucose}_n + P_i \xrightarrow[\text{Phosphorylase}]{\text{Glycogen}} \text{Glucose}_{n-1} + \alpha\text{-D-Glucose-1-phosphate}$$

Scheme 1.1

GP removes glucose units sequentially from the outer branches but ceases to act some 4 units before a branch point. This is because the active site of the enzyme lies within a deep cleft, into which the more bulky parts of the glycogen cannot go. Eventually, only the core of the original glycogen molecule remains, approximately 50% of the glucose units. At this point, a de-branching enzyme is needed to allow access to the remaining glucose. There are two such enzymes which work alongside one another. The first removes the linear α-1,4-chain and adds those glucose units onto another chain, creating a longer linear chain which can access the active site and therefore be digested by the GP. The second enzyme cleaves the 1,6-linkage, releasing glucose, not glucose-1-phosphate, resulting in slightly elevated glucose levels (**Scheme 1.2**).[85]



Scheme 1.2: Glucose degradation.[85]

In muscle, glucose-1-phosphate is used in glycolysis to provide energy for muscle contraction. In the liver it is dephosphorylated to provide glucose as fuel for other tissues.

1.3.3 Glycogen Phosphorylase

Glycogen Phosphorylase is a large, complex enzyme, made up of two subunits, each containing 841 amino acids and having a molecular weight of 97412 daltons.[86] It exists in two inter convertible states, a and b, where 'a' is the active form, having high substrate affinity and activity, whilst the 'b' form is less active with a lower substrate affinity; one subunit of GPb is shown by **Figure 1.18**. These two states are sometimes referred to as the T and R forms, where the R is the active type.

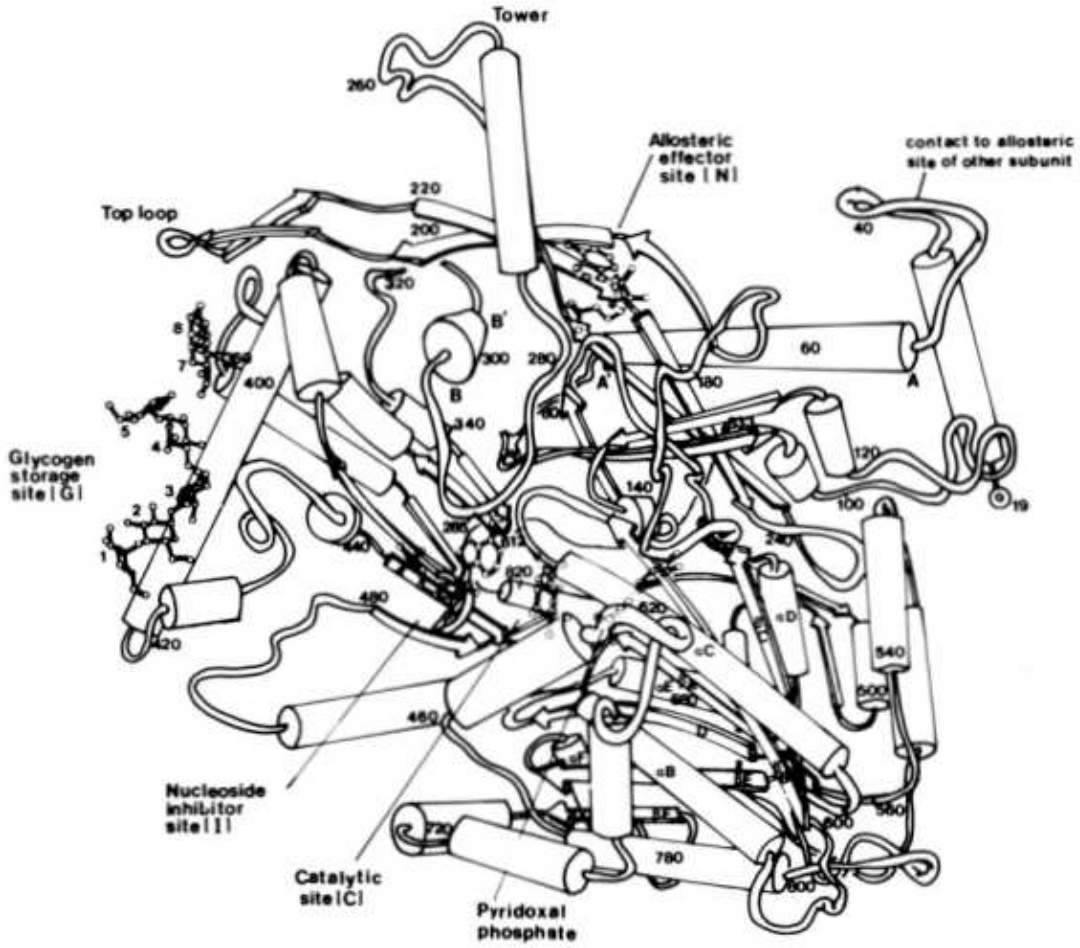
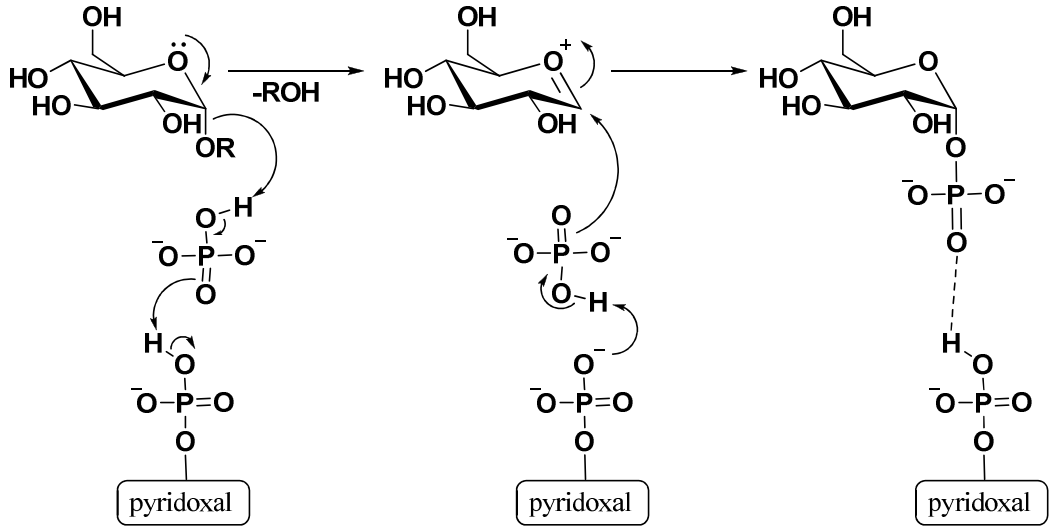


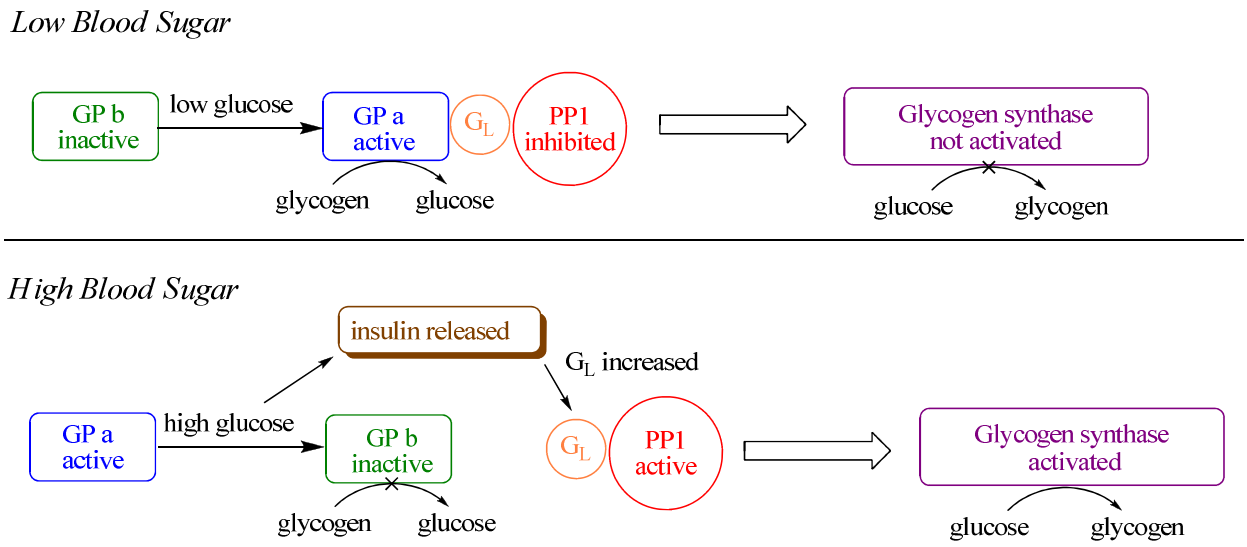
Figure 1.18: One subunit of GPb. α-helices and β-sheets are illustrated by cylinders and arrows respectively.

Each subunit contains a pyridoxal 5'-phosphate moiety in the active site. Given that the glucose subunits in glycogen are α-linked, and the glucose-1-phosphate is also α-linked, either a double inversion at C1 must have occurred in the substrate to retain stereochemistry or an S_N1 type mechanism with retention. As glucose-1-phosphate is likely to be required quickly, a single step S_N1 type mechanism was proposed by McLaughlin *et al.* in which pyridoxal 5'-phosphate acts as a general acid-base catalyst (**Scheme 1.3**).[87]



Scheme 1.3: Catalytic mechanism of GP.

The inactive form of GP can be converted to the active form through phosphorylation of a single serine residue. Hydrolysis of this phospho-serine bond by protein phosphatase-1 results in deactivation of GPa. When blood sugar levels are low, GPb is converted into GPa. This active form can release glucose-1-phosphate from stored glycogen. GPa is also able to bind to G_L, the glycogen binding subunit of protein phosphatase-1 (PP1). This inhibits PP1, making it unable to dephosphorylate glycogen synthase (GS), rendering it in turn inactive. These mechanisms mean that while glucose is released by GPa, it is not simultaneously being removed from the blood stream by glycogen synthase (**Scheme 1.4**). When blood sugar levels are high, GPa is converted into the less active GPb format, stoping the release of stored glycogen. Simultaneous release of insulin raises the likelihood of G_L binding to PP1, making it active. This activates GS, so glucose is removed from the blood and stored as glycogen.



Scheme 1.4: Schematic of how the systems interact.[88]

In a patient suffering from insulin resistant diabetes, G_L levels will not be raised. PP1 will remain inactive, and GS will not be activated. This could quickly lead to dangerously high blood sugar levels, and depleted hepatic glycogen stores.

1.3.4 Glycogen Phosphorylase Inhibition to Treat Hyperglycaemia in Type 2 Diabetes?

Given its key role in control of blood sugar levels, it was predicted that inhibition of glycogen phosphorylase should prohibit hyperglycaemia caused by type 2 diabetes. Early animal studies in mice showed that diabetic mice had increased hepatic glycogen phosphorylase activity compared with non-diabetic mice.[89,90] It was also noted that weak inhibitors of glycogen phosphorylase are weakly hypoglycaemic.[91] Thus, inhibition of GP has been proposed as a promising new strategy for the treatment of type 2 diabetes.[92,93]

Several binding sites for physiological and pharmacological ligands have been identified by crystallography studies (**Figure 1.18**). They include:[94]

(1) The active site that binds glucose and glucose analogues at the glycogen recognition site;

(2) An allosteric AMP-binding site that also binds glucose 6-phosphate;

(3) A glycogen storage site that accounts for the association of GP with the glycogen particle;

(4) An inhibitor site described as the nucleoside site that binds flavopiridols and methylxanthines;

(5) A novel site at the interface between the two monomers that binds various pharmacological compounds and is described as the 'indole carboxamide' site.

This work will focus on a strategy based on active site inhibition, through the synthesis of a novel glucose analogue. Pharmacological inhibitors have been identified for all sites other than the glycogen storage site. For these alternative GP inhibition strategies, Baker *et al.* provides a comprehensive review of recent patents.[95]

1.3.5 Glucose as GP inhibitor

Since glycogen, a glucose homopolymer, is the natural substrate of GP, it is anticipated that glucose should be a competitive inhibitor of GP. This was indeed found to be the case, with K_i values for the inhibition of GPb of 1.7 mM for α-D-glucose and 7.4 mM for β-D-glucose.[96] K_i is a measure of the binding affinity of a substrate; the smaller a K_i value is the better an inhibitor a substance is. However, unlike an antibiotic agent, it is not necessary to totally inhibit GP. It has been noted that when GPa activity was lowered to a certain threshold, small reductions in GP activity were accompanied by large increases in glycogen synthase activity. Clearly though a molecule must be reasonably active, to keep the dosage size sensible. Thus it has been estimated that for an adult dose of 300 mg, a K_i of 1 μM would be required. However, as all of the compound is unlikely to reach its target due to absorption and metabolism issues, a more realistic K_i would be in the region of 0.01 μM.

1.3.6 Glucose anomeric analogues as GP inhibitors

Glucose binds at the catalytic site which crystallographic studies have indicated is situated at the centre of the subunit, about 15 Å from the surface. There is extensive hydrogen bonding to the hydroxyl groups of the sugar from amino acid residues surrounding the active site. With the exception of the anomeric hydroxyl, all hydroxyl groups are a donor and acceptor for 1 hydrogen bond. It was anticipated that analogues of glucose that were able to hydrogen bond to the active site

through the anomeric position would bind more effectively than glucose and therefore exhibit better GP inhibition. A range of inhibitors of the type **30** were synthesised and evaluated (**Figure 1.19**).[97]

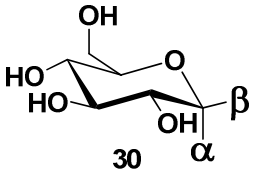


Figure 1.19

A selection of the most interesting results is discussed here (**Table 1.1**).

	Substituents at C1		**K_i (mM)**
	α	**β**	
31	OH	H	1.7
32	$C(=O)NH_2$	H	0.37
33	CH_2OH	H	1.5
34	CH_3	H	53
35	H	OMe	24.7
36	H	$C(=O)NH_2$	0.44
37	H	$C(=O)NHCH_3$	0.16
38	H	$C(=O)NHNH_2$	0.4

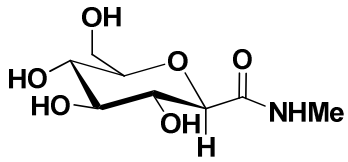
Table 1.1: Results from tests on rabbit muscle GPb.

Substitution of an amide group at the anomeric position was found to enhance activity, regardless of whether it was in the α- (**32**) or β-position (**36**, **37** and **38**), as it allowed for an additional hydrogen bond to the active site. By removing the possibility of a hydrogen bond at the anomeric position (*i.e.* **34**), inhibition was significantly worse. In the case of methyl amide **37**, the additional activation is thought to derive from a lipophillic interaction between histidine and threonine residues, coupled with an entropic release of a water molecule.

1.3.7 Specificity for Hepatic GP

Glycogen stores are found in tissues with high energy needs; thus there will be glycogen phosphorylase in these tissues. As such there are three isoforms of GP designated liver, muscle and brain. From an inhibitor design perspective, the significant homology between these forms poses a complex challenge. If GP inhibition is to be a successful strategy for the treatment of type 2 diabetes, it must be selective for hepatic GP. Inhibition of muscle GP would limit glycogen degradation, leading to impaired muscle function and therefore reduced exercise tolerance and patient mobility. Since patients with NIDDM are typically inactive, an increase in the level of physical activity is usually advised by a medical practitioner. Furthermore, early trials have indicated that unselective GP inhibitors show cardiac effects, by limiting the energy available to heart muscle.[92]

Glucose analogue type drugs are able to utilise the specific pathways which exist *in vivo* for the transport of glucose to the liver.[98] Glucose is transported to muscles *via* an insulin dependent mechanism,[99] which means the glucose concentration in muscle tissue is lower than the blood glucose concentration. Thus, in diabetics, where blood insulin levels are low, glucose transport to the muscles is also low, and therefore a glucose analogue type GP inhibitor was predicted to travel more quickly to the liver to inhibit hepatic GP than to the muscle where it would inhibit skeletal GP. This was indeed found to be the case and glucose analogue **37**, exhibited specificity for hepatic over muscular GP (**Figure 1.20**).[100]



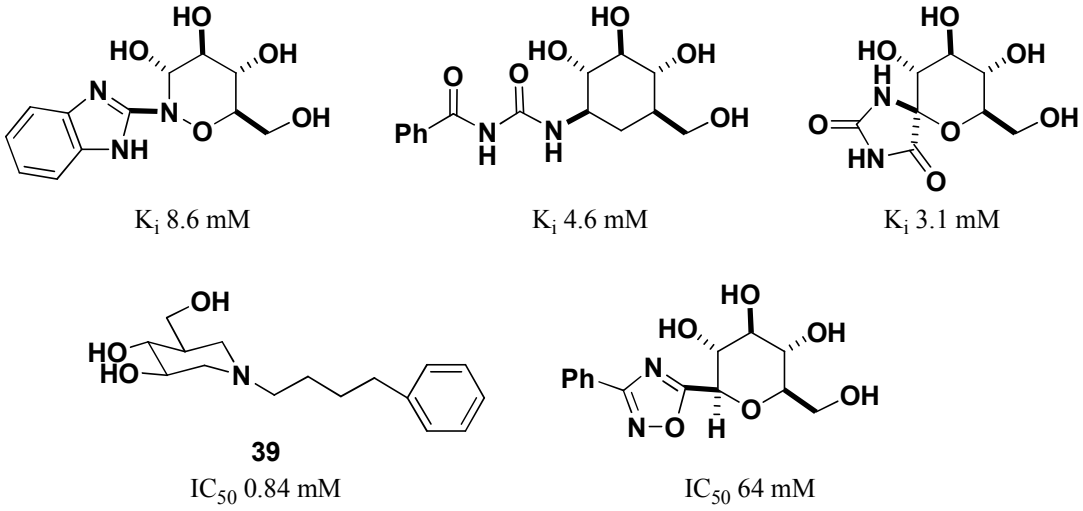
37

K_i 0.16 mM

Figure 1.20: 37, a potent and specific GP inhibitor.

1.3.8 Second Generation Glucose Analogues

A second generation of more complex glucose analogues showed that a wide range of modifications could be tolerated well, including removal of the ring oxygen (**Figure 1.21**). In some cases, K_i values were not reported, in favour of the IC_{50} value. This value represents the concentration of a drug that is required for 50% inhibition *in vitro*, and is related to K_i through the following formula: $IC_{50} = K_i(1 + [S]/K_m)$ where [S] denotes the substrate concentration and K_m is the Michaelis constant.



K_i 8.6 mM K_i 4.6 mM K_i 3.1 mM

IC_{50} 0.84 mM IC_{50} 64 mM

Figure 1.21: Glucose analogues as GP inhibitors (tested on rabbit muscle GPb)[101,102,103]

These analogues illustrate the variety of structural variation from glucose that can be tolerated, however the degree of inhibition exhibited is still some way from the target of 0.01 μM. **39** is more correctly classified as an imino sugar, and these will be considered in more detail in **Section 1.5**.

1.3.9 Lipophillic glucose analogues as GP inhibitors?

The molecules discussed in **Table 1.1** were synthesised largely because molecular modelling had predicted them to show inhibition. Modelling studies had been undertaken to find a molecule that might bind more tightly to the inactive GPb form than active GPa, pushing the equilibrium between these two in favour of the inactive GPb, thus inhibiting glycogen phosphorylase. Added binding could come from either an additional hydrogen bond, or a Van der Waals interaction. For example **33** (**Table 1.1**) had been predicted to be a good inhibitor because an additional hydrogen bond could

be formed to the active site, and this was indeed found to be the case upon testing. The predictions were found to be largely accurate in several other cases,[104] and molecular modelling had shown itself to be a good predictor of inhibition.

After modelling simple glucose derivatives, it was observed that part of the active site cavity, under the glucose ring, was not occupied by any of the previously designed structures. In the native enzyme, two water molecules bind here, but it was noticed that the site was large enough to accommodate several other atoms as well. Continually adding atoms to the underneath of the glucose molecule creates a problem because the molecule will be flexible, and thus binding studies become progressively more difficult. Thus a rigid glucose framework was proposed based on adamantane **40** and its derivatives **41**, **42** and **43** (**Figure 1.22**).[97]

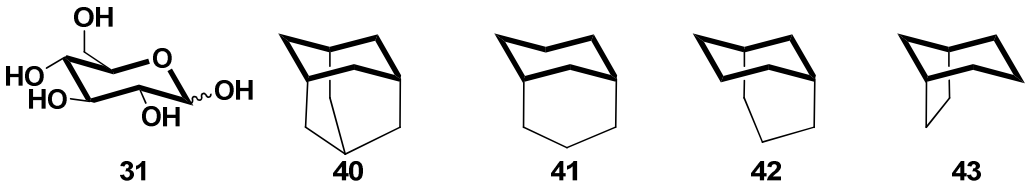
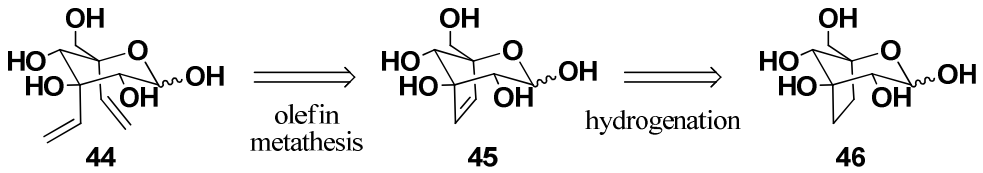


Figure 1.22: Adamantane and derivatives that were modelled as potential GP inhibitors. The bold cyclohexane ring represents a glucose moiety **31**.

Chemically however, practical considerations made the synthesis of any one of these unmanageable, as there was no literature precedent for the synthesis of glucose molecules with two or more branch points. The synthesis of a 3,5 ethyl bridge **46** could be envisaged *via* a ring closing metathesis reaction from vinyl branched sugar **44**, giving **45**, with subsequent hydrogenation. However, installation of vinyl groups at the 3 and 5 positions of glucose is far from trivial (**Scheme 1.5**).



Scheme 1.5: Proposed synthesis of **46** from 3,5-di-*C*-vinyl glucose **44**.

It was therefore proposed to work towards the synthesis of 3,5-di-*C*-methyl-D-glucose **1** in the hope this could adopt a similar conformation to the bicyclic system when enveloped in the active site (**Figure 1.23**). The methodology employed in this synthesis could then be extended to incorporate vinyl groups, leading to fused bicyclic systems.

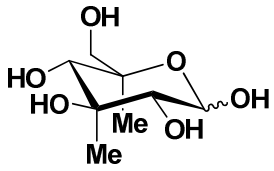


Figure 1.23: 3,5-Di-*C*-methyl-D-glucose **1**, the primary target in this investigation.

1.3.10 Glycogen phosphorylase inhibitors beyond diabetes

There is evidence to suggest that GP inhibitors may be therapeutically useful beyond treatment of type 2 diabetes. Use of GP inhibitors to treat and/or prevent cardiac disease in non-diabetic patients has been reported by Birch and Morley.[105] In particular, they drew attention to the potential utility in stabilising cardiac arrhythmias, protection against ischemic injury and increasing the survival of transplant hearts. Combination drugs, using a GP inhibitor with an anti-arrhythmia and/or anti-hypertensive agent is also claimed to target specific cardiovascular problems. These claims are supported by evidence that GP inhibitors are cardio protective.[106] GP inhibitors may be useful in preventing tumour growth.[107] It has been reported that cancerous cells possess higher levels of glycogen than normal cells,[108,109] and studies on various cancer cell types has shown that the slowest growth was observed in the cells with the highest glycogen content.[110] This ability of a GP inhibitor to withhold energy from a cell has been applied to treatment of infections. By combining the antibiotic azithromycin with a GP inhibitor, it was possible to impede the growth of a bacterial infection, helping the antibiotic to clear infection faster.[111] These effects have not yet been supported by *in vivo* testing.

1.4 New Foods from Novel Monosaccharides

The WHO indicated that globally in 2005 approximately 1.6 billion adults were overweight, with an additional 400 million being obese. At least 20 million children under the age of 5 years were overweight in 2005.[112]

Rising global obesity and medical conditions related to it has increased pressure on the search for low calorie sugar substitutes.[113] The market research company Freedonia has estimated that the US sweetener industry can expect to grow by 4% each year, to reach over $1 bn in 2010.[114] Sweeteners are able to activate taste receptors and taste 'sweet', but cannot be metabolised *in vivo*, making them low or zero calorie alternatives to more conventional sources such as sucrose and honey. Artificial sweeteners also have implications for oral health,[115] as the link between excessive sugar consumption and tooth decay has been noted for centuries. As far back as 1911, substitution of saccharin for sugar was recommended to protect teeth.[116]

One of the first recorded examples of an artificial sweetener comes from ancient Rome, where lead(II) acetate was regularly used to enhance food and wines.[117] The toxicity of lead compounds is now well known, and lead(II) acetate subsequently disappeared from use. Similarly, beryllium chloride is sufficiently sweet to be considered as a potential sugar substitute but again the high toxicity of beryllium is problematic.[118] With the exception of these heavy metal salts, most sweet tasting compounds are organic compounds, in particular dipeptides and organosulphur salts. (**Figure 1.24**).

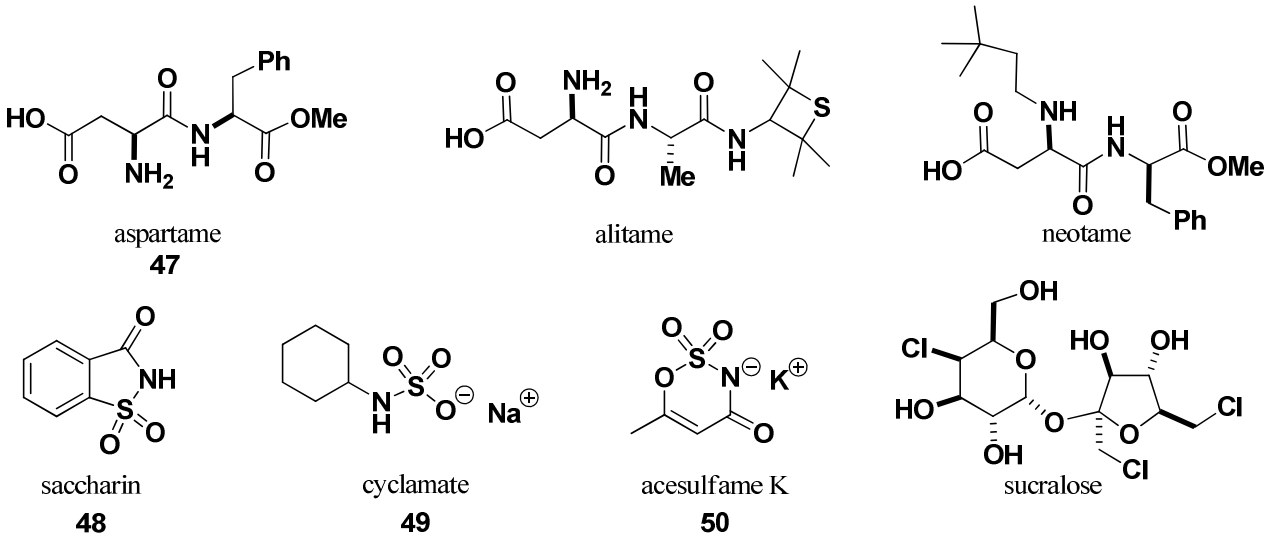


Figure 1.24: Examples of sugar substitutes.

Given the heteroatom and structural variety between the sweet tasting compounds, one of the largest problems facing a synthetic chemist trying to generate a sweet compound is that the mechanism of taste is still poorly understood. Structure and placement of different functional groups is clearly important, as shown by **Figure 1.25**.

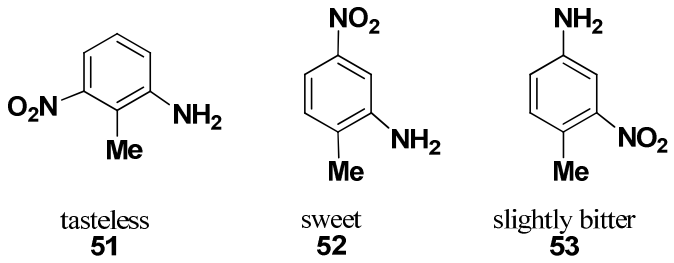


Figure 1.25: The complex relationship between taste and structure.

When all three functional groups are on the same side of the molecule, **51**, it has no taste, whereas placing either the nitro group or amine *para* to the methyl group i.e. **52** or **53**, generates a compound with a distinct taste.[118] This simple example illustrates how complex a mechanism taste is. Other factors affecting taste include size, as molecules that are too big simply won't fit the receptor and possibly hydrophobic/ hydrophilic interactions. It has also been suggested that a hydrogen bonding group is important in enhancing binding and/or recognition.[119] However, these are merely guidelines, and exceptions to the rule exist, for example the sugars gentiobiose and β-D-mannose are bitter tasting.[120]

1.4.1 Continued Controversy

The development of artificial sweeteners has been dogged with health concerns since 1906, when the potential 'dangers' of saccharin **48** were first debated (**Figure 1.24**). Many studies on the safety of saccharin were carried out and the issue was widely debated; President Roosevelt backed its use declaring it harmless in the early twentieth century. However in the 1970s, new research appeared to link saccharin to an increased risk of bladder cancer.[121] These findings were often inconsistent,[122] and although its use has been prohibited in Canada since 1977,[123] saccharin is still in widespread use today in most other countries. A link between cyclamate **49** and the development of bladder cancer in mice was reported,[124] but again, inconsistent results mean it is still in use in many countries.[125] The safety of aspartame **47** is still widely debated,[126,127] and similar doubts hang over the use of acesulfame K **50**.[128]

1.4.2 Recent advances

With both the rising demand and the continuing ambiguity over the safety of existing sugar alternatives comes a growth area in food chemistry. Rare and new monosaccharides have potential as healthy dietary alternatives,[129,130] and recent advances in food chemistry have lead to the increased availability of the once rare sugar D-tagatose **54**, now in widespread use in soft drinks and cereals (**Figure 1.26**).[131]

In contrast to the sweeteners mentioned previously, D-tagatose has been shown to possess health *benefits* beyond being just a low calorie sugar substitute.[132] It was found to have prebiotic properties, which are beneficial to the maintenance of healthy gut bacteria.[133] There is also evidence to suggest it may have a wider range of medical applications for conditions such as type 2 diabetes, hyperglycaemia, anaemia, and haemophilia as well as showing an improvement in foetal development.[134] Even more surprisingly, D-allose **55** has been shown to inhibit cancer cell proliferation[135,136] and even have cryoprotective effects, like the disaccharide trehalose.[137] D-Psicose

56 is another novel no-calorie sweetener;[138] once a rare sugar, it is now readily available *via* an enzymatic transformation of D-fructose (**Figure 1.26**).[139,140,141] It has been shown to be an effective antioxidant,[129] and also to lower the plasma glucose level in rats, whilst simultaneously raising insulin levels, making it a potential therapeutic agent for type 2 diabetes.[142]

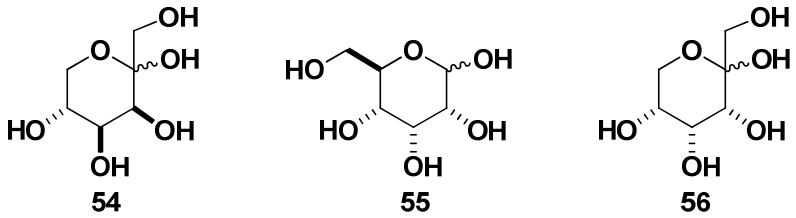


Figure 1.26: Once 'rare sugars' as new sweeteners with added medicinal benefits.

From a commercial perspective, the use of monosaccharides as sweeteners presents large advantages over the other classes. Monosaccharides are usually highly crystalline, and crystallinity often is needed to impart the correct 'mouth feel' to a given foodstuff. They will undergo Maillard browning when heated,[143] giving foodstuffs an authentic look and taste. Also, their sweetness is more closely comparable to that of table sugar (**Table 1.2**), making them easy to substitute like-for-like, requiring no drastic amendments to recipes, of particular importance in baked goods.[144]

Substance	***Relative Sweetness***
Sucrose (table sugar)	1
Aspartame **47**	150-200[145]
Saccharin **48**	300-500[145]
D-Tagatose **54**	0.92[146]
D-Psicose **56**	0.70[147]

Table 1.2: Relative sweetness of selected sugar alternatives.

Given that this work focused on the synthesis of novel monosaccharides, it was anticipated that their relative sweetness and therefore their suitability as sugar substitutes could be assessed with the added potential of exciting medicinal properties.

1.5 Imino sugars

Imino sugars are defined as sugars in which the endocyclic oxygen atom has been replaced by nitrogen. The occurrence of this atom replacement is widespread, and imino sugars have been discovered in a range of plants and micro-organisms.[148] They exist in five distinct classes, piperidines, pyrrolidines, indolizidines, pyrrolizidines and nortropanes. The monocyclic piperidines and pyrrolidines bear a clear structural resemblance to the corresponding monosaccharides; the relationship of the bicycles, indolizidines, pyrrolizidines and nortropanes to sugars is less clear but is a reflection of hydroxyl stereochemistry. Examples of naturally occurring molecules in each class are shown (**Figure 1.27**).

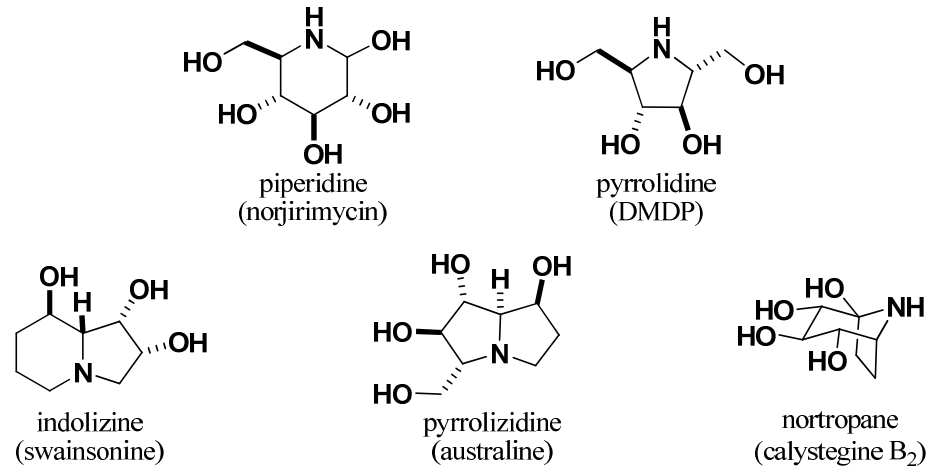


Figure 1.27: Classes of naturally occurring imino sugars.

The bio-significance of this class of molecules has already been alluded to with the introduction of isofagomine analogue **39**, a glycogen phosphorylase inhibitor and miglitol **25**, an α-glucosidase inhibitor, used for the treatment of postprandial hyperglycaemia (**Figure 1.28**).

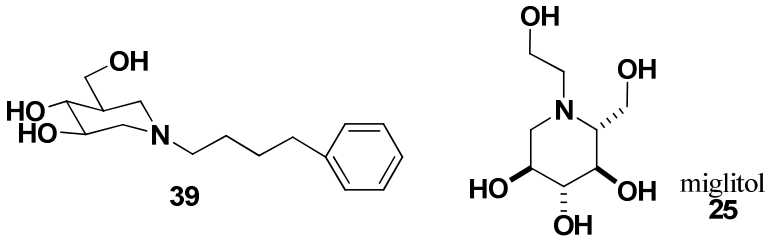
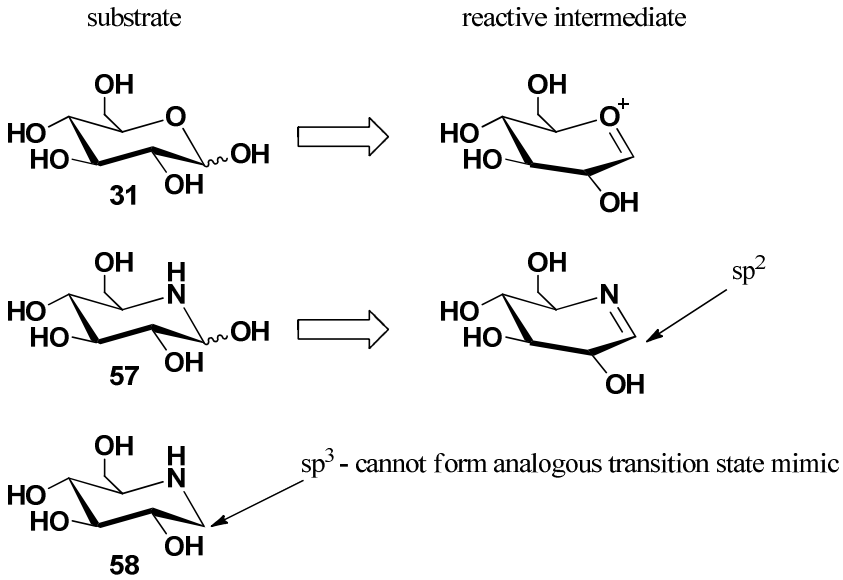


Figure 1.28: Biologically important imino sugars.

Many imino sugars have been found to be potent and highly selective glycosidase inhibitors.[149,150] Glycosidases catalyse the hydrolysis of glycosidic bonds in complex carbohydrates and glycoconjugates, and are therefore involved in a wide range of biological processes including digestion, glycoprotein synthesis and quality control in the endoplasmic reticulum.[151] The possibility of blocking or modifying these pathways has lead to the suggestion that imino sugars could be therapeutic targets for a diverse range of diseases, including cancer, diabetes, lysosomal storage disorders and obesity.[152] Furthermore, viral envelope glycoproteins are often essential for assembly, secretion and infectivity thus compounds which interfere with the glycosylation process of viral glycoproteins can be expected to be anti viral agents.[153]

The similarity in size and electronegativity between oxygen and nitrogen means that imino sugars closely structurally resemble the enzyme's natural substrate.[154] At physiological pH the endocyclic nitrogen will be protonated, leading to the widely accepted theory that the most potent glycosidase inhibitors are transition state mimics of the enzymatic process in question (**Scheme 1.6**).



Scheme 1.6: Imino sugars as transition state analogues?

Withers *et al* undertook analysis of transition-state mimicry of two major classes of glycosidase inhibitors of the type **57** and **58** (**Scheme 1.6**). Their findings suggest that in certain enzymes imino sugars with an sp^2 hybridised 'anomeric centre' are good transition state mimics whereas sp^3

hybridised sugars, although equally potent, do not mimic transition states.[155] This debate is central to imino sugar design since transition state analogues should be the best starting point for inhibitor optimisation.[151]

Naturally occurring pyrrolidine 1,4-dideoxy-1,4-imino-D-arabinitol (DAB, **59**) was isolated from the hyacinth plant and found to be a potent inhibitor of a wide range of glycosidases.[156,157,158] Furthermore, it has been shown to be an effective glycogen phosphorylase inhibitor and therefore a potential therapeutic agent for type 2 diabetes, with a unique mode of action (**Figure 1.29**).[159,160]

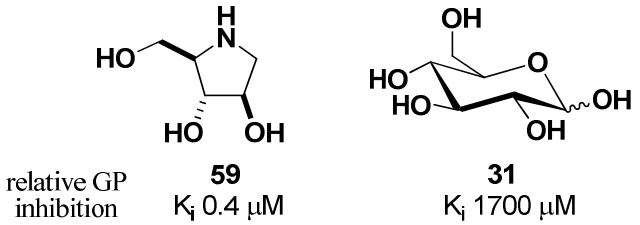


Figure 1.29: Imino sugar **59** is a powerful glycosidase and GP inhibitor.

The naturally occurring piperidine, nojirimycin **60**, was found to be a glycosidase inhibitor and its deoxygenated analogue **61**, 1-deoxynojirimycin DNJ, also a natural product, was found to have greater inhibitory properties.[161] Interestingly, DNJ was one of a selection of imino sugars isolated from the leaves of the mulberry bush, used in traditional Chinese medicine for the treatment of diabetes.[151] Several analogues were then synthesised and it was discovered that *N*-butyl deoxynojirimycin (*N*Bu-DNJ) **62** was more potent still.[162] In addition, *N*-Bu-DNJ is known to act as a ceramide glucosyltransferase inhibitor and is currently used for the treatment of Gaucher disease, a lysosomal storage disorder.[163164] The closely related compound Miglitol (**25**), has already been noted as a therapeutic agent for type 2 diabetes (**Figure 1.30**).[165]

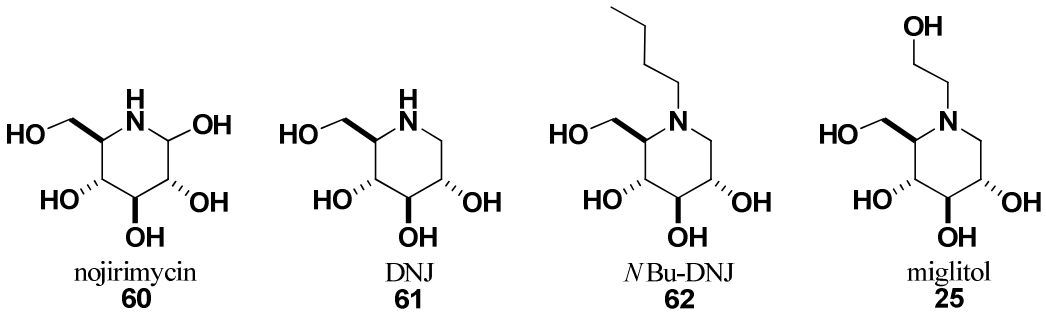


Figure 1.30: Examples of imino sugar therapies already in use.

Biological activity is not restricted to the *gluco* stereochemistry. 1-Deoxygalactonojirimycin (DGJ) **63** and 1-deoxymannojirimycin **64** (**Figure 1.31**) have been shown to be good inhibitors of a range of glycosidases.[166,167,168,169] Moving further away from parent compound nojirimycin, *N*-acetyl-DGJ **65** is a known hexosaminidase inhibitor (**Figure 1.31**). Elevated levels of β-*N*-glucosaminidases have been observed in cancerous cells, thus inhibition of these enzymes is an area of considerable therapeutic interest.[170]

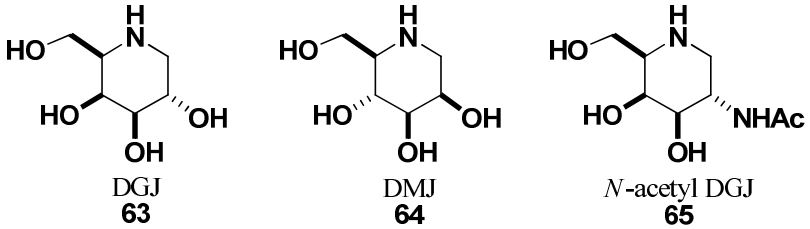


Figure 1.31: Analogues of DNJ **61**.

Since many unnatural imino sugars have been found to be potent glycosidase inhibitors, their synthesis has become an area of growing interest, in particular, the introduction of branch points. Many unnatural hydroxymethyl and methyl branched imino sugars have been synthesised so far (**Figure 1.32**).[171,172,26] Not all of these were found to be effective inhibitors, however, through their synthesis and biological evaluation comes a greater understanding of how these compounds act *in vivo*.

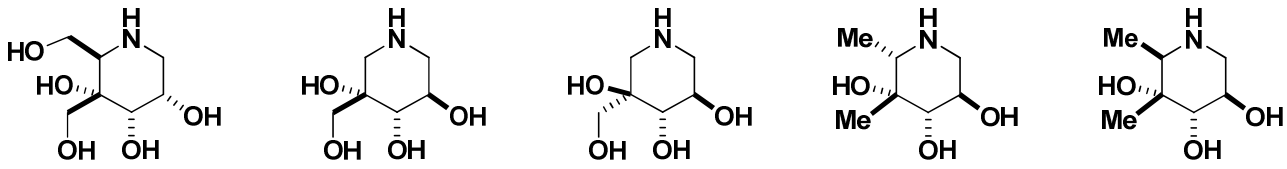


Figure 1.32: Recently synthesised methyl and hydroxymethyl branched imino sugars.

1.5.1 L-*Imino Sugars*

As most glycosidases act as competitive inhibitors, it would be easy to assume that the enantiomeric imino sugars, derived from L-sugars, would possess little biological activity. Most L-sugars are scarce, and as such have received little attention. Synthesis of D-glucose, fructose and mannose was reported in 1890;[173,174] it took nearly 100 years until the synthesis of the L-hexoses was reported.[175] The lack of suitable syntheses of L-sugars has meant there have been limited investigations into L-imino sugars, however early biological studies have been encouraging.

Naturally occurring pyrrolidine 1,4-dideoxy-1,4-imino-D-arabinitol (DAB, **59**) has already been mentioned as a potent glycosidase inhibitor, acting in a competitive manner (**Figure 1.29**). Its enantiomer LAB **66** (**Figure 1.33**) was also found to be an even more effective glycosidase inhibitor with a non-competitive mode of action.[176] *N*-Acylated analogues also displayed this trend as again the D-enantiomers were found to be weak competitive inhibitors whilst the unnatural L-enatiomers were potent allosteric inhibitors. Addition of the benzyl group in each case enhanced inhibitory activity, this trend of increasing inhibition occurring alongside increased lipophilicity has already been illustrated with DNJ **61**, where activity significantly increased with the introduction of a butyl group.[177] Similarly, L-DMDP **67** is a more powerful and specific α-glucosidase inhibitor again with a non competitive mode of action, over the naturally occurring competitive inhibitor D-DMDP **68**.[175]

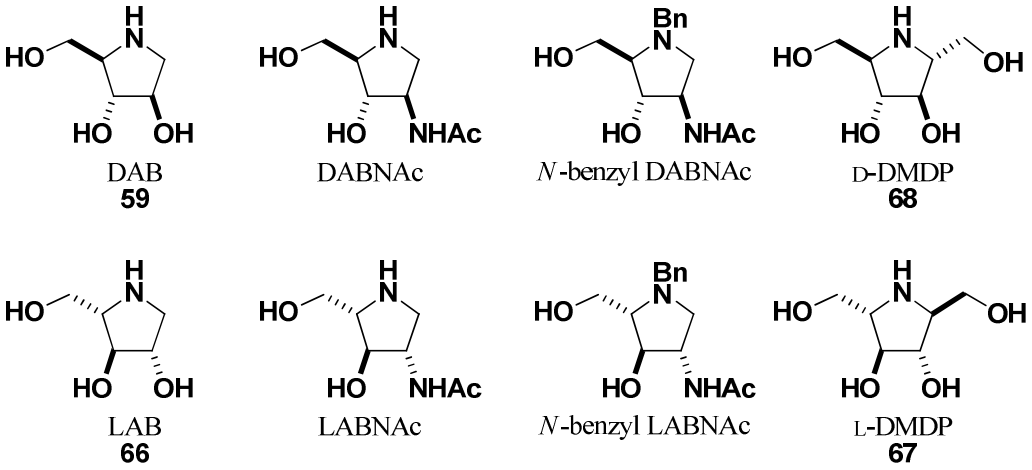


Figure 1.33: Examples of L-imino sugars which are potent glucosidase inhibitors.

D-Swainsonine **69** and 1,4-dideoxy-1,4-imino-D-mannitol (DIM, **70**) are both α-mannosidase inhibitors; in both cases the enantiomers show limited mannosidase inhibition, but do inhibit naringiase (**Figure 1.34**).[178]

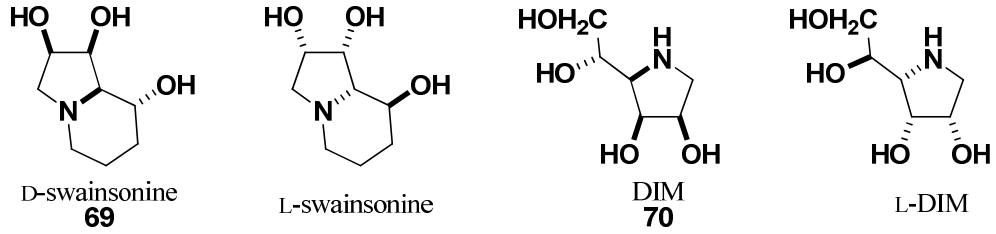


Figure 1.34: Examples of L-imino sugars which inhibit different enzymes than their D-enantiomers.

1.6 Proline Analogues

Proline is unique amino acid and a key determinant of protein structure, being the only secondary amine and possessing a saturated ring.[179] Therefore, modified proline analogues could affect peptide binding patterns, and several proline analogues have been synthesised for this purpose.[180] Proline analogues are also of interest as proline itself has been used as a chiral catalyst in asymmetric synthesis, for example in the Knoevnagel reaction.[181]

Proline analogue kainic acid **71** has received much attention in the literature (**Figure 1.35**). [182,183,184] Isolated from marine alga *Dignenea Simplex*, it displays neuroexcitatory properties and therefore may be useful in the treatment of epilepsy, Alzheimer's and Huntington's disease.[185] Inclusion of **72** into a peptide has been shown to form almost exclusively *cis* amide bonds and as such accelerate protein folding.[186] *cis*-4-Hydroxy-L-proline **73** was found to inhibit collagen biosynthesis in tissues,[187] which has implications for the treatment of the rare but potentially fatal condition scleroderma, characterised by hardening of the skin and/or organs.[188] It has also been used successfully as a chiral catalyst in aldol reactions.[189]

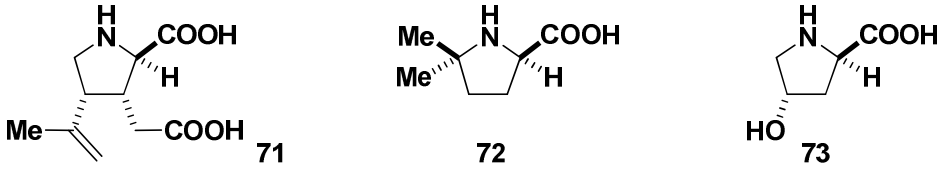
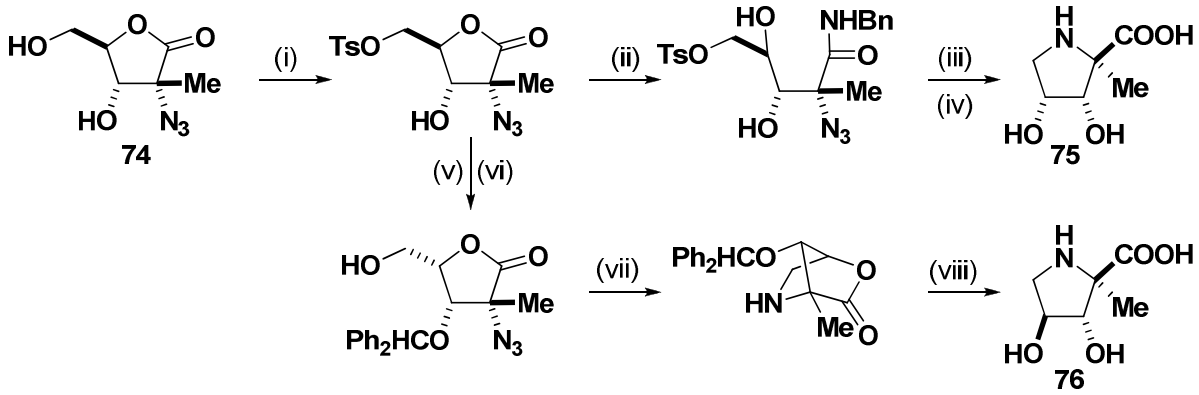


Figure 1.35: Biologically active proline analogues.

Many substituted proline derivatives have been synthesised; alkylation at the α-carbon of proline is perhaps the simplest way to access a library of compounds,[190] but clearly the scope is limited. Substitution at other centres however often involves multi-step syntheses and/or use of expensive chiral catalysts.[191] Recently, an expedient synthesis of novel branched hydroxy prolines **75** and **76** was reported by da Cruz *et al.* (**Scheme 1.7**).[192] This elegant synthesis circumvents the use of chiral catalysts by starting from a functionalised aldonolactone **74** and requires few functional group manipulation steps.



Reagents and Conditions: (i) TsCl, Py, 68% (based on recovered starting material); (ii) NH_2Bn, THF, 82%; (iii) H_2, Pd/C, 1,4-dioxane; (iv) 37% $HCl_{(aq.)}$, 83% over 2 steps; (v) Ph_2CN_2, 1,4-dioxane, heat, 60%; (vi) KOH, 1,4-dioxane, then Dowex (50W X8 H^+ form), 72%; (vii) Tf_2O, Py, DCM then H_2 Pd/C, 1,4-dioxane, 42%; (viii) NaOH, 1,4-dioxane, H_2O then H_2, Pd/C, H_2O, 90%.

Scheme 1.7: Synthesis of novel branched hydroxy proline analogues.

Steps (v) and (vi) are of particular interest as a protecting group is employed followed by inversion of stereochemistry at C4, allowing access to the *lyxo* compound. The $CHPh_2$ or 'benzhydryl' group has only recently come to the fore as a suitable protecting group for carbohydrate syntheses, particularly suitable as it is stable to strong base as well as moderately acidic conditions.[193] Use of this protecting group will be further explored in **Chapter 2**.

1.7 Content of this Thesis

In **Chapter 2** routes towards 3,5-di-*C*-methyl-D-glucose are explored. During these investigations, work was carried out on the synthesis of the L-enantiomer as a test system for the methodology and in both cases the corresponding 3,5-di-*C*-methyl mannose is also reported. As part of these investigations, the synthesis of 3,5-di-*C*-methyl-L-fructose was examined. Glycogen Phosphorylase inhibition studies on 3,5-di-*C*-methyl-D-glucose was carried out by Prof. A. Kato.

Chapter 3 investigates the chemistry of branched sugars further, by probing the reactivity of 2,4-di-*C*-methyl-L-arabinonolactone, synthesised as part of the investigations in to the synthesis of 3,5-di-

C-methyl sugars. This culminated in the synthesis of a novel dibranched proline analogue, two piperidines, a pyrrolidine and a deoxy sugar. Routes towards a third piperidine are discussed.

The **Appendix** contains the results of detailed NMR analyses on 3,5-di-*C*-methyl glucose and mannose, a key intermediate in their synthesis, 2,4-di-*C*-methyl arabinose and 2-deoxy-2,4-di-*C*-methyl arabinose, kindly performed by Dr M. Wormald.

The **CD Appendix** contains crystallographic information from a range of structures run during the course of this work. X-Ray crystallography has been very valuable to this project; the existence of two quaternary centres, and therefore a lack of hydrogen atoms around the carbohydrate ring meant that establishing stereochemistry with nOe experiments was frequently unreliable.

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Chapter 2

Synthesis of Di-C-Methyl Branched Chain Sugars

2.1 Synthesis of branched chain sugars

Most carbohydrates are straight chain molecules;[1] However, singly branched sugars, with one methyl or hydroxy methyl branch are found in nature, often with significant roles to play.[2,3,4,5] With the discovery of more and more branched carbohydrates in natural products comes an interest in their synthesis and biological evaluation. As such, there are now several different methods for the synthesis of branched carbohydrate derivatives. These were briefly assessed for their suitability in the synthesis of doubly branched sugar 3,5-di-*C*-methyl glucose **1** (**Figure 2.1**).

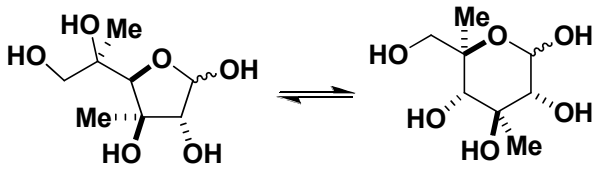
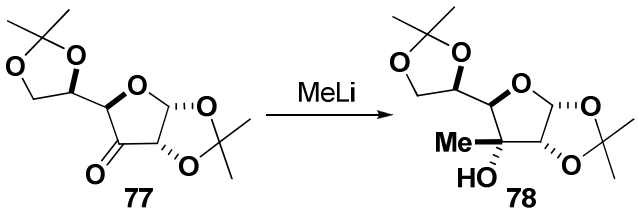


Figure 2.1: 3,5-Di-*C*-methyl-D-glucose **1**.

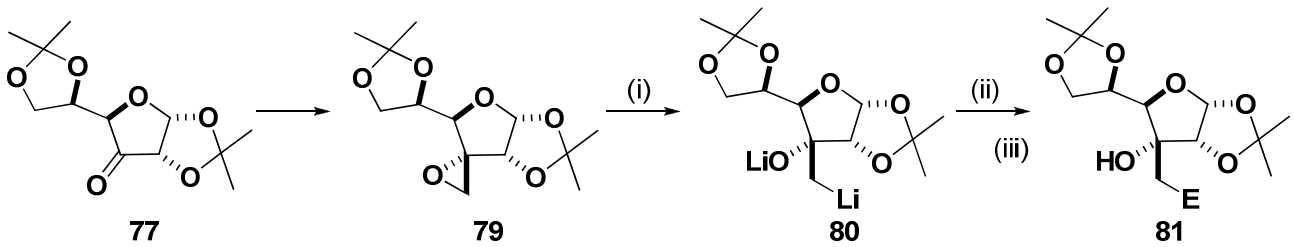
2.1.1 Nucleophilic approaches

Perhaps the simplest way to indroduce a branch point is by the oxidation of a hydroxyl group on the carbohydrate backbone, followed by addition of a nucelophilic carbon species. In the example below, diacetone glucose is oxidised at the 3 position, giving ketone **77** and methyl lithium added, giving 3-*C*-methyl allose **78** (**Scheme 2.1**).



Scheme 2.1: Using nucleophiles to generate branched sugars.

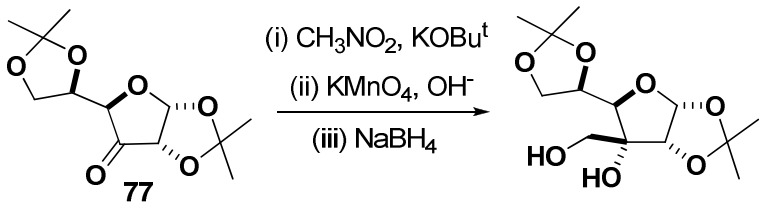
This method is completely steroselective, however, it gives exclusively the *allo* rather than the *gluco* stereochemistry, and would therefore be unsuitable in the synthesis of a 3-*C*-methyl branched glucose analogue. Epoxidation of this type of ketone, *via* sulphur ylid chemistry, can be used to access a wide range of branched sugars. Excess lithium and a catalytic quantity of DTBB (4,4'-di-*tert*-butylbiphenyl) opens the epoxide ring **79** to dianion **80**; subsequent treatment with an electrophile and aqueous work up can give a access to a wide range of branched and functionalised sugars of the type **81** (**Scheme 2.2**). [6,7]



Reagents and Conditions: (i) Li, DTBB (5%), THF, -78°C, 2 hrs. (ii) E^+ = H_2O, D_2O, Me_3SiCl, PhCHO, Me_2CO, $(CH_2)_5CO$, -78°C, 10 min. (iii) H_2O, -78 to 20°C.

Scheme 2.2: Branched sugars from epoxides.

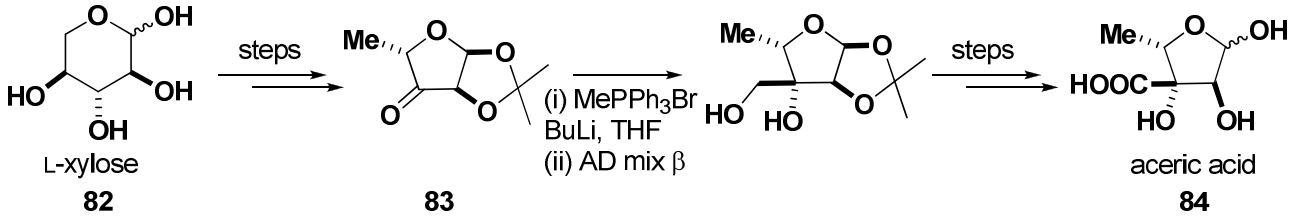
Henry reactions[8,9] have also been used in the synthesis of branched sugars, taking a ketone such as the one derived from diacetone glucose **77** with the nitromethane anion (S**cheme 2.3**). Whilst addition of the anion is generally achieved in high yields; subsequent hydrolysis of the nitro group can require forcing conditions, often incompatible with carbohydrates, and subsequent reduction to give a hydroxy methyl branch. Both yield and stereoselectivity in these types of syntheses can be high, however they rely heavily on protecting group manipulations to selectively access the desired hydroxyl group.



Scheme 2.3: Use of Henry reactions for branched sugar synthesis.

2.1.2 Wittig Chemistry

The formation of carbon-carbon bonds from carbonyl compounds and phosphorus ylides is known as the Wittig Reaction. Since the discovery of this transformation in the early 1950s it has become one of the most commonly used methods to synthesise alkenes and nearly 40 reviews on the subject have been written to date.[10] Its use in the synthesis of branched chain sugars is through addition of an ylid to a carbonyl on the carbohydrate scaffold;[11,12,13] this was the approach adopted by Jones *et al* in their synthesis of aceric acid **84**, from ketone **83**, itself derived from L-xylose **82** (**Scheme 2.4**).[14]

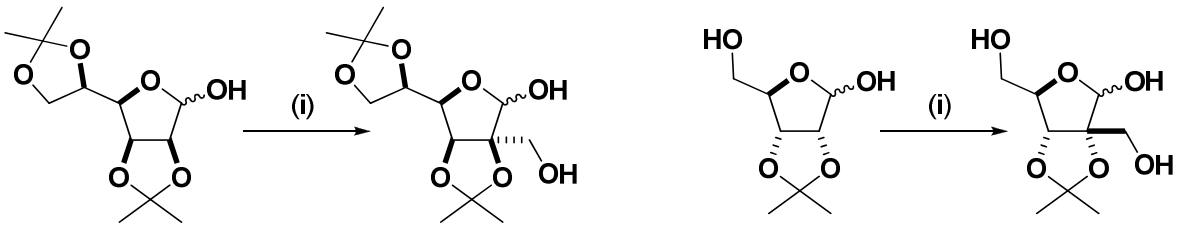


Scheme 2.4: Wittig reaction followed by dihydroxylation to introduce a hydroxy methyl branch.

A silicon, rather than phosphorus ylid, has also been employed in the synthesis of branched chain sugars, in a Peterson type reaction.[15] By use of a branched Wittig reagent of the type Ph_3P=C(R)COMe with a glyceraldehyde derivative, C1 branched sugars have been successfully produced, where 'R' could be either a hydrogen atom to synthesise straight chain sugars, or an alkyl chain to introduce a branch point.[16]

2.1.3 Modified aldol procedure

Introduction of a hydroxymethyl branch could alternatively occur *via* addition of a carbon electrophile in an aldol type reaction. This requires formation of a ketone enolate which subsequently acts as a nucleophile towards an aldehyde. Given that a wide variety of aldehydes are readily available, this should present a straightforward method of generating a vast library of branched monosaccharides. Traditional aldol reactions are carried out using a strong base to generate the enolate, such as lithium di*iso*propylamide (LDA) or potassium hexamethyldisilazane (KHMDS), however rapid degradation of reducing sugars in strong base is often observed. The reaction can present difficulties when performed on aldonolactones due to the strength of base required, so it generally must be performed on aldoses. Ho carried out a significant amount of work in this area, taking sugars with formaldehyde in the presence of mild base (**Scheme 2.5**).[17,18,19]



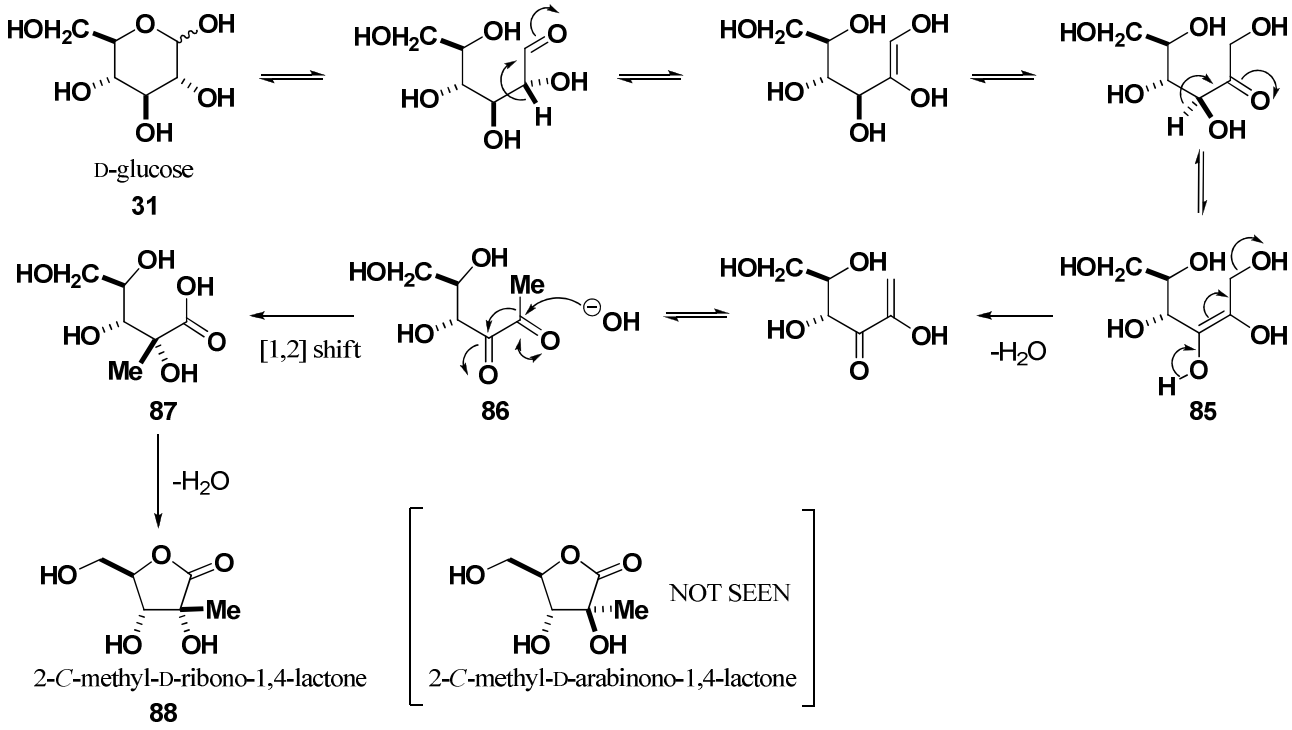
Reagents and Conditions: 37% aq. HCHO, K_2CO_3, MeOH, heat.

Scheme 2.5: Aldol reactions on carbohydrates.

The relatively mild conditions needed to effect this transformation are believed to derive from the stability of the intermediate anion to decomposition. Degradation of this anion in an E_1cB type pathway requires a Baldwin disfavoured 5-*endo*-trig opening of the isopropylidene ring. However, despite the stability of the anion to decomposition, the aldol reaction as a method of introducing branch points has considerable drawbacks. In order to fix the stereochemistry in the products, the 2,3-position must be protected in a five ring form and this requires OH-2 and OH-3 to be *cis*, limiting substrate variety. Aditionally, crossed Cannizarro products have been reported as a side product in this reaction.[20]

2.1.4 Treatment of sugars with strong base and the Amadori Rearrangement

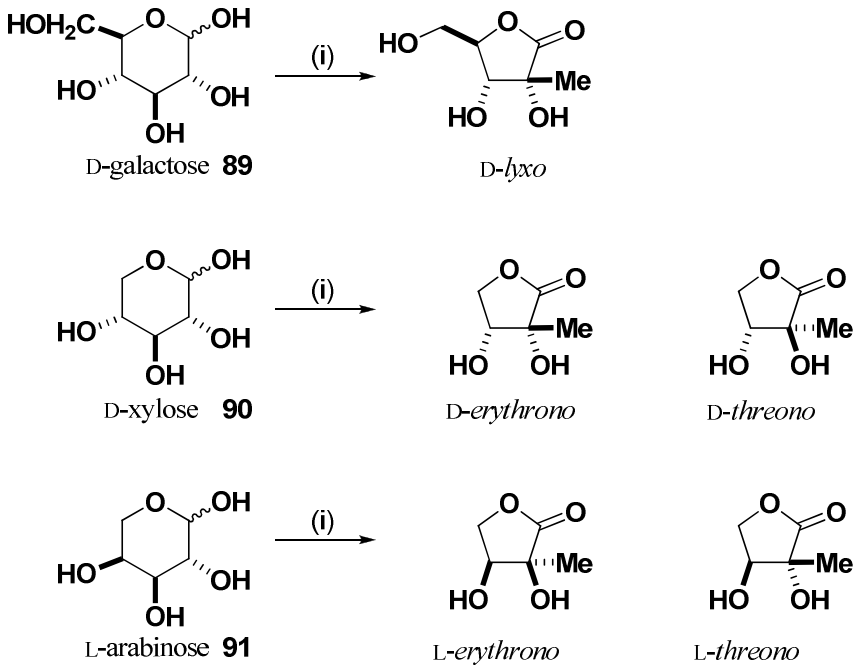
The reaction of glucose with strong aqueous base is one of the oldest and most complex reactions known.[21,22] Treatment of glucose with calcium hydroxide results in the formation of over 50 compounds, one of which was found to be the branched saccharinic acid **87**; this is believed to arise from a Lobry-de-Bruyn-Alberda-van-Ekenstein rearrangement (**Scheme 2.6**).[23] The elimination of water from **85** to form diketone **86** and the subsequent [1,2] shift are key step in this process. The [1,2] shift proceeds completely stereospecifically, generating exclusively the *ribo* product **88**.



Scheme 2.6: Base catalysed isomerisation of glucose to give the branched saccharinic acid **87**. Hydroxide attack of intermediate **86** at the other carbonyl group will also result in the formation of saccharinic acid **87**.

Despite the complexity and low yield (0.5 %) of this reaction, it was found that treatment of fructose in an analogous fashion could afford, after careful optimisation, an 11% yield of lactone **88**.[24] Whilst a clear improvement, this procedure was impractical for the synthesis of large quantities of **88**, as it required a reaction time of six weeks and the work up procedure involved removal of very large quantities of water. The elimination of water from **85** to form diketone **86** is a critical step in this sequence. Hotchkiss *et al.* demonstrated that by the introduction of a nitrogen

based leaving group at the anomeric centre of **85**, and thereby employing an Amadori rearrangement prior to base catalysed isomerisation,[25] significantly higher yields could be achieved with a greatly reduced reaction time; 19% in only 24 hours.[26] This sequence of treating sugars with amines then calcium hydroxide was also successfully applied to D-galactose **89**, D-xylose **90** and L-arabinose **91** (**Scheme 2.7**).[27] Despite the moderate yields obtained by these transformations, this methodology provides rapid access to 2-*C*-methyl branched sugar lactones in a single step, following a green, environmentally friendly procedure using inexpensive starting materials.

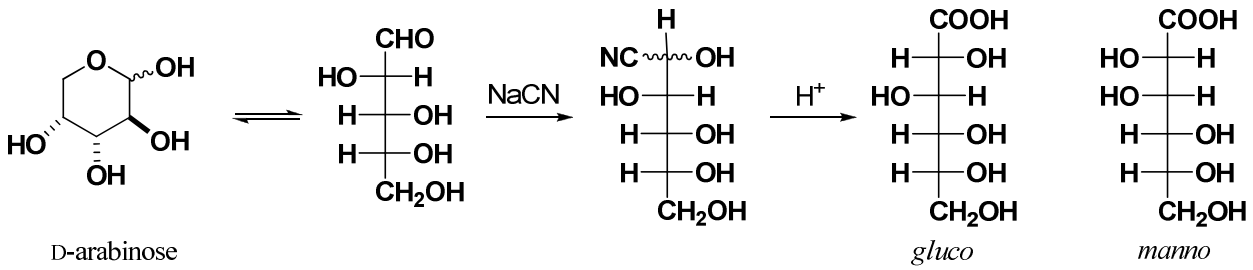


Reagents and Conditions: (i) Me_2NH, AcOH then $Ca(OH)_{2(aq)}$ then H^+.

Scheme 2.7: Synthesis of 2-*C*-methyl branched sugar lactones *via* treatment of Amadori ketoses with calcium hydroxide.

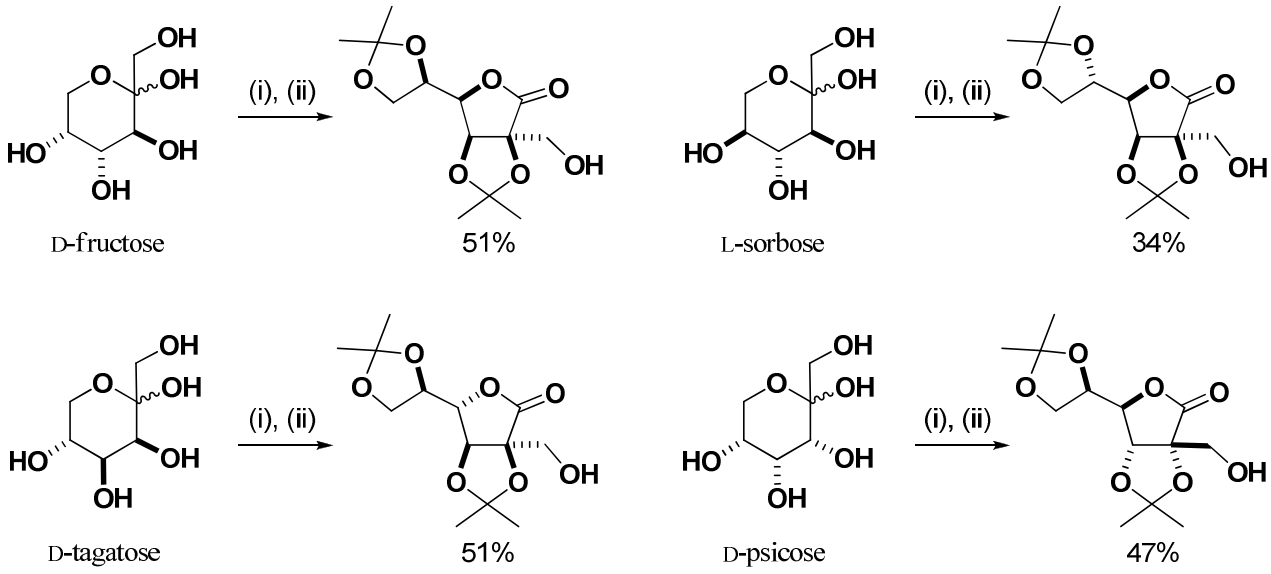
2.1.5 The Kiliani Reaction

Over 120 years ago, it was demonstrated that treatment of a sugar with sodium or potassium cyanide resulted in addition of the cyanide anion to the aldehyde. If followed by acid hydrolysis, this would give a carboxylic acid, which should then close to give a lactone with one extra carbon in its scaffold.[28,29,30] This process became known as the Kiliani-Fischer synthesis, and has been applied in the synthesis of sugars with linear carbon chains. For example, elongation of the *arabino* sugar can give a mixture of the *gluco* and *manno* sugar lactones (**Scheme 2.8**).[31,32,33,34]



Scheme 2.8: Using the Kiliani-Fisher reaction to generate hexoses

Unitl relatively recently, the procedure was limited to aldoses, however recent developments have shown that treatment of a ketose sugar in an analogous fashion can yield branched sugars. Efficient, green and inexpensive routes to a range of branched sugars have been established in this way.[35,36,37] In most cases, direct re-crystallisation of the branched sugar product arising from the Kiliani sequence is not possible, however crystalline sugar lactones can be obtained *via* a Kiliani-acetonation sequence (**Scheme 2.9**).[38]

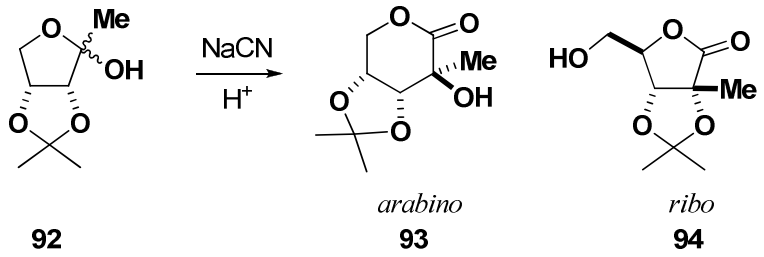


Reagents and Conditions: (i) NaCN, H_2O; (ii) $(CH_3)_2CO$, $CuSO_4$, c.H_2SO_4

Scheme 2.9: Kiliani reactions on ketoses.

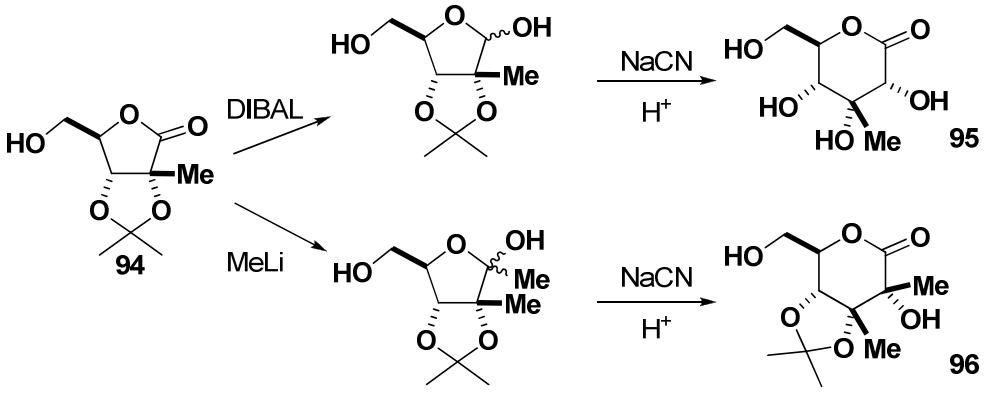
Although the yields are moderate, the cost of the ketoses is low and facile crystallisation of the major products makes this a practical approach to the synthesis of sugars possessing a hydroxy methyl branch.

Whilst hydroxy methyl branches can be easily converted in to methyl branches *via* halogenation and hydrogenation, it would be preferable to start with the methyl branch in place, thereby minimising the number of steps in the total synthesis. This approach was demonstrated by Hotchkiss *et al.* in their synthesis of 2-*C*-methyl-D-arabinono **93** and ribono **94** lactones from 1-deoxy ribulose **92** (**Scheme 2.10**).[39]



Scheme 2.10: Synthesis of 2-*C*-methyl sugars by Kiliani reaction.

Curran *et al.* extended this work by treating the protected 2-*C*-methyl-D-ribono-1,5-lactone **94** with DIBAL then sodium cyanide, to generate 3-*C*-methyl branched sugar lactone **95** and by taking **94** with methyl lithium then sodium cyanide, generating 2,3-di-*C*-methyl branched lactone **96** (**Scheme 2.11**).[40,41]

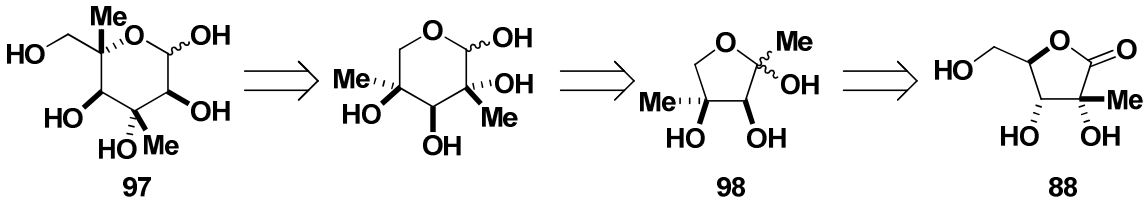


Scheme 2.11: 3-*C*-Methyl and di-*C*-methyl branched sugars from the Kiliani reaction.

It is of interest to note here that by use of a protected starting material, the acetonation step can be avoided, although in some cases, deprotection of the products is observed during the acidic work up of the Kiliani reaction, necessary to hydrolyse the cyanohydrin.[42]

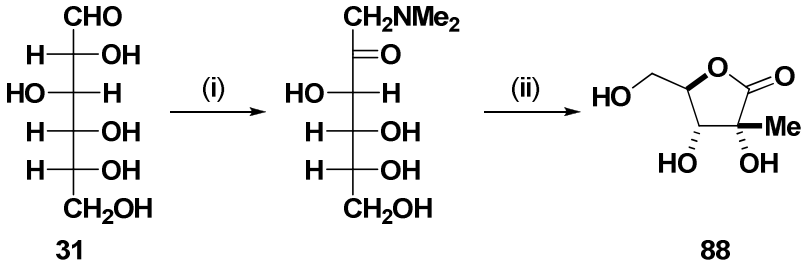
2.2 Synthesis of 3,5-di-C-methyl glucose

Given the success of the Kiliani reaction in the synthesis of di-C-methyl sugar **96** (**Scheme 2.11**), a retrosynthesis was proposed using two sequential sodium cyanide additions. From the addition of a carbon atom by treatment with sodium cyanide, it should be possible to generate a 3,5 branched sugar from a 2,4 branched molecule. Since no 2,4 branched chain sugars are commercially available, a convenient precursor would be a '1,3 branched sugar'. It was envisaged that the '1,3 branched sugar' **98** could come from the readily available branched sugar **88** (**Scheme 2.12**).[43] The synthesis will provide the L-enantiomer of 3,5-di-*C*-methyl glucose **97**, which could be used as a test sytem for the synthesis of the desired D-enantiomer.



Scheme 2.12: Retrosynthesis of 3,5-di-*C*-methyl-L-glucose from 2-*C*-methyl-D-ribono-1,4-lactone.

Synthesis of 2-*C*-methyl-D-ribono-1,4-lactone **88** from D-glucose **31** *via* an Amadori rearrangement has previously been discussed. As large quantitites of this material were now required, a modified and largely anhydrous work up procedure was adopted, reducing the quantities of water used and allowing for the direct crystallization of **88** (**Scheme 2.13**). Use of jacketed reactors controlled by Julabo heater/chiller units allowed for more precise temperature control than had been previously possible, and therefore slightly improved yields were observed. 157 g of D-glucose **31** gave 38 g of **88**, 27%; 1260 g of **31** gave 229 g of **88**, 20%, compared with 19% on smaller laboratory scale without the modified procedure or more tightly controlled conditions. Additional ribonolactone **88** was present in the mother liquors and residues but further isolation of the material proved to be cumbersome and not cost effective.[44]

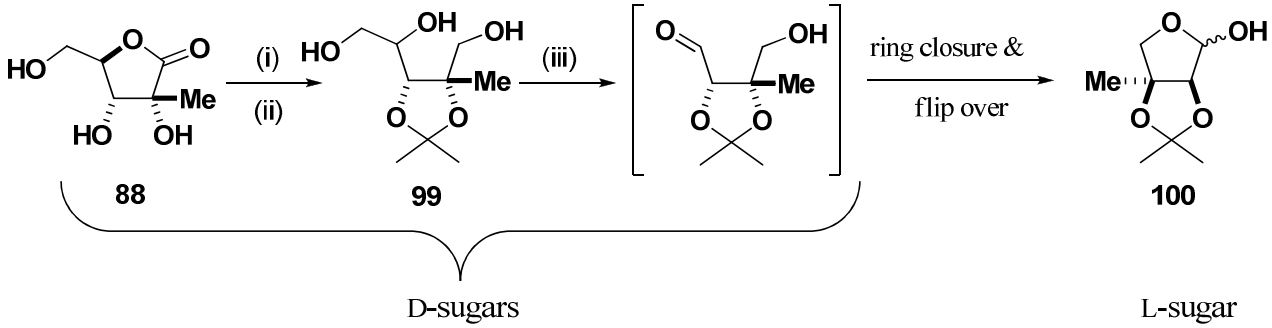


Reagents and Conditions: (i) Me_2NH, AcOH, EtOH; (ii) CaO, H_2O then H^+.

Scheme 2.13: The synthesis of 2-*C*-methyl-D-ribono-1,4-lactone **88** in a scaleable one pot reaction.

2.2.1 Synthesis of 1-deoxy ribulose

Isopropylidene protection of **88** with acetone, copper(II) sulfate and acid was achieved in quantitative yield.[45] To form the branched erythrose derivative **100**, it was necessary to reduce the protected ribose to the open chain alcohol and cleave the terminal hydroxy methyl group. Reduction of the lactone to the triol could be achieved in high yield with either lithium or sodium borohydride. The crude polyol **99** can be treated directly with sodium periodate; this cleaves the terminal diol generating an aldehyde, which closes *in situ* to give lactol **100** in upto 94% yield from protected 2-*C*-methyl-D-ribono-1,4-lactone (**Scheme 2.14**). This sequence has changed the most oxidised position on the backbone from C1 to C4, making it now an L-sugar.



Reagents and Conditions: (i) $(CH_3)_2CO$, $CuSO_4$, H_2SO_4, RT, 16 h; (ii) $LiBH_4$, THF, RT, 1.5 h; (iii) $NaIO_4$, MeOH, H_2O, RT, 45 min.

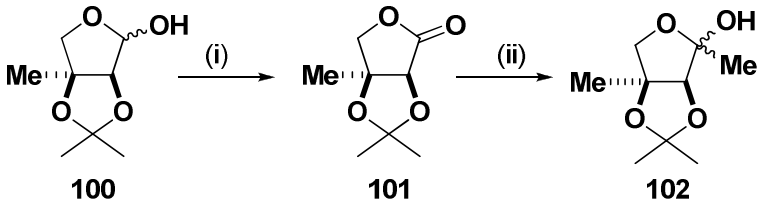
Scheme 2.14: Synthesis of 3-*C*-methyl-L-erythrose **100**.

A literature procedure exists for the oxidation of **100** to the corresponding lactone **101**, using *N*-iodosuccinimide, but affords moderate yields (45-61%).[46] An oxidation with bromine was tried, using potassium carbonate as a base in aqueous conditions;[47] t.l.c. analysis of the reaction mixture showed a single product, however, after work up *via* a DCM/water wash, only 7% of the desired

lactone was obtained. The failure of the bromine oxidation procedure was surprising as Fleet *et al* have applied this method to the synthesis of a wide varity of sugar lactones.[48,49,50]

Deprotection of lactol **100** was attempted in the hope that the product would be more soluble in aqueous systems, improving the yield of oxidation. Lactol **100** was dissolved in water and treated with a large quantity of acidic resin. Despite heating, the reaction took 7 days to go to completion, and it was therefore decided to proceed with the protected form.

Repetition of the initial conditions with barium carbonate gave a similar result, however, it was noticed that the aqueous phase from the work up was found to be very strongly acidic, and it is thought that prolonged contact during work up may have caused the lactone to open to the saccharinic acid, which would then remain in the aqueous phase. The aqueous phase was concentrated, in the hope that the acid would close upon dehydration. The residue was dissolved in an organic solvent and t.l.c. analysis clearly showed the lactone. Therefore, when the reaction was repeated, the DCM/water wash step was avoided; the reaction mixture was simply diluted with ethyl acetate and the organic phase removed *via* pipette, giving the lactone in high yield (94%). The final step in the synthesis of ketone **102** was the addition of a methyl branch, using methyl magnesium bromide (87%). This sequence from 2-*C*-methyl-D-ribono-1,4-lactone **88** to ribulose **102** could be repeated on up to a 20 gram scale with no significant reduction in yield (**Scheme 2.15**).

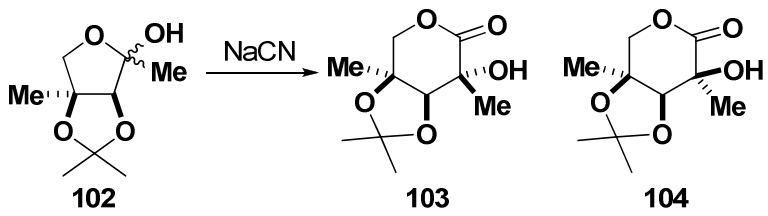


Reagents and Conditions: (i) Br_2, $BaCO_3$, H_2O, 0°C → RT, 18 h; (ii) MeMgBr, THF, -78°C, 45 min.

Scheme 2.15: Synthesis of protected 1-deoxy-4-*C*-methyl-L-ribulo furanose, **102**.

2.2.2 Synthesis of 2,4-di-C-methyl-L-arabinono, 103, and ribono, 104, lactones

Deprotection of ketone **102** prior to treatment with sodium cyanide was briefly investigated, however, it quickly became apparent that the strongly acidic conditions required to remove the isopropylidene group degraded the sugar. Treatment of 605 mg of **102** with Dowex® (50W X8 H^+ form) acidic resin for several days afforded only 80 mg of the desired deprotected ribulofuranose, which was shown by ^{1}H NMR spectroscopy to be heavily contaminated, even after repeated attempts at purification *via* column chromatography. It was therefore decided to pursue direct treatment of ketone **102** with aqueous sodium cyanide; subsequent hydrolysis could give two possible products, **103** or **104** (**Scheme 2.16**). It was not possible to tell prior to carrying out the reaction which would be favoured (if either) however in the analgous singly branched case, the anti Felkin-Ahn *arabino* product was found to predominate.[51]



Scheme 2.16: Proposed Kiliani reaction to form 2,4-di-*C*-methyl branched sugar lactones, **103** and **104**.

Ketone **102** was treated with aqueous sodium cyanide at room temperature for 24 hours, and then heated to 100°C for 24 hours. The crude reaction mixture was then acidified by eluting through a column of Amberlite IR-120 (H^+ resin) with water. Evapouration of solvents results in a creamy coloured crystalline crude, from which the major product could be recrystallised directly. Column chromatography of the mother liquor was necessary to retrieve the minor component. A striking degree of selectivity was observed; the major product was obtained in up to 80% yield, whilst the minor was only acquired in a 1-4% yield. This is an extremely good result for a reaction of this kind as frequently Kiliani reactions on protected substrates suffer reduced yields due to isopropylidene deprotection of the sugar lactones during work up, and subsequent difficulties in recovering deprotected sugar lactones from aqueous media.[52] Whilst the highly stable nature of this

isopropylidene group is advantageous in this instance, it posed significant problems in other synthetic routes, in particular the synthesis of 3,5-di-*C*-methyl-L-fructose (please see **Subsection 2.4**).

The stereochemistry of the major product was confirmed by X-ray crystallographic analysis to be the *arabino* lactone, **103** (**Figure 2.2**), as anticipated from the singly branched case.[53]

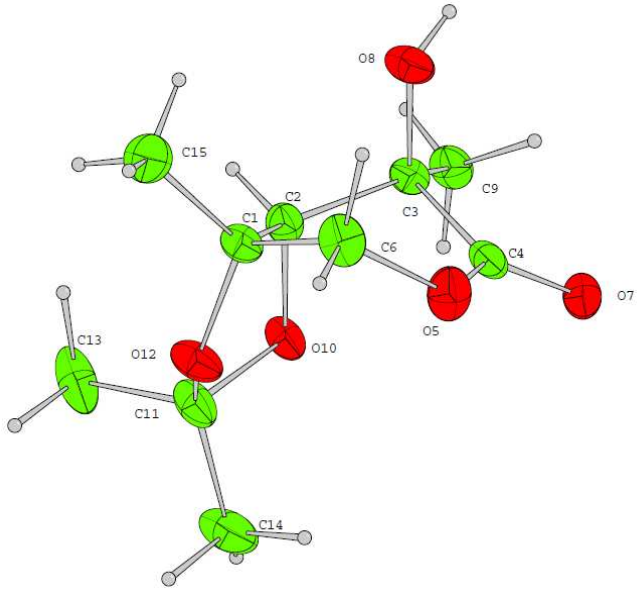
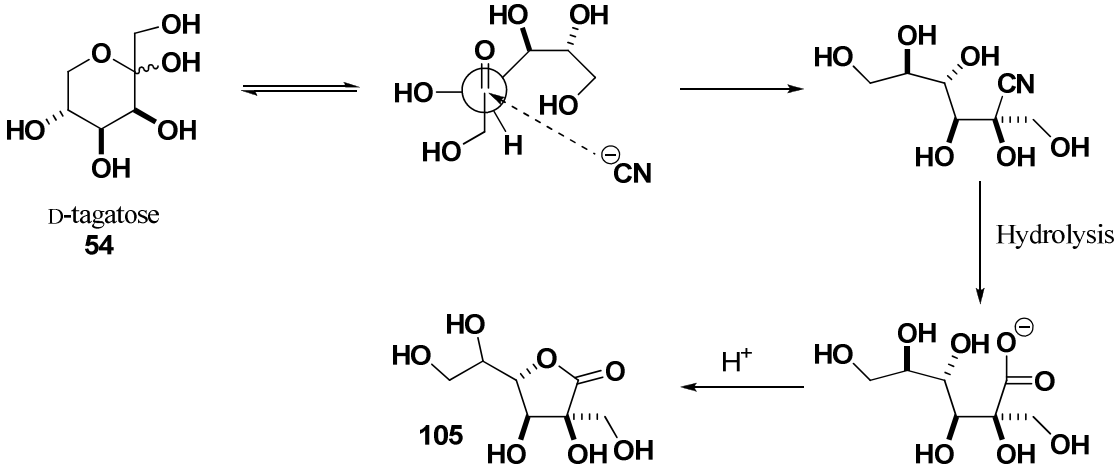


Figure 2.2: 2,4-Di-*C*-methyl-L-arabino-1,5-lactone **103** with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as spheres of arbitary radius. N. B. The labelling is in accordance with the published crystallographic labelling scheme not standard carbohydrate numbering systems.

This structure clearly shows the twist boat configuration, with hydroxyl at C2 (C3 in the crystallographic labelling scheme) adopting the flagpole position, rather than the methyl group, C9. This twisted shape, coupled with the lack of protons around the ring provides unreliable nOe enhancements, so these alone could not be used to determine the stereochemistry at C2. Indeed, the nOe data was initially suggestive of a *ribo* chair. The minor product had the *ribo* stereochemistry and from the nOe enhancements obtained, it is also likely to adopt a twist-boat shape.

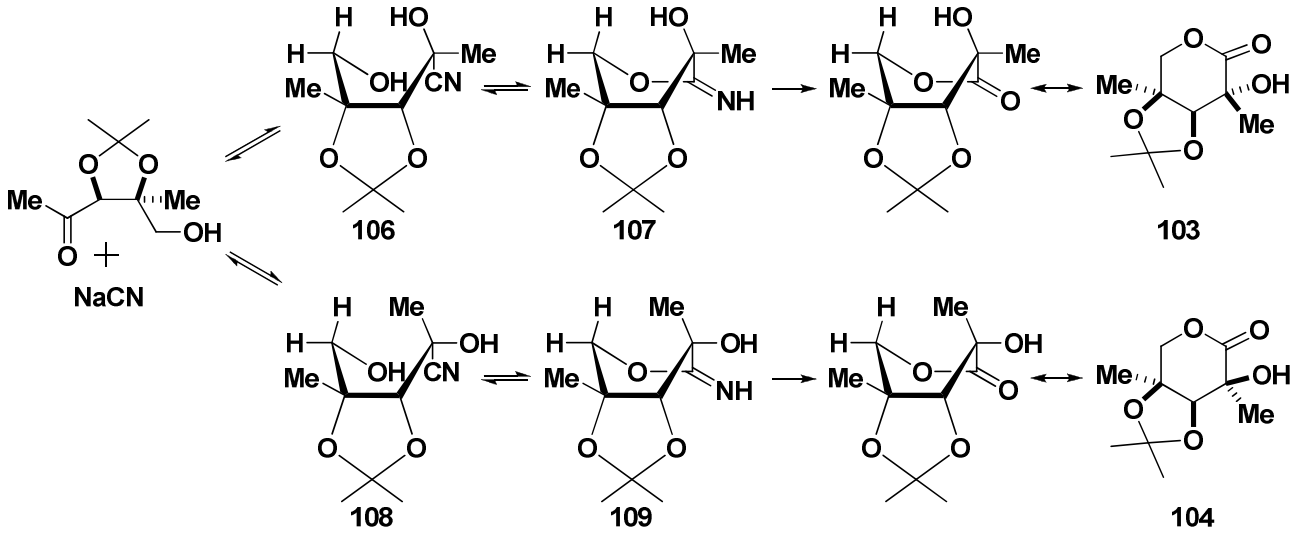
The dominance of the *arabino* stereochemistry is extremely fortunate, as treatment of arabinose with sodium cyanide gives *gluco* and *manno* sterochemistries; Kiliani extension of the *ribo* compound would afford *altro* and *allo* lactones. Indeed, the Felkin-Ahn model of nucleophilic

susbstitution predicts the *ribo* stereochemistry to be dominant, and this model is usually followed in Kiliani reactions in substrates with *cis* vicinal hydroxyl groups at C2 and C3.[54] For example, the analogous reaction on D-tagatose **54**,[55] gives exclusively the corresponding branched talose derivative **105** (**Scheme 2.17**), following attack by the nucleophile on the lowest energy conformation of the open chain aldehyde.



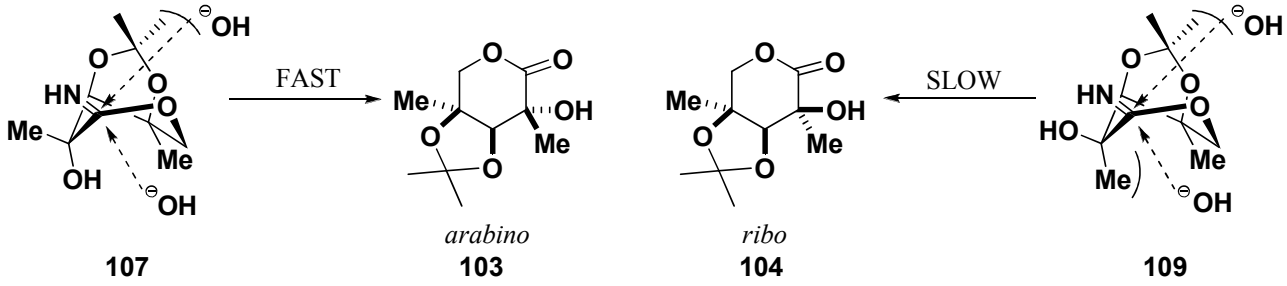
Scheme 2.17: Selectivity of the Kiliani reaction on D-tagatose

There is no one clear explanation as to why the Felkin-Ahn predicted compound is disfavoured in this case. One possibility is that the formation of the imidate intermediate **107**/**109** is rate limiting (**Scheme 2.18**).



Scheme 2.18: Formation of the imidate as the rate determining step.

The unfavourable steric clash in the *ribo* configuaration **109**, between flagpole methyl and hydrogen groups may mean the imidate is slower to form, than the less hindered *arabino* intermediate **107**. As all steps in imidate formation are reversible, this may push the equibilrium in favour of the *arabino* lactone. A second possible explaination has the hydrolysis as the rate limiting step (**Scheme 2.19**). Approach of hydroxide is blocked on the lower face by the C2 methyl group and on the upper face by the isopropylidene group methyls in the *ribo* imidate. In the *arabino* case, the hydroxide anion can attack the imidate by passing the smaller hydroxyl group, and hydrolysis is therefore faster in this configuration. In both these possible scenarios, the location of the hydroxyl group in the flagpole position seems key.



Scheme 2.19: Hydrolysis of the imidate as the rate determining step.

It is also possible that electrostatic interactions may play a role here too. By looking at intermediate **108** (**Scheme 2.18**), which leads ultimately to the *ribo* product, the unprotected OH groups both have their dipoles aligned towards the right of the page, as drawn. In the *arabino* case **106**, these two point in almost opposing directions, reducing the net dipole moment, rather than increasing it, as in the *ribo* case **108**. Without detailed mechanistic studies, it is difficult to know which, if any, of these arguements exerts the greater influence.

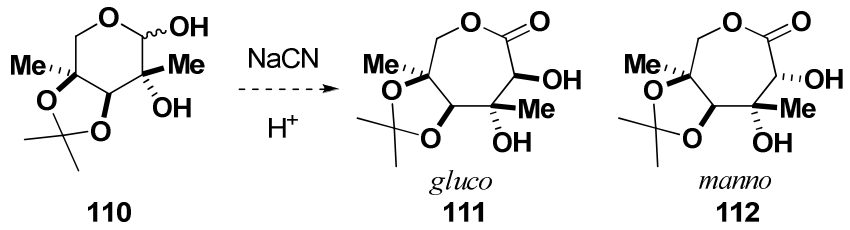
2.2.3 Towards 3,5-di-C-methyl-L-glucose

With easy access to the 5 carbon di-*C*-methyl branched sugar, it was now necessary to extend the carbon chain by one unit. It was envisaged that treatment of the aldehyde corresponding to lactone **103** with sodium cyanide as before should give, after hydrolysis, 3,5-di-*C*-methyl-L-glucono and

mannono lactones. Again, prior to carrying out the reactions, it would not be possible to tell which stereochemistry would dominate.

Synthesis of *gluco* and *mannono* lactones from arabinose *via* cyanide chain elongation chemistry has been subject to much investigation in the past on unbranched systems. Treatment of arabinose with HCN under anhydrous conditions gave almost exclusively the *manno* product.[56] However, using sodium cyanide and a selection of metal salts, Isbell attained a 73:27 ratio of *gluco* to *manno* stereochemistry in this reaction.[57]

Unlike in the previous case, no singly branched precedent exists from which to draw any assumptions. Hoping to keep protecting group manipulations to a minimum, and given the success of the Kiliani reaction on an isopropylidene protected sugar in the previous step, it was anticipated that repetition of the conditions on aldehyde **110** would provide the protected lactones, **111** and **112** (**Scheme 2.20**).



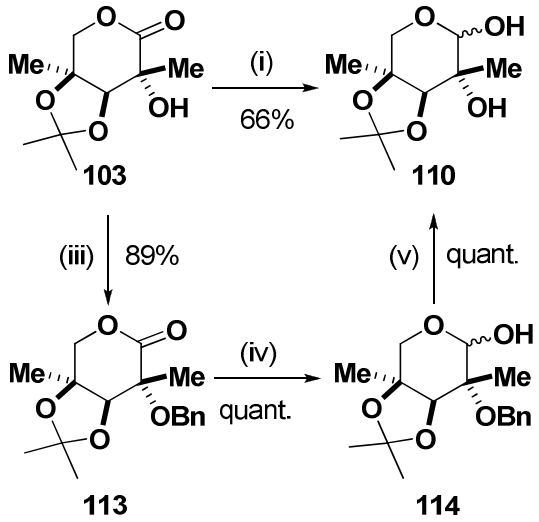
110 **111** **112**

Scheme 2.20: Proposed synthesis of protected 3,5-di-*C*-methyl sugars.

Synthesis of aldehyde **110** by reduction of **103** (**Scheme 2.21**) was attempted with di*iso*butylaluminium hydride solution (DIBAL) at -78°C. The highest yield of lactol **110** isolated in this way was 66%, with 20% of starting material recovered. Attempts to drive the reaction to completion by either warming the reaction mixture and/or adding more reagents led to a reduced yield and considerable decomposition.

It was hoped that by protection of the free hydroxyl, less reducing agent would be needed, and the reaction would be more efficient. Since the ideal protecting group is one that can be both put on

easily and be orthogonal to other protecting groups, a *tert*butyldimethylsilyl group was proposed. The protection was attempted with TBDMS chloride, *N*,*N*-dimethylformamide and pyridine at room temperature to no effect. Upon prolonged heating, only decomposition products were observed. The more reactive reagent, TBDMS trifluoromethanesulphonate was also employed, but that too failed, presumably due to the severe steric hindrance imposed by the methyl group at the same position. An alternative protecting group, the benzyl group, was investigated using benzyl bromide and sodium hydride as base. The use of strong base with sugar lactones is generally avoided due to the likelihood of epimerisation caused by deprotonation α to the carbonyl group. In this case however, the presence of a methyl group at the 2-position prohibits epimerisation, and the benzylated lactone **113** could be obtained quickly and in high yield (S**cheme 2.21**).

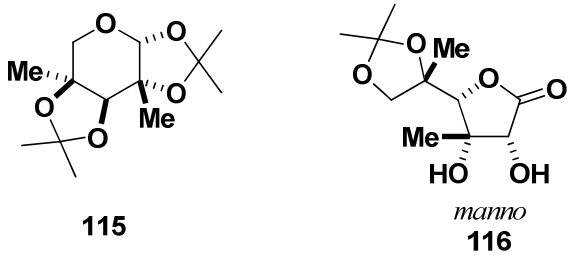


Reagents and Conditions: (i) DIBAL, DCM, -78°C, 100 min; (ii) NaCN, H_2O, RT 24 h then 90°C 48 h; (iii) BnBr, NaH, 4Å molecular sieves, DMF, RT, 2 h; (iv) DIBAL, DCM, -78°C, 1.5 h; (v) $Pd(OH)_2$, EtOH, H_2, RT, 2 h.

Scheme 2.21: Two routes to lactol **110**, and the Kiliani reaction.

Reduction of lactone **113** to lactol **114** could now be achieved quickly in a spot-to-spot reaction; subsequent removal of the benzyl group to give desired lactol **110** was achieved in quantitative yield. With a synthesis of isopropylidene protected lactol **110** in place, the Kiliani reaction was attempted (**Scheme 2.20**). After treatment with sodium cyanide at room temperature for 24 hours and heating to 90°C for 48 hours, the dark brown crude was acidified using Amberlite IR-120 acidic resin. No products could be identified from the resulting oil, and so it was dissolved in a mixture of

1,4-dioxane and water and stirred with Dowex® (H^+) resin to fully deprotect the products, as it was thought that the presence of the isopropylidene group was preventing ring closure. Once the reaction was judged to be complete, the resin was removed by filtration and the crude re-protected using acetone and copper(II) sulfate. Several fractions were obtained following column chromatography, the major one of which was unreacted starting material in which an extra isopropylidene group had been added to the molecule in the 1,2 position **115** (**Fig. 2.3**).[58] The small amount of the anticipated 3,5-di-*C*-methyl mannono lactone **116** with a 5,6-*O*-isopropylidene was obtained, but accounted for only 5% of the products. Extensive decomposition was observed, demonstrated by the very dark colour of the crude material before purification.



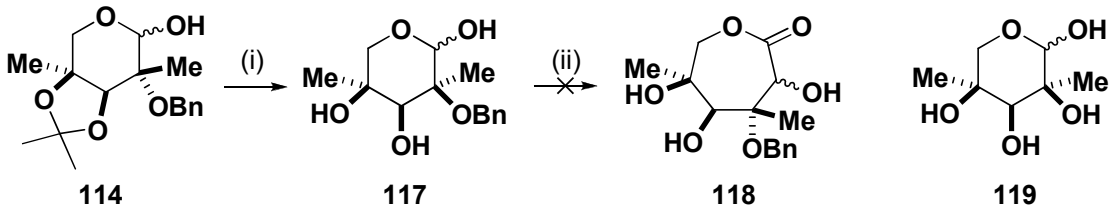
115 *manno* **116**

Figure 2.3: The only products isolated form the Kiliani reaction on protected lactol **110**.

Cyanide extension of benzyl protected lactol **114** was next attempted. Removal of the isopropylidene group from **113** would then allow the synthesis of lactones **117** and **118**, with benzyl functionality at the 3 position (**Scheme 2.22**). Deprotection of **114** was achieved using acidic resin at high temperature. Upon cooling the reaction mixture to room temperature, a white film formed on top of the reaction mixture, which was shown to be the product. Its insolubility in water meant that when treated with sodium cyanide, a water/dioxane solvent mixture was required, alongside gentle heating to 50°C to be fully solubilised.

After 24 hours at 50°C, the mixture was heated to reflux for 48 hours. The crude was passed down an acidic resin column and, after concentration of solvents, partitioned between water and dichloromethane. The phases were separated, and in the aqueous portion some completely debenzylated lactol **119** was seen alongside a small portion of an unidentified lactone, shown by ^{13}C

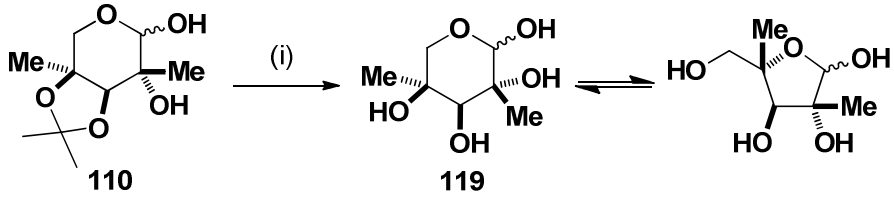
NMR and IR spectroscopy, but the mass was too low to proceed with. The organic portion contained no useful products.



Reagents and Conditions: (i) Dowex® (50W X8 H^+ form), H_2O, 70°C, 2h; (ii) NaCN, 1,4-dioxane, H_2O, 50°C 24h then 90°C 48 h.

Scheme 2.22: Proposed synthesis of protected 3,5-di-*C*-methyl glucono and mannono lactones **118**.

It was becoming clear that the Kiliani reaction on a protected substrate is a complex reaction. With only limited success thus far, it was decided to proceed with fully deprotected arabinose **119**, and follow a Kiliani/acetonation protocol, which had been adopted by Soengas *et al*.[59] Deprotected arabinose **119** could be produced in large quantitites by taking lactol **110** with acidic resin in water at high temperature (**Scheme 2.23**).

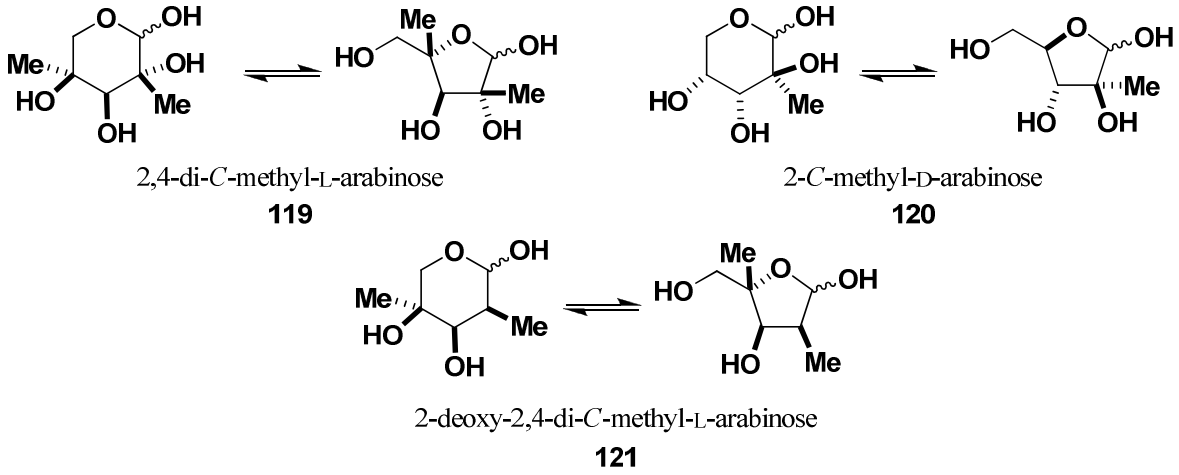


Reagents and Conditions: Dowex® (50W X8 H^+ form), H_2O, 90°C, 90 min.

Scheme 2.23: Synthesis of 2,4-di-*C*-methyl-L-arabinose, **119**.

2.2.4 NMR Analysis of C*-Branched Arabinoses*

Detailed NMR analysis of 2,4-di-*C*-methyl-L-arabinose **119** was undertaken by Dr M. Wormald, and this novel di-*C*-methyl branched sugar compared with the data obtained from 2-*C*-methyl-D-arabinose **120** and 2,4-di-*C*-methyl-2-deoxy-L-arabinose **121** (**Scheme 2.24**). A co-worker prepared 2-*C*-methyl-D-arabinose **120** whilst working on the synthesis of branched nucleosides;[60] the synthesis of 2,4-di-*C*-methyl-2-deoxy-L-arabinose **121** will be discussed in **Chapter 3**.



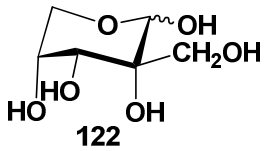
Scheme 2.24: Three branched arabinoses for NMR analysis.

Each sugar could exist in any combination of five possible systems; as the open chain aldehyde, the α and β pyranose and the α and β furanose forms. In aqueous systems, most sugars show a preference for one or two of these forms, for example it is well known that glucose exists almost exclusively in a pyranose form, and that the β-pyranose ring accounts for approximately 64% of the material, with the α-pyranose accounting for the remaining material. Only traces of the other forms are seen.[61,62,63] Arabinose preferentially adopts the pyranose form in aqueous solution, accounting for 95% of the material.[64] One proposed explanation of the predilection for the pyranose form is that water preferentially stabilises the pyranoses through hydrogen bonding. This is based on evidence that the structure of the water itself suffers little disruption when solvating a pyranose chair form and intermolecular association can easily take place.[65] Mackie and Perlin[66] tested this hypothesis by showing that when a less ordered solvent was used to solvate the sugars, dimethylsulfoxide, the furanose form was seen in greater proportions; 33% versus only 3% in water.

The discovery of methyl branched sugars in antibiotics[67] has resulted in the isolation of a number of them but given the difficulties in extraction and purification, and the additional complications that arise from multiple signals in NMR spectra of free sugars, most NMR spectra belong to their derivatives, such as glycosides and acetates.[68] As such, few free sugars have been subjected to detailed analysis.

From the few studies that have been performed however, it is generally true that in deprotected branched monosaccharides the acyclic form is present in far lesser concentration than in the parent monosaccharide.[69] This is largely due to the Thorpe-Ingold effect, whereby the addition of susbtituents reduces the entropy of the open chain form, so the entropy change is less negative upon ring closure.[70] This is demonstrated by 3-*C*-methyl erythrose, with 0.2% aldehyde, whilst unbranched erythrose contains 2% of the open chain form.[71]

Studies on hamamelose **122** (2-*C*-hydroxymethyl-D-ribose) show that the hydroxy methyl branch adopts the equatorial position, forcing two hydroxy groups into axial positions (**Figure 2.4**).



122

Figure 2.4: Preferred conformation of hamamelose in water.

This lessens the stability of the pyranose form, and the furanoses therefore preponderate. The ratio (at 23°C) found was as follows 12: 21: 38: 29 (α-pyranose: β-pyranose: α-furanose: β-furanose).[72] Ribose (at 30°C) was shown to exist in the following ratio: 21.5: 58.5: 6.5: 13.5;[73] clearly, the effect of introducing branches can be very significant. Detailed analysis on C2 or C4 methyl branched sugars has not, to the best of our knowledge, been carried out. It was therefore of interest to determine whether the methyl group added to the sugar backbone affected the pyranose/furanose equilibrium.

The results of these investigations are summarised by **Figure 2.5**. Further detail can be found in the appendix at the rear of this work.

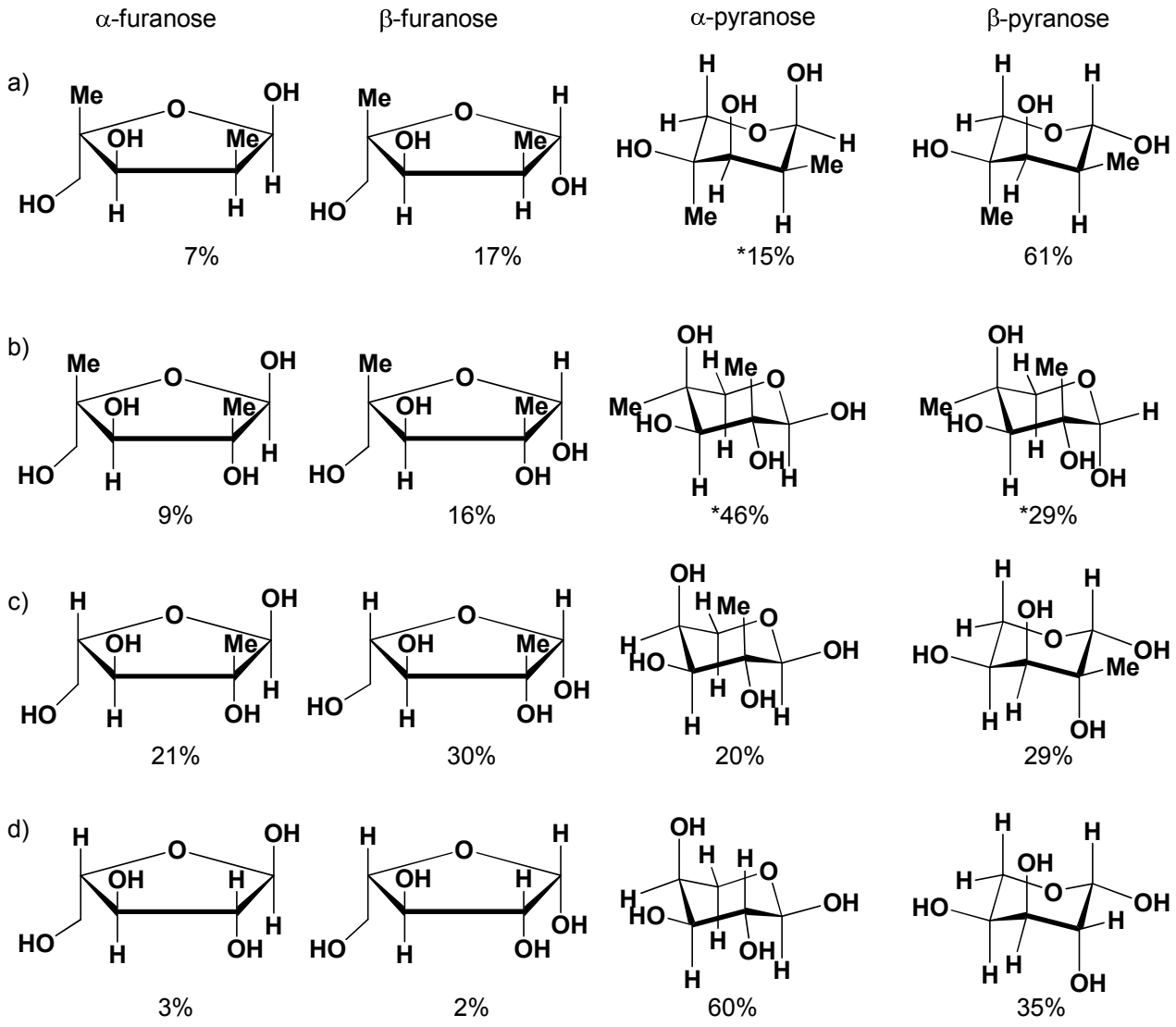


Figure 2.5: Ring sizes and relative populations for the α-furanose, β-furanose, α-pyranose and β-pyranose forms. (a) 2,4-di-*C*-methyl-2-deoxy arabinose **121**; (b) 2,4-di-*C*-methyl arabinose **119**; (c) 2-*C*-methyl arabinose **120**; (d) arabinose.[74] * - the ring size cannot be unambiguously determined by NMR.

The introduction of methyl branches to the carbohydrate scaffold of arabinose causes an increase in the preponderance of the furanose forms; this effect is more pronounced in the singly branched species **120**. Of the two possible furanose forms, the β-type is stabilised more than the α-, and 2,4-di-*C*-methylation stabilises the β-anomer still further as seen for **121** and **119**. This is as expected as both methyl groups are on the same side of the ring as the C1OH in the α-anomer. For the pyranose forms, *C*-methylation causes more complex changes in ring conformation and stability. C2-Methylation does not alter the stable ring conformations of the α- and β- pyranose anomers relative

to arabinose, but makes the β- pyranose anomer more stable than the α-anomer. 2,4-Di-*C*-methylation alters the ring conformation of the β-anomer relative to arabinose and again increases its stability relative to the α-anomer, although the latter is still more stable. 2,4-Di-*C*-methylation combined with loss of the C2-hydroxyl changes the ring conformation of the α-anomer and makes the β-anomer far more stable.

The solid state conformation of 2,4-di-*C*-methyl-L-arabinose **119** was also determined. X-Ray crystallographic studies show it exists solely as the α-pyranose monhydrate (**Figure 2.6**). As the deprotected sugar, a chair shape is adopted unlike the twist boat seen in the protected lactone. Regretably, recrystallisation of the 2-*C*-methyl-D-arabinose **120** gave poor quality crystals, and the deoxy sugar **121** exists as an oil, so their solid state conformations could not be compared.

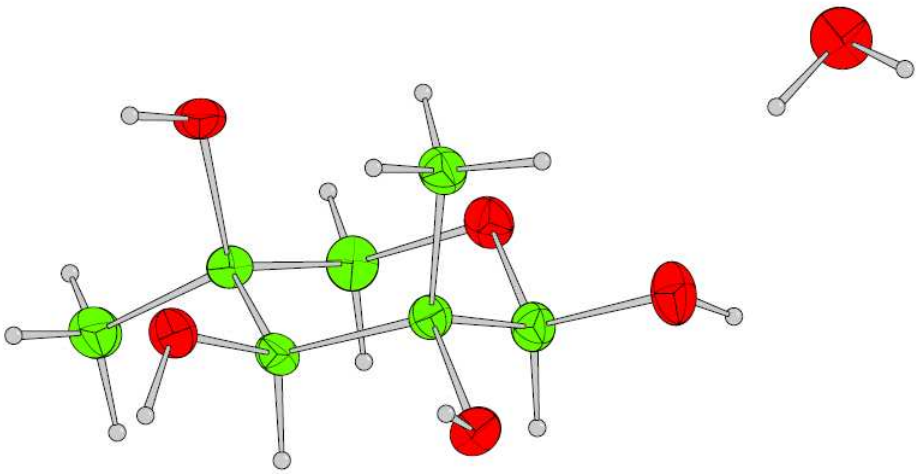


Fig 2.6: 2,4-Di-*C*-methyl-α-L-*arabino* pyranose with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

*2.2.5 To 3,5-Di-*C*-Methyl Glucose*

2,4-Di-*C*-methyl-L-arabinose **119** was treated with aqueous sodium cyanide at room temperature for 24 hours and then heated to 90°C for 24 hours. The reaction mixture was acidified by elution through acidic resin (Amberlite IR 120) with water, and the solvents concentrated to give a crude brown oil. This was shown to contain a mixture of 4 compounds by NMR, assumed to the *gluco* and *manno* sugars as both 5 and 6 ring lactones (**Figure 2.7**).

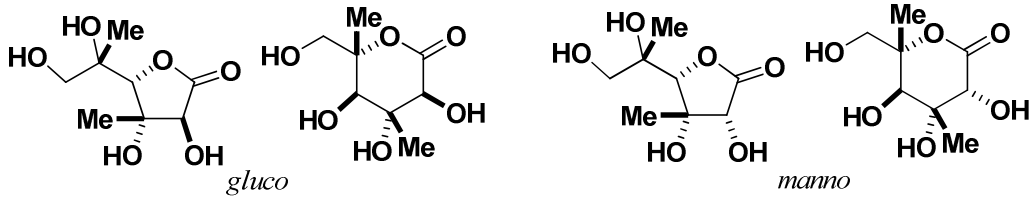


Figure 2.7: The four possible products from the reaction of 2,4-di-*C*-methyl-L-arabinose **119** with aqueous cyanide.

As anticipated, separation of these 4 lactones was not possible, so the crude was treated directly with acetone, copper(II) sulfate and a catalytic quantity of sulfuric acid to isopropylidene protect the diols. The three products isolated from the reaction are the mono isopropylidene protected lactones **123** and **124** and the di*iso*propylidene protected lactone **125** (**Figure 2.8**). The stereochemistry at C2 of these sugars was elucidated by X-ray crystallography (**Figure 2.9**).[75,76,77]

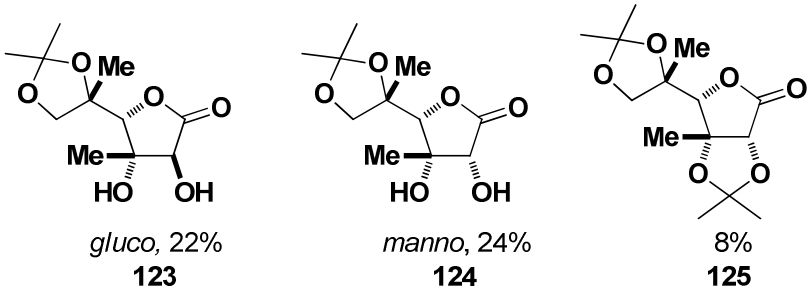
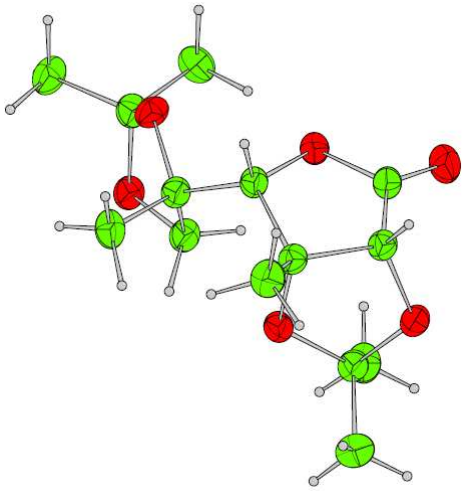
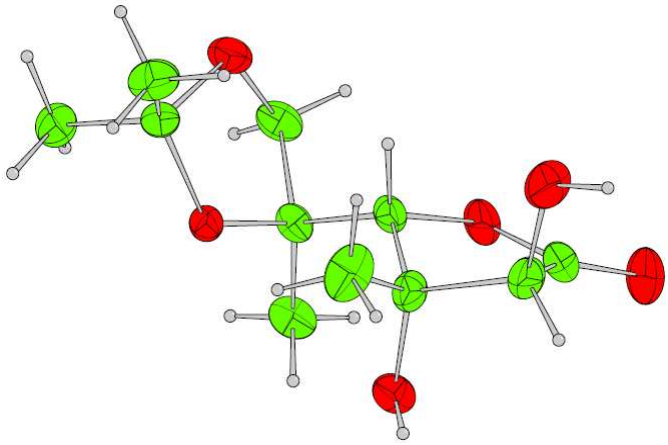


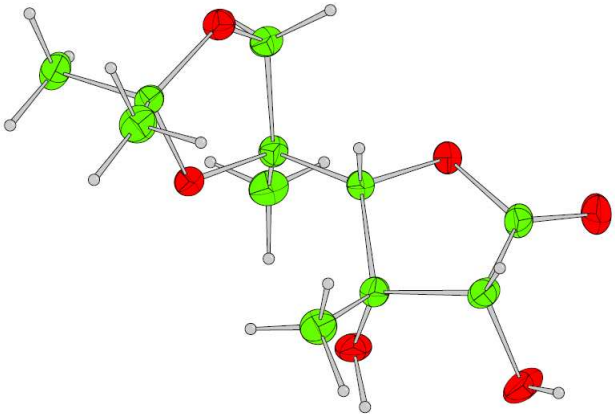
Figure 2.8: Protected 3,5-di-*C*-methyl glucono and mannono lactones.



2,3:5,6-Di-*O*-isopropylidene-3,5-di-*C*-methyl-L-mannono lactone **125**



3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-glucono lactone **123**

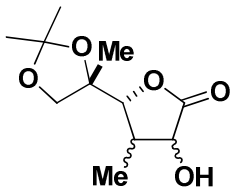


3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-mannono lactone **124**

Figure 2.9: Displacement ellipsoids drawn at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

Repetition of the above sequence, using sodium cyanide followed by isopropylidene protection of the crude to generate more of the di-*C*-methyl branched lactones proved to be problematic. Regrettably, most changes in reaction conditions lead to little or no useful material being generated, as it proved itself to be a temperamental reaction, sensitive to both time and temperature. Increasing the length of time the reaction mixture was heated for yielded none of the desired lactones **123** or **124**. The longer the reaction mixture was heated for, the darker in colour it became, suggesting

more decomposition had occured. Increasing the temperature and refluxing the cyanohydrin, as is usual practice with these reactions,[78,79] yielded none of the lactones. Instead, a large number of decomposition products were seen. IR and HMBC analyses were strongly suggestive that one of these products was a 5 ring lactone and the presence of peaks in the 2.5 ppm region of the ^{1}H NMR spectrum is indicative of a deoxy centre. Thus the structure **126** was proposed, with the support of LRMS data (**Figure 2.10**). These sugars exist as an inseparable mixture and as such further structural confirmation was not possible.



126

Figure 2.10: Evidence that the cyanide addition step was occurring, but the product was becoming deoxygenated and decomposing during the work up/ purification process.

This result, although not the sugar that was anticipated, was nevertheless encouraging, as it must have been formed through the loss of hydroxide from either the *gluco* or *manno* lactone. It was therefore proposed that the problems were not with the initial Kiliani reaction, but with either the work up of the reaction or the subsequent isopropylidene protection conditions. Alternative protection conditions were attempted, using 2,2-dimethoxy propane and *p*TSA, to allow more control over the quantity of acid added to the protection step, as it was thought that lack of precision with the quantity of sulfuric acid that was added might have been catalysing the decomposition of the Kiliani products. However, no improvement was seen by adopting more tightly controlled conditions.

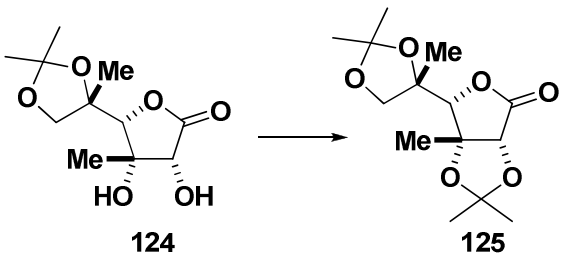
Alternative protecting groups were tried for the protection of the crude mixture, including silyl and actate groups. Peracetylation was attempted with acetic anhydride and pyridine, however t.l.c. analysis of the reaction mixture revealed a very large number of products, assumed to be the mono, di, tri and tetra acetylated forms of both the *manno* and *gluco* configurations. Forcing conditions

were used to give only the tetra acetylated products, but unfortunaltely once this was accomplished it was clear from t.l.c analysis that no separation of products would be readily achievable. Triethylsilyl chloride was also attempted, but, whilst some separation of lactones was seen by t.l.c. analysis, in practice, little separation was achieved after column chromatography. Performing column chromatography at a slower elution rate, whilst giving better separation, resulted in removal of the protecting groups on silica, compromising the yield. To circumvent this problem, a larger protecting group, *tert*butyldimethylsilyl was proposed, but it was found that its increased bulk hindered the protection reaction, resulting in very little of the desired material being recovered. Similarly, mono protection on the primary hydroxyl with *tert*butyldiphenylsilyl chloride was also unsuccessful.

Turning to the work up procedure, it was discovered that by replacing the Amberlite IR-120 (acidic resin) column at the end of the Kiliani reaction with a more controlled amount of acid a much cleaner crude could be obtained. Thus a revised procedure was proposed; fully deprotected arabinose **119** was dissolved in water and stirred with sodium cyanide at room temperature for 24 hours. After this time, it was heated to 90°C for a further 24 hours, and then the reaction mixture was concentrated under reduced pressure to an off-white residue. Once completely dry, this was dissolved in anhydrous methanol, and treated with acetyl chloride in the presence of 4Å molecular sieves. Use of measured, anhydrous acid resulted in a clean reaction and purification of the crude by column chromatography afforded mixed clean lactones; these conditions were repeated several times giving consistent results. With the lactones in hand, isopropylidene protection could be carried out using copper(II) sulfate and acetone in catalytic sulfuric acid, giving protected lactones **123** and **124**, which were readily separable. Interestingly, although this sequence was carried out many times, none of the di*iso*propylidene protected *manno* species **125** was seen (**Figure 2.8**).

2.2.6 From Lactones to Free Sugars

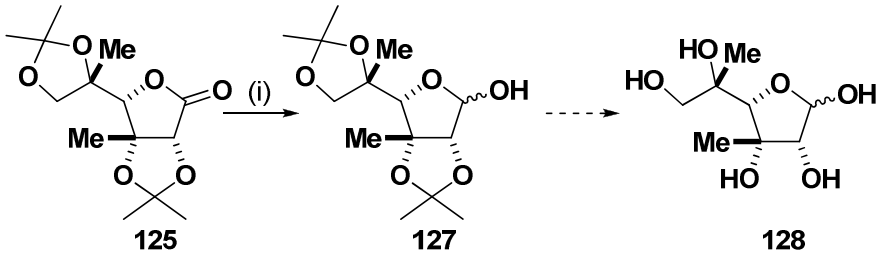
It was now necessary to convert the protected lactones into their free sugars *via* reduction and deprotection. As seen in the reduction of 3,4-*O*-isopropylidene-2,4-di-*C*-methyl-arabinono-1,5-lactone **103**, the direct reduction of a sugar lactone possessing a free hydroxyl group can be poorly yielding and requires a large number of equivalents of reagent (see **Scheme 2.21**, page 61). In the arabinonolactone case, the absence of an acidic proton α to the carbonyl group allowed for the use of a strong base (sodium hydride) and benzyl bromide to protect OH-2. Unfortunately, this could not be repeated here, due to the presence of a proton α to the carbonyl group, as treatment with base was likely to deprotonate and thereby epimerise this centre. It was therefore decided to take fully protected lactone **125** with di*iso*butylaluminium hydride. Unfortunately, after characterisation and crystallographic analysis of lactone **125** there was only a very small quantity of material with which to test the reduction to the lactol, and as previously mentioned, repetition of the Kiliani sequence had failed to generate any more of this material. Protection of the mono isopropylidene protected *mannono* lactone was thus attempted to generate more of this di*iso*propylidene protected sugar (**Scheme 2.25**).



Scheme 2.25: Anticipated straightforward isopropylidene protection of **124**.

Simple repetition of the conditions used previously, using acetone, copper(II) sulfate and catalytic sulfuric acid for 48 hours at room temperature were unsuccessful, yielding only unreacted starting material. Heating this reaction lead to extensive decomposition and no useful products could be obtained. Alternative conditions, using 2,2-dimethoxy propane and catalytic *para*-toluenesulfonic acid in anhydrous DMF were also attempted, but none of the desired product was observed. The most probable reason for this is that the addition of a second isopropylidene is disfavoured by the

steric effect of possessing two methyl branches, and no further conditions were investigated. Protection with an excess of trimethylorthoformate and catalytic acid was attempted, but only unreacted staring material was recovered. The small quantity of **125** which was available was treated with DIBAL as before, to afford **127** in 99% yield (**Scheme 2.26**), but there was insufficient material to carry out the deprotection to the free sugar.

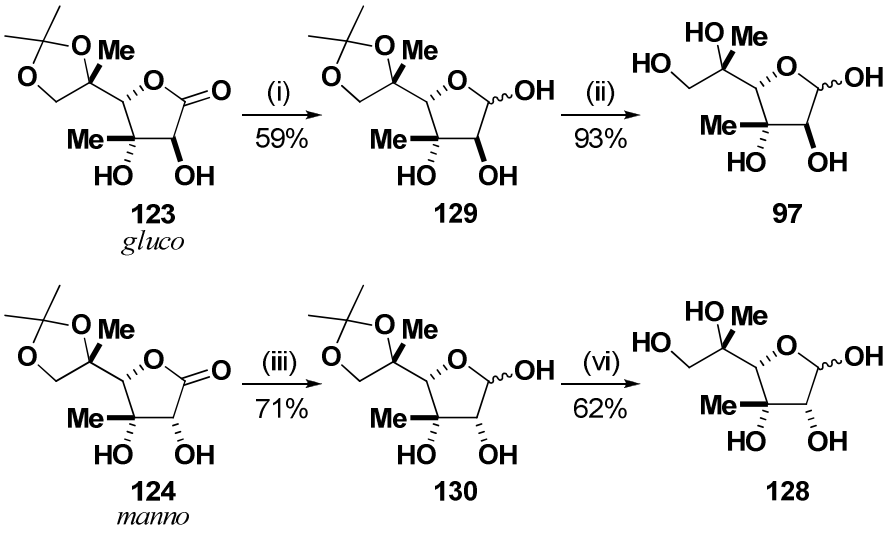


Reagents and Conditions: (i) DIBAL, DCM, -78°C, 1 h.

Scheme 2.26: Anticipated route towards 3,5-di-*C*-methyl-L-mannose.

It had already been demonstrated that protection of the *manno* and *gluco* lactones with silyl groups was difficult. For the larger silyl groups, protection could not be achieved in adequate yields, and the smaller silyl groups were found to be highly labile on silica gel, hindering purification. As, for reasons outlined above, protection strategies requiring strong base were inappropriate, protection in neutral or acidic conditions would be preferable. A brief investigation into protection with trichloroacetimidates, both benzyl and *para*methoxybenzyl[80] was performed, to no avail. Problems of this sort are common in carbohydrate syntheses, where the use of strong acids or bases is not compatible due to the possibility of dehydration and/or epimerisation. Recent work by Best *et al*[81] has shown a novel strategy for the protection of alcohol groups under neutral conditions, using diphenyldiazomethane. The $CHPh_2$ group ('OBzh') is particularly suitable for synthesis as it can be removed in acidic conditions, but is stable to base and mild hydrogenation. Whilst the literature reports consistently high yields on a range of hindered substrates, it was not possible to affect the same transformation in either the *gluco* or *manno* cases.

As finding a suitable protecting group was proving far from trivial, it was decided to proceed with the partially protected lactones **123** and **124**. The *gluco* lactone **123** was dissolved in tetrahydrofuran, cooled to -78°C and treated with di*iso*butylaluminium hydride solution, however the reaction was found to be sluggish and, despite the use of four equivalents of reagent and warming to -20°C, little of the desired product was found after work up. The work up procedure chosen was an aqueous wash using DCM and sodium potassium tartrate solution, and it is believed that the low yield reflected the poor solubility of **123** in an organic solvent. After changing the solvent to tetrahydrofuran and adopting an aqueous work up procedure, using potassium dihydrogen orthophosphate, it was found that the protected glucose **129** could be obtained in 59% yield, and the mannose **130** in 71% (**Scheme 2.27**).



Reagents and Conditions: (i) DIBAL, THF, -60°C → -50°C, 2 h; (ii) Dowex (50W X8) H^+ resin, H_2O, 2 h; (iii) DIBAL, THF, -78°C → -60°C, 2.5 h; (vi) Dowex (50W X8) H^+ resin, H_2O, 16 h.

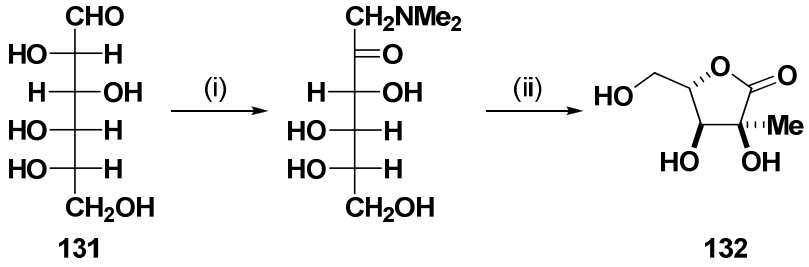
Scheme 2.27: From lactones to free sugars

The slightly lower yield of the *gluco* compound (59%) may be a reflection on the instability of the isopropylidene group on silica, as it was found that this acetonide was particularly acid sensitive, and full deprotection to free sugar **97** could be achieved in only two hours with acidic resin at room temperature. In contrast, acid catalysed deprotection of the *manno* compound **130** in similar conditions took 16 hours; the reduced yield (62%) of the mannose sugar **128** is most likely due to

the fact that it was necessary to purify the crude reaction mixture after deprotection by column chromatography, and therefore some material may have been lost in this process.

2.2.7 Synthesis of 3,5-di-C-methyl-D-glucose and mannose

In the synthesis of the L-series sugars, 2-*C*-methyl-D-ribono-1,4-lactone **88** was used as a convenient readily available starting material, generated on a multigram scale by treatment of D-glucose first with dimethylamine in ethanol and then with calcium oxide (see **Scheme 2.13**, page 53). As mentioned previously, the yields obtained for this process are satisfactory at best, but the synthetic utility of the product, combined with the low cost of D-glucose makes this a very valuable transformation. Whilst 5 Kg of D-glucose is currently available for $130, the L-enantiomer **131** is considerably more expensive, at $220 for 5 g.[82] Clearly, synthesis of the corresponding 2-*C*-methyl-L-ribono-1,4-lactone **132** in the same process carries unacceptable costs and was carried out on a small scale using the previous methodology as proof of concept only (**Scheme 2.28**).



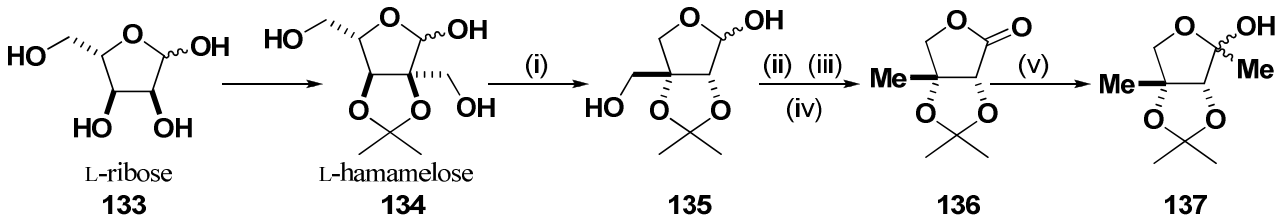
131 **132**

Reagents and Conditions: (i) Me_2NH, AcOH, EtOH; (ii) CaO, H_2O then H

Scheme 2.28: Synthesis of 2-*C*-methyl-L-ribono lactone **132** from L-glucose **131**.

To make the synthesis of 3,5-di-*C*-methyl-D-glucose **1** financially viable, an alternative strategy was needed. Two potential alternative routes were found, the first utilised L-ribose **133**, to which a hydroxyl methyl branch at C2 was added *via* an aldol reaction to generate L-hamamelose **134**.[83] Use of a borohydride reduction followed by treatment with sodium periodate as before generated the corresponding branched D-erythrose **135**. Oxidation of the lactone followed by a triflation/iodide displacement/hydrogenation sequence converted the hydroxyl methyl branch into a methyl branch to give common lactone **136**, to which methyl magnesium bromide was added to give protected 1-

deoxy-4-*C*-methyl-D-ribulofuranose **137** (**Scheme 2.29**). This work was carried out by D. Best, using hamamelose supplied by Dextra Laboratories, Reading.[84]

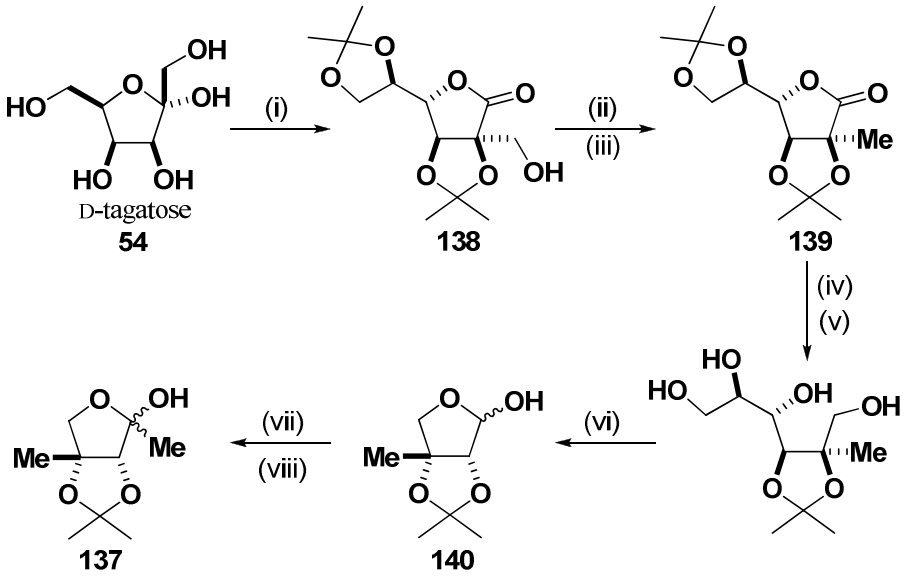


Reagents and Conditions: (i) $NaBH_4$, H_2O, RT, 80 min; then $NaIO_4$, AcOH, RT, 60 min, 85%; (ii) Br_2, $BaCO_3$, H_2O, 0°C to RT, 3 h, 82%; (iii) Tf_2O, Py., DCM, -30°C, 90 min; then TBAI, DMF, RT, 24 h, 85%; (iv) Pd/C (10%), H_2, TEA, RT, 3 h, 76%; (v) MeMgBr, THF, -78°C, 45 min, 95%.

Scheme 2.29: Route towards common intermediate, protected 1-deoxy-4-*C*-methyl-D-ribulo furanose.

Whilst this is a considerably cheaper altenative, L-ribose **133** is still relatively expensive at around $85 for 5 g.[85] The second alternative route proposed uses the once rare sugar, D-tagatose **54**. D-Tagatose is found in small amounts in certain cheeses;[86] its scarcity and the difficulty in extraction made it very expensive. Although the price of D-tagatose in 2007-2008 Aldrich catalogue is still high at £331.00 for 5 g,[87] it is available cheaply in large quantities (around £5 per kg) by either chemical[88] or biotechnological[89,90] procedures. It has since been developed as a low calorie sweetener,[91] and its potential as a chiral building block is beginning to be recognized.[92,93]

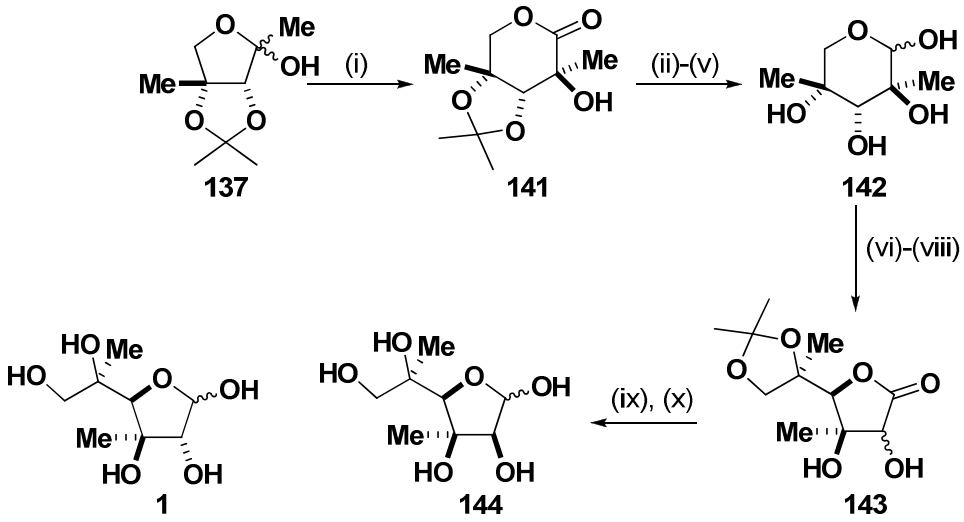
The Kiliani reaction of D-tagatose **54** followed by isopropylidene protection generates the 2-*C*-hydroxymethyl branched D-talono lactone **138**, in 51% yield.[94] The hydroxymethyl branch was then reduced to a methyl group and selective deprotection of the primary acetonide, followed by reduction of the lactol to the polyol and subsequent periodate cleavage of the terminal triol gave 3-*C*-methyl-D-erythrose **140**, in 79% yield over 5 steps. Bromine oxidation and methyl addition as before gave the desired 1-deoxy-4-*C*-methyl-D-ribulofuranose **137** (**Scheme 2.30**).



Reagents and Conditions: (i) NaCN, H_2O, RT, 24 h, then 100°C, 24 h; then acetone, $CuSO_4$, cat. H_2SO_4, RT, 18 h, 51%; (ii) I_2, PPh_3, Imidazole, toluene, 85°C, 2 h, 93%; (iii) Pd/C (10%), H_2, EtOH, TEA, RT, 48 h, 99%; (iv) $LiBH_4$, THF, 0°C to RT, 16 h, 99%; (v) AcOH, H_2O, RT, 16 h, 90%; (vi) $NaIO_4$, H_2O, RT, 4 h, 96%; (vii) Br_2, $BaCO_3$, H_2O, 0˚C to RT, 18 h 75%; (viii) MeMgBr, THF, -78°C, 45 min, 95%.

Scheme 2.30: Route towards common intermediate, protected 1-deoxy-4-*C*-methyl-D-ribulo furanose.

1-Deoxy-4-*C*-methyl-D-ribulofuranose **137** was dissolved in water and treated with sodium cyanide to give protected 2,4-di-*C*-methyl-D-arabinono-1,5-lactone **141** in high yield, 79% (**Scheme 2.31**). The converstion of D-tagatose **54** to 2,4-di-*C*-methyl-D-arabinono-1,5-lactone **141** was performed by F. Fernandez-Nieto, following much of the methodology laid down by myself during the synthesis of the enantiomer.[95] The free hydroxyl group was protected as a benzyl ether, before reduction of the lactone functionality with di*iso*butylaluminium hydride, and deprotection to afford the free arabinose sugar **142** in 77% over three steps. This was treated directly with sodium cyanide, to generate the 3,5-di-*C*-methyl-D-*glucono* and *mannono* lactones, which were isolated as the 5,6-*O*-isopropylidene protected lactones **143**. As previously, the lactones were readily separable at this stage and they were individually taken with di*iso*butylaluminium hydride, and deprotected to give the free sugars **1** and **144** (**Scheme 2.31**).



Reagents and Conditions: (i) NaCN, H_2O, RT, 24 h, then 100°C, 24 h, 79%; (ii) BnBr, NaH, DMF, 4Å sieves RT, 2 h, 90%; (iii) DIBAL, DCM, -78°C, 1.5 h, 86%; (vi) $Pd(OH)_2$, H_2, EtOH, RT, 2 h, quant.; (v) Dowex® (50W X8 H^+), H_2O, 90°C, 1.5 h, quant.; (vi) NaCN, H_2O, RT, 24 h, then 90°C, 24 h; (vii) MeOH, AcCl, 4Å sieves, RT, 24 h, 53% over two steps; (viii) $(CH_3)_2CO$, $CuSO_4$, cat. H_2SO_4, RT 16 h then 50°C 4 h, 32% *gluco*, 27% *manno*; (ix) DIBAL, DCM, -50°C, 64% *gluco*, 32% *manno*; (x) Dowex (50W X8 H^+ form), H_2O, 43% *gluco*, 72% *manno*.

Scheme 2.31: Synthesis of 3,5-di-*C*-methyl-D-glucose **1** and mannose **144**.

Interestingly, in the D- sequence most yields are as high, if not higher than in the analogous L-sequence, until the final two steps, the DIBAL reduction and isopropylidene deprotection (**Table 2.1**). As previously mentioned, lower yields in the deprotection step may be due to problems freeing all the compound from silica gel following column chromatography. The reduced yield for the D-*manno* DIBAL reduction may be related to difficulties encountered during the work up procedure, resulting in the compond being in contact with silica for an unusually long period of time, possibly removing the isopropylidene group and accounting for the low yield.

Substrate	***DIBAL reduction***	***Deprotection***
L-*gluco*	59%	93%
D-*gluco*	64%	62%*
L-*manno*	71%	62%*
D-*manno*	32%	72%*

Table 2.1: D- *vs* L- yields.

* Purification by column chromatography was necessary

2.2.8 NMR Analysis of 3,5-Di-C-Methyl Glucose 1 and Mannose 144

NMR studies on 3,5-di-*C*-methyl glucose **1** and mannose **144** were undertaken by Dr M. Wormald to establish the relative amounts of the pyranose and furanose forms in aqueous solution. In order to act as a competitive glycogen phosphorylase inhibitor, the modified glucose molecule must adopt a predominantly pyranose shape, as glucose itself does, so it will sit in the active site.

Unlike the C2 and C4 case, limited data exists in the literature on the NMR analyses of C3 and C5 methyl branched sugars. 5-*C*-Methyl-D-glucose **145** was synthesised by Driver and Stevens, who undertook an investigation of the solution phase conformation.[96] They found that the α-pyranose conformation was disfavoured compared with unbranched glucose (**Figure 2.11**). This is unsurprising, given that the trans diaxial strain is greatly increased.

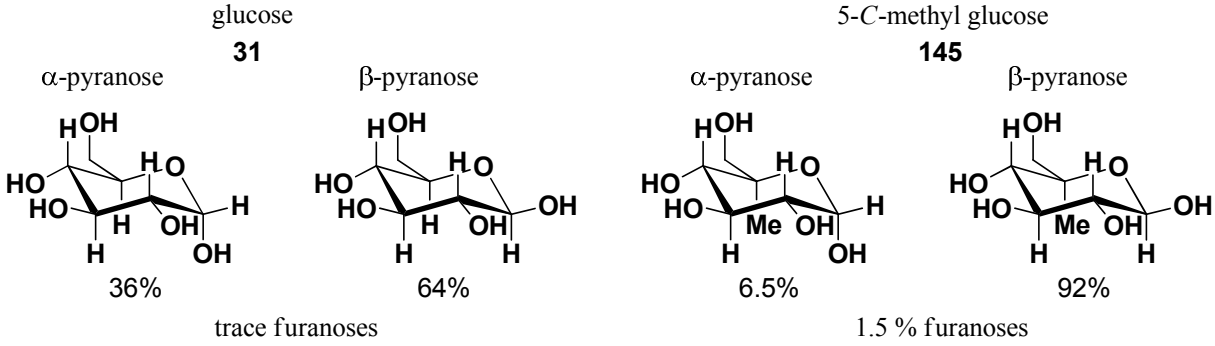


Figure 2.11: Relative pyranose/furanose forms of 5-*C*-methyl glucose **145** compared with glucose **31**.

Also, whilst in the case of the branched arabinoses, very large increases in the amount of furanose forms were observed, here, the increase observed was slight. Studies on the 3-*C*-methyl branched sugar evermicose (2,6-dideoxy-3-*C*-methyl-D-*arabino* hexose) **146** have indicated that the methyl on the 3-position also disfavours the α-pyranose conformation for the same reasons of unfavourable *syn*-axial interactions (**Figure 2.12**).[97] Again, no marked increase in the furanoses was observed.

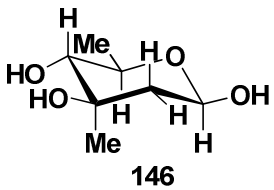


Figure 2.12: 3-*C*-Methyl branched sugar, evermicose **146**.

The presence of two methyl groups however may disfavour the pyranose form, as was the case with 2,4-di-*C*-methyl arabinoses (see **Figure 2.5**, page 66). In order to most accurately predict the *in vivo* structure, NMR analyses were carried out at physiological pH, with the *gluco* sample at pH 7.5, and the *manno* at 7.3. NMR spectra are shown by **Figure 2.13**; the results of these investigations are summarised in **Figure 2.14**.

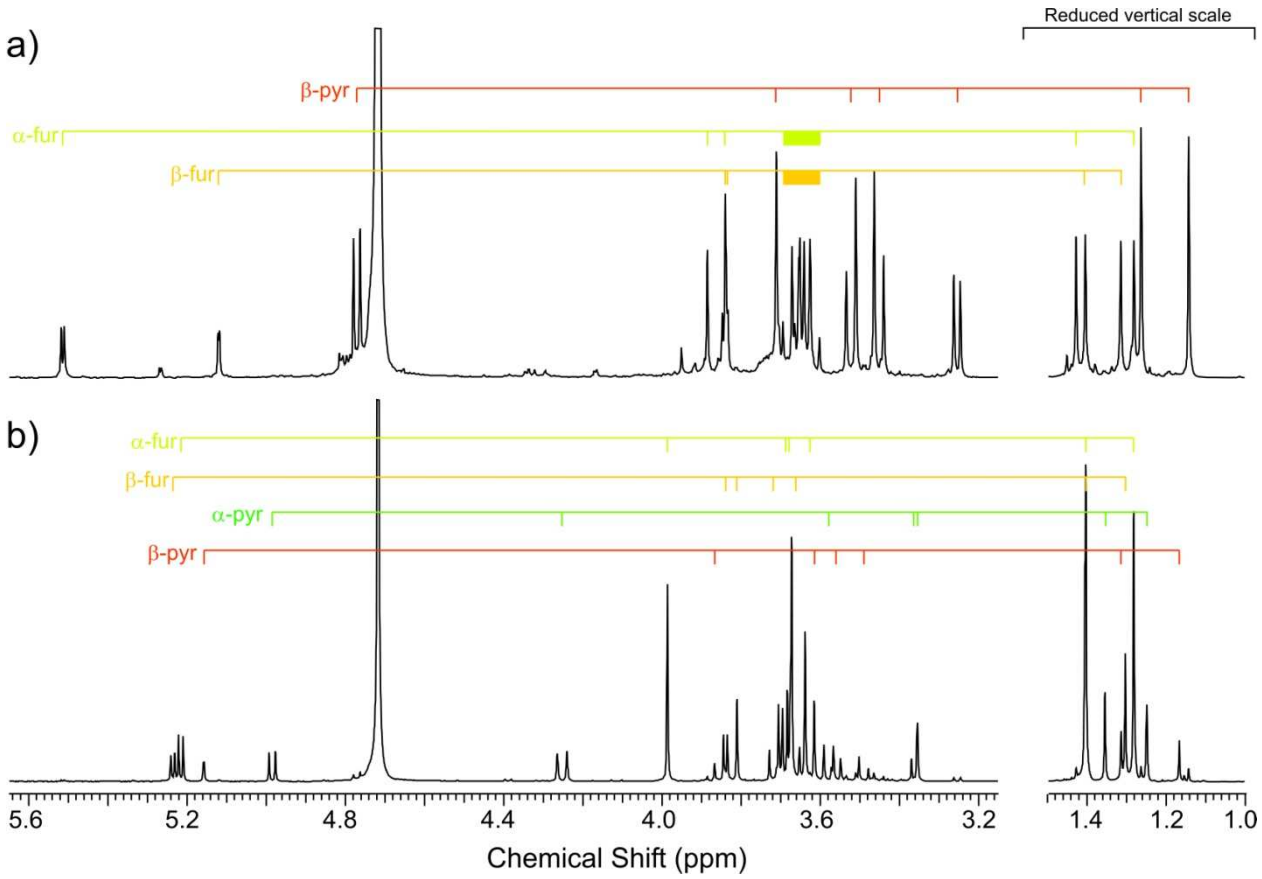


Figure 2.13: 1D ^{1}H NMR spectra (a) 3,5-di-*C*-methyl-glucose **1**; (b) 3,5-di-*C*-methyl-mannose **144**. The vertical scales of the methyl regions have been reduced for clarity.

The two methyl groups had a very marked effect on ring conformation. In the di-*C*-methyl glucose sample, the amount of the β-furanose form increased 250 fold from unbranched glucose, whilst the previously absent α-furanose form now accounts for 25% of the material. The α-pyranose structure is completely disfavoured, presumably due to the increased strain incurred by positioning both methyl and the anomeric hydroxyl groups in the axial position if the more common $^{4}C_{1}$ form was to be adopted. In contrast, the β-pyranose configuration escapes relatively unchanged, and still

manages to account for 50% of the material, down only slightly from 64% in straight chain glucose. This result is extremely positive, as it suggests that *in vivo*, half the material will exist in the pyranose configuration, and therefore be able to sit within the active site of glycogen phosphorylase.

In stark contrast, branching radically effected the prevelance of the pyranose conformation of the *manno* sugar; it dropped from accounting for 99% of the material in unbranched mannose, to just 25% in 3,5-di-*C*-methyl mannose. Both the α- and β-pyranose forms fared equally badly, reduced to just 25% of their original value, whilst both furanoses increased by roughly the same amount. The position of the anomeric hydroxyl in the α- position is favourable for both the pyranose and furanose forms, and in each case consists of roughly twice as much material; this may be attributable to the anomeric effect.[98]

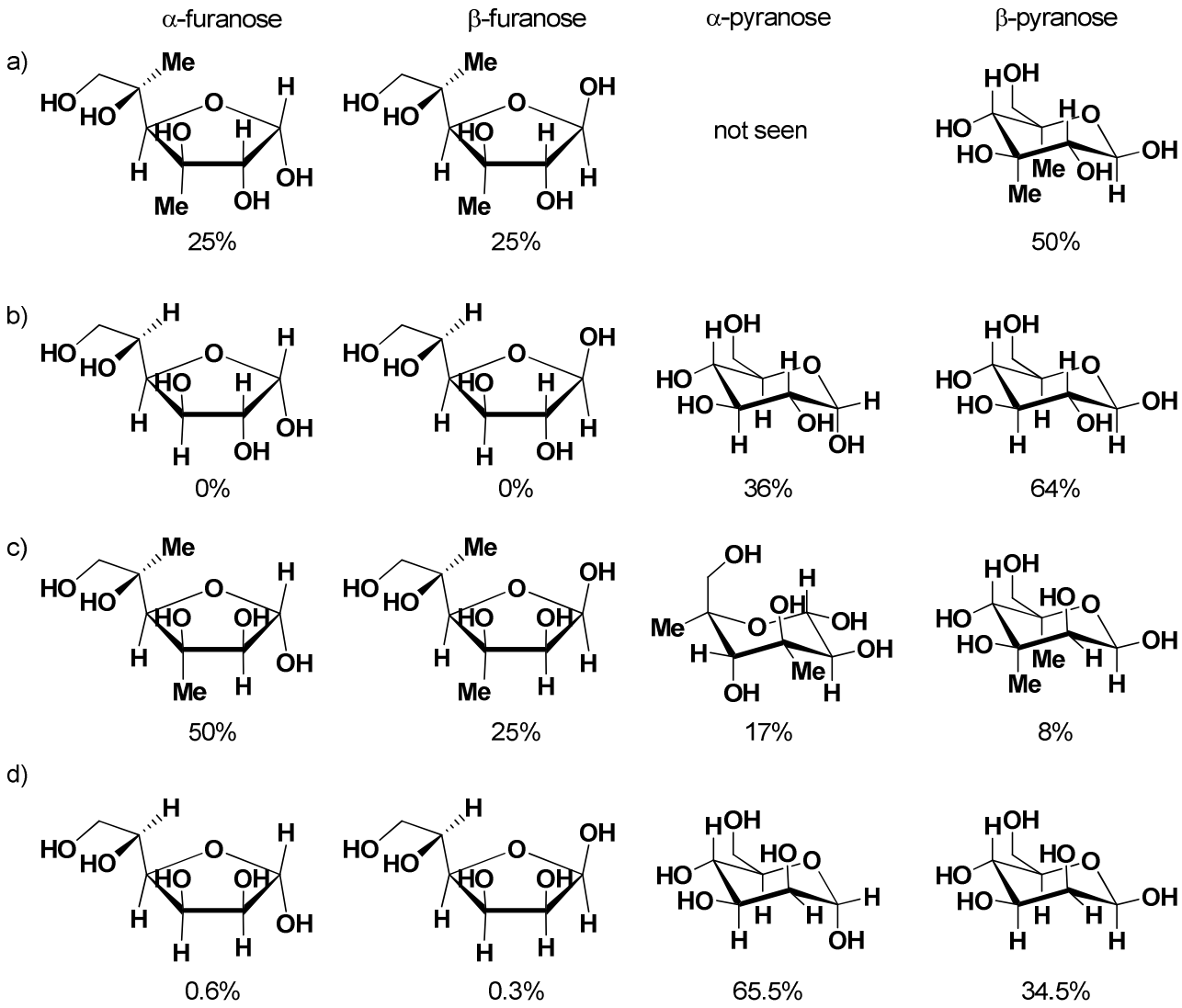
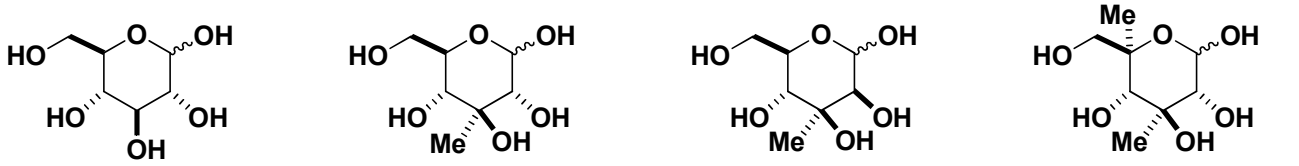


Figure 2.14: Ring sizes and relative populations for the α-furanose and β-furanose, α-pyranose, β-pyranose forms. (a) 3,5-di-*C*-methyl glucose **1**; (b) D-glucose (at 31°C) **31**; (c) 3,5-di-*C*-methyl mannose **144**; (d) D-mannose (at 44°C).

2.2.9 GP Inhibition: Biological Results

Biological testing of GPb inhibition by 3,5-di-*C*-methyl-D-glucose **1** was carried out by Prof. A. Kato, at the University of Toyama, Japan. This result is compared with 3-*C*-methyl-D-glucose **147** and 3-*C*-methyl-D-mannose **148**, prepared by a co-worker (**Figure 2.15**).



D-glucose **31**

3-*C*-methyl-D-glucose **147**

3-*C*-methyl-D-mannose **148**

3,5-di-*C*-methyl-D-glucose **1**

Figure 2.15: Sugars tested for GPb inhibition.

Encouragingly, all three branched sugars are better inhibitors than D-glucose **31**, thus the presence of a lipophilic group, in ths case a methyl group has enhanced binding through a hydrophobic interaction, as predicted by the molecular modelling studies. The results are summarised in **Table 2.2**.

Inhibition % at 5 mM			
D-glucose **31**	3-*C*-methyl-D-glucose **147**	3-*C*-methyl-D-mannose **148**	3,5-di-*C*-methyl-D-glucose **1**
14.2	64.2	71.0	17.4
IC_{50} not measured	IC_{50}=3.5 mM	IC_{50}=2.5 mM	IC_{50} not measured

Reagents and Conditions: GPb activity was measured in the direction of glycogenolysis using 2 mg/mL glycogen as the substrate in 45 mM phosphate buffer at pH 6.8, containing 0.1 mM EDTA, 0.34 mM $NADP^+$, 4 mM glucose-1,6-bisphosphate, 15 mM magnesium chloride, 1 mM AMP, phosphoglucomutase (0.8 units/mL), glucose 6-phosphate (glucose-6-P) dehydrogenase (3 units/mL) and GPb. The rate of enzyme-catalysed reaction in the medium can be followed as the increase in absorbance at 340 nm and 25°C due to the formation of NADPH.

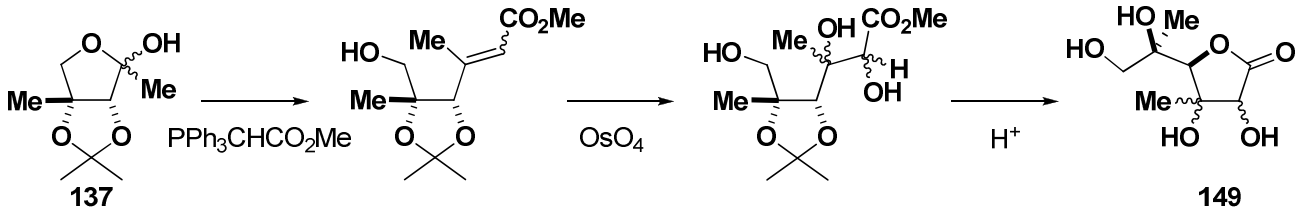
Table 2.2: Results of GPb inhibition studies

It is disappointing to note that 3,5-di-*C*-methyl glucose **1** is a poorer inhibitor than either of the singly branched moieties. Without other doubly branched sugars or singly branched 5-*C*-methyl sugars with which to compare this result, it is impossible to draw any firm conclusions, however the poorer result for **1** may be linked to the preferred conformations in aqueous solution of this molecule. From the NMR studies, it is known that 50% of the material exists in the β-pyranose form with the remainder as the two furanoses. The furanose form would not be expected to be biologically active, so it might be possible that inhibition is being masked by 50% of the material being inert. Also, studies on D-glucose have shown better inhibition for the α-pyranose form than the β, with Ki values of 1.7 mM and 7.4 mM respectively.[99] This may go some way to explain the moderate inhibition observed.

2.3 Alternative Approaches to 3,5-Di-C-Methyl-L-Glucose

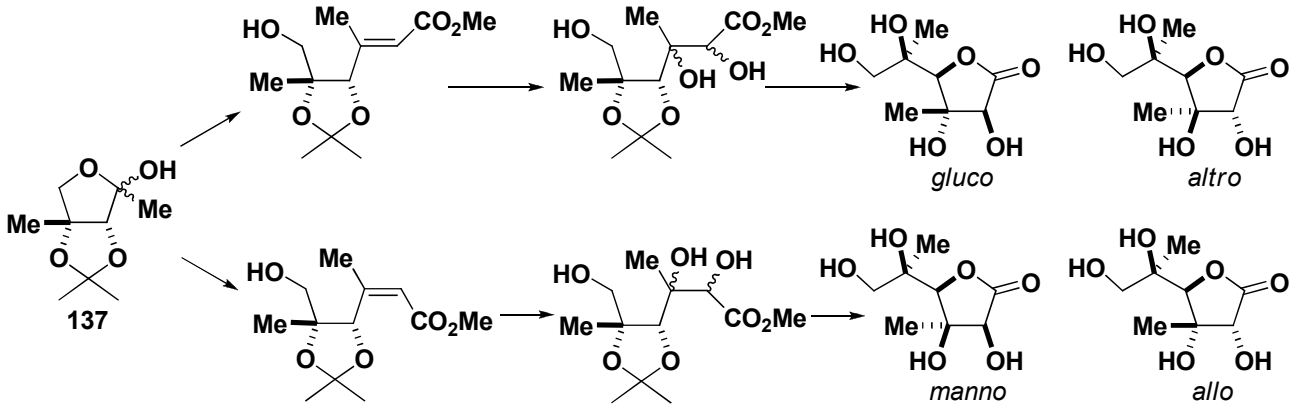
2.3.1 Wittig approach

The synthesis described above, whilst successful, produces an approximately 50/50 mixture of the desired *gluco* product and the *manno* isomer. It would clearly be desirable to find an alternative synthesis in which the *gluco* configuration of the product dominates. The Wittig reaction has been previously used to introduce branch points into sugars by addition of the phosphorous ylid to a ketone functionality on the carbohydrate backbone (see **Section 2.1.2**, page 46). It was envisaged that the Wittig-dihydroxylation reaction sequence could instead be applied to sugar lactols to simultaneously affect a two carbon chain extension and introduce a branch point. This could be carried out with branched 1-deoxy-sugars which, after dihydroxylation and subsequent ring closure would give di-*C*-methyl branched sugar lactones (**Scheme 2.32**).



Scheme 2.32: General scheme for the synthesis of di-*C*-methyl branched sugars *via* a Wittig approach.

This approach will give rise to four branched lactones, as the Wittig reaction could give both *E* and *Z* alkenes and subsequent the *syn* stereospecific dihydroxylation would give rise to 4 possible esters which on ring closure would allow access to four lactones (**Scheme 2.33**). The formation of the *E* alkene should lead to the generation of *gluco* and *altro* lactones whereas the *Z* alkene should give rise to the *allo* and *manno* lactones. Therefore, by stereocontrol of both the Wittig and the dihydroxylation reactions all four products would be accessible in both series leading to four possible branched lactones. Application of this methodology to the corresponding starting materials derived from threose would give access to the remaining four lactones in each series *i.e. gulo*, *ido*, *galacto*, and *talo*.



Scheme 2.33: Wittig approach to di-*C*-methyl branched sugar lactones.

It was decided to test this methodology on a singly branched system before applying it to the di-branched system, by using 1-deoxy ribulose **92** (F**igure 2.16**). This sugar was chosen as it is readily available in two steps from erythrono lactone, which is itself easily accessed *via* Humphlett oxidation of arabinose[100] or from D-isoascorbic acid.[101]

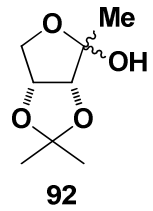
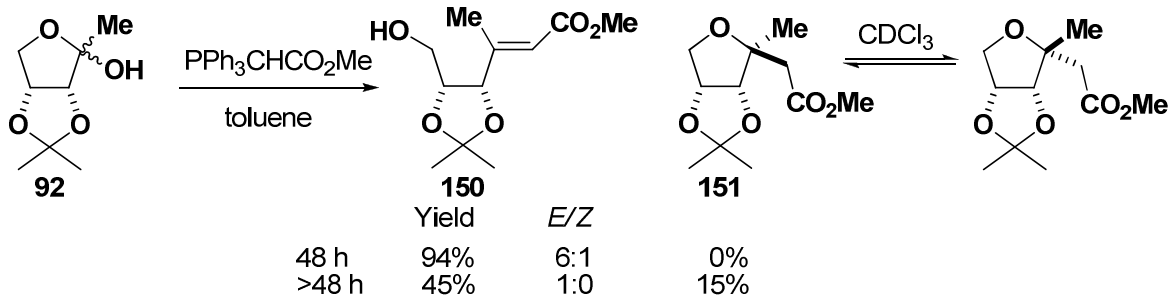


Figure 2.16: Test lactol for the Wittig approach to branched sugar synthesis.

Ketone **92** was taken with Bu_3PCHCO_2Me in DCM; no reaction was observed at room temperature, and due to the nature of the solvent, the reaction could not be subjected to prolonged heating. Use of a higher boiling solvent, 1,4-dioxane allowed the reaction temperature to be raised to 40°C, however, again the reaction did not go to completion and contamination with starting material remained an issue. Keeping the temperature the same but changing solvent to toluene gave an excellent yield of a mixture of alkenes **150**, in a 6:1 *E*/*Z* ratio (**Scheme 2.34**). If the reaction mixture was allowed to remain at 40°C then a second product **151**, arising from an intra-molecular Michael addition reaction, began to form. Interestingly the Michael addition product **7** was found to be unstable and on standing in $CDCl_3$ and the formation of an additional product was observed. This

was identified, by nOe experiments, as the epimer at C-3 arising from a reverse Michael reaction in the slightly acidic conditions followed by closure on the opposite face of the alkene.



Scheme 2.34: Wittig reaction on ketone **92** and Michael addition side product **151**.

As anticipated for the use of a stabilised ylid, the *E* stereochemistry dominated, which would lead to the *gluco* and *altro* sugar lactones. There was limited evidence that changing from the tributyl to the triphenyl ylid could affect the *E*/*Z* ratio,[102] however, in this instance, changing the ylid produced little effect on ratio, but was severly detrimental to the yield.

Stereoselective dihydroxylation of the *E* alkene should give the desired *gluco* compound. Dihydroxylation reactions proceed *syn* stereospecifically to generate *cis* diols. Therefore it was expected that two products would be formed from the *E* alkene **150**. Standard Upjohn conditions[103] (potassium osmate and 4-methylmorpholine-*N*-oxide (NMO) were applied to alkene **150** and the reaction was seen to proceed in a very poor yield giving only 16% of a single product, later found to be **153**. Modified Sharpless conditions, using a chiral ligand,[104] were next investigated and alkene **150** was reacted with potassium osmate, NMO and $(DHQ)_2PHAL$ (**Scheme 2.35**). The yield of the reaction was improved to a total of 46% and two products were observed in a 1:3 ratio (**152**:**153**). It has been shown in the literature[105,106] that dihydroxylation may show a pH dependency and addition of certain acids can increase the rate of reactivity and alter the selectivity of the reaction, therefore a number of acids were investigated. In the case of L-malic acid (**Table 2.3**) although the reaction proceeded at a faster rate there was little change in either the yield or selectivity. Addition of citric and L-tartaric acid, however, greatly increased both the overall yield and the rate of the reaction.

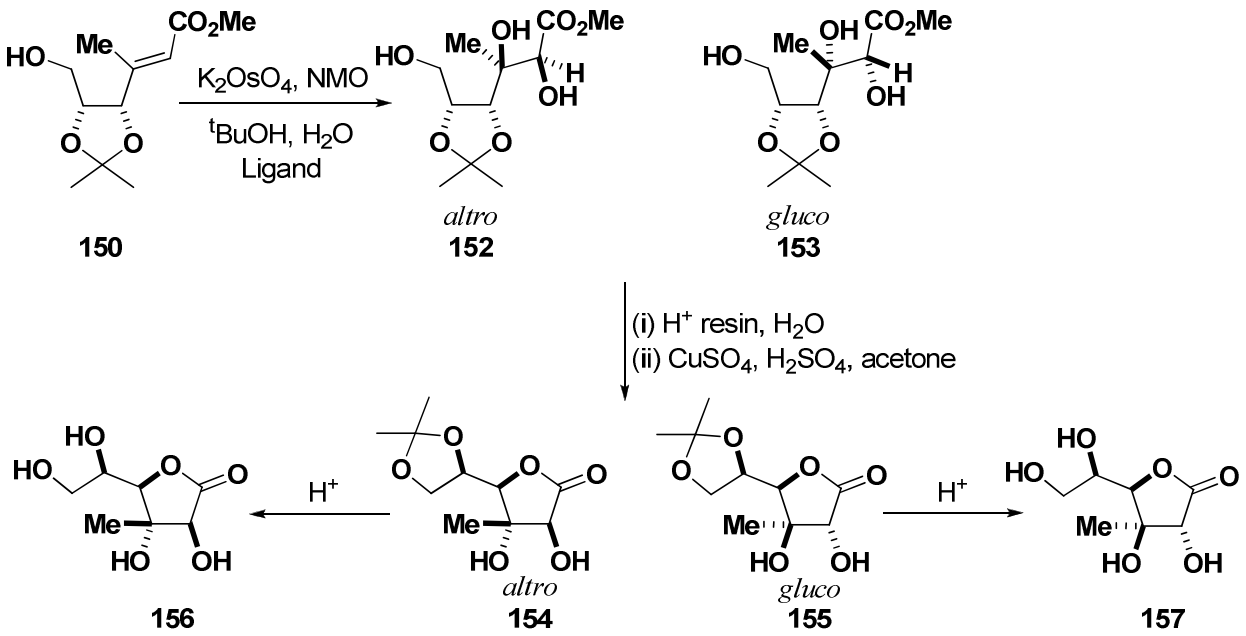
Interestingly the selectivity of the reaction was reversed with both of these reactions favouring the formation of triol **152** (**Table 2.3**). As L-tartaric acid is chiral it was wondered whether use of the enantiomeric D-tartaric acid would reverse the selectivity but maintain the high yields. This, however, was not seen to be the case. The reaction with D-tartaric acid was high yielding but again gave triol **152** as the major product, with the selectivity only slightly decreasing from 6:1 to 4:1. It should be noted that although there were initial concerns that excess acid in the reaction mixtures would cause the intramolecular Michael addition reaction, in none of the above cases was any of this molecule isolated.

Ligand	***Quantity***	***Yield of 152 (altro)***	***Yield of 153 (gluco)***
$(DHQ)_2PHAL^{\dagger}$	2 mol%	14%	32%
L-malic acid	0.5 eq	9%	28%
citric acid*	0.5 eq	42%	15%
citric acid	0.75 eq	63%	21%
L-tartaric acid	0.5 eq	61%	10%
L-tartaric acid	0.75 eq	56%	13%
D-tartaric acid	0.5 eq	55%	14%

Ratios determined by integration in ^{1}H NMR, reactions ran for 18 h

† 72 h reaction time; * 38% starting material was recovered from this reaction.

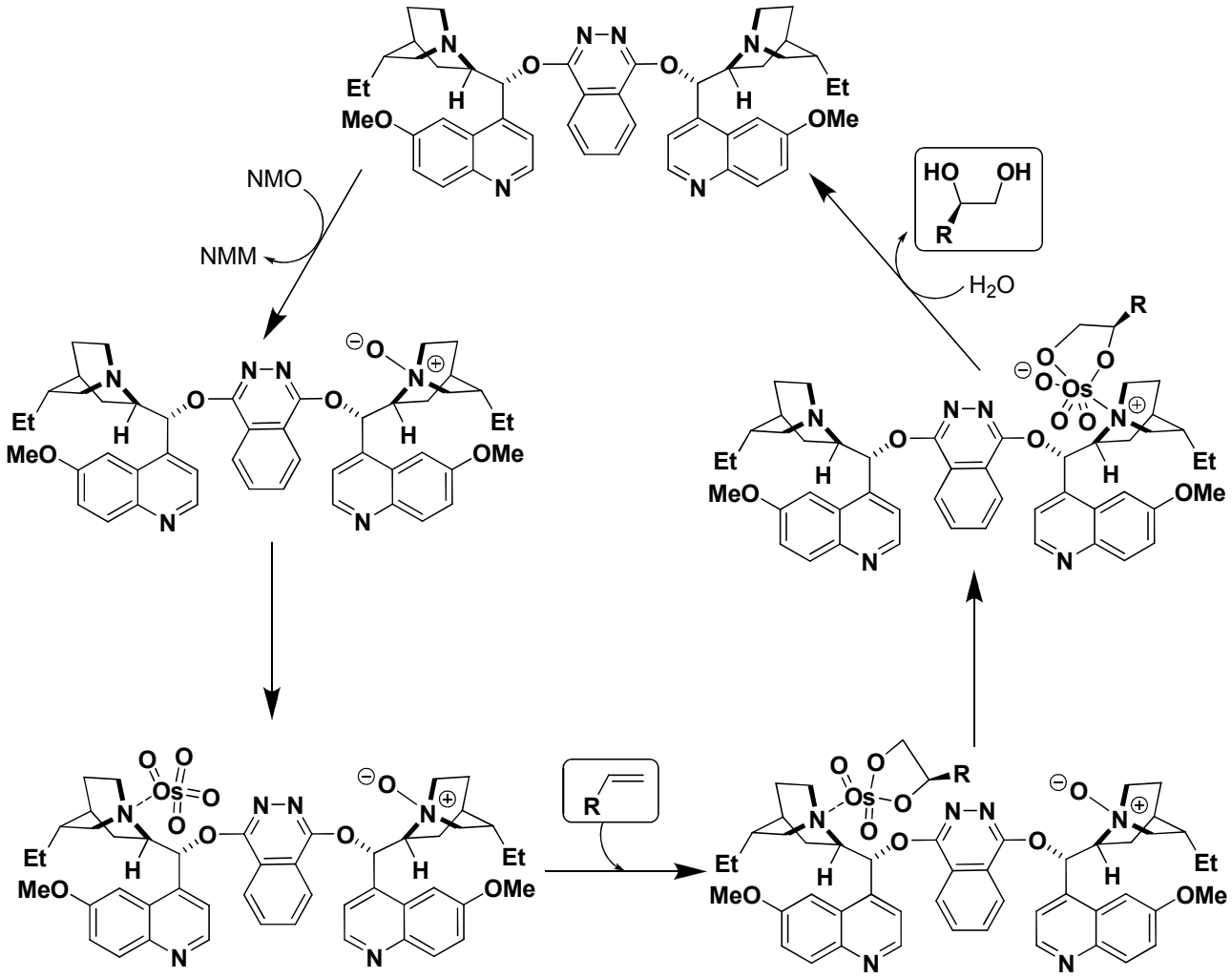
Table 2.3: The effect of ligand choice on the outcome of the dihydroxylation reaction.



Scheme 2.35: A deprotection/reprotection strategy was necessary to separate the lactones.

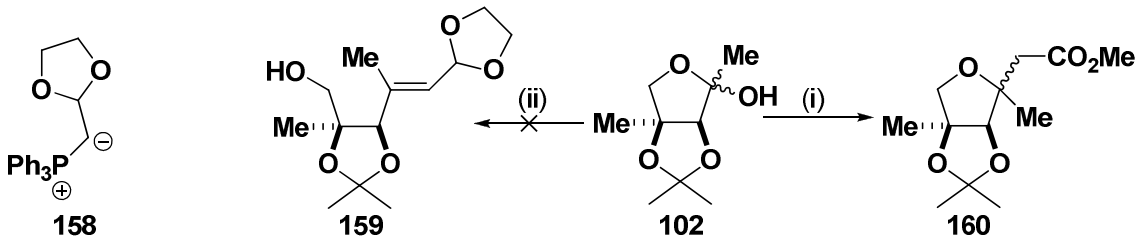
Separation of the triols **152** and **153** by column chromatography was difficult and therefore the mixtures were treated with acidic resin to deprotect the acetonide protecting group and effect ring closure to the lactone. The mixture of lactones was then reprotected with anhydrous copper(II) sulfate, acetone and catalytic sulfuric acid to give a both the altronolactone **154** and the gluconolactone **155** (**Scheme 2.35**) which could be cleanly isolated and the stereochemistry firmly determined by nOe experiments. Subsequent deprotection using Dowex (H^+) resin in water gave the fully deprotected altronolactone **156** and gluconolactone **157**.

Regrettably, in all but the poorest yielding cases, the major product was seen to have the *altro* stereochemistry. This may be due to crowding on the lower face of the molecule, blocking the approach of the osmium. The selectivity observed with $(DHQ)_2PHAL$ may be rationalised by the catalytic cycle (**Scheme 2.36**), which suggests that the bulk of the chiral ligand is far in excess of that of the substrate and therefore the selectivity of dihydroxylation is reversed, allowing access to the most hindered face of the substrate.



Scheme 2.36: Catalytic cycle of the enantioselective dihydroxylation of alkenes using $(DHQ)_2PHAL$

With the methodology in place, it was decided to proceed with the di-branched system, using 1-deoxy-4-*C*-methyl-L-ribulofuranose **102**. Again, the more readily available L-enantiomer was used for the initial investigations. Ketone **102** was treated with Bu_3PCHCO_2Me in toluene at 40°C, as before, however the major product isolated from this reaction did not reveal any UV activity. It was shown by ^1H and ^{13}C NMR analysis to be a furan **160** formed by nucleophilic attack of the hydroxyl into the alkene, in a Michael fashion (**Scheme 2.37**). Attempts to isolate the alkene before this side reaction could occur were unsuccessful. With the related triphenyl ylid, only unreacted starting material was recovered.



Reagents and Conditions: (i) Bu_3PCHCO_2Me, toluene, 40°C, 48 h.

Scheme 2.37: Wittig approach to di-*C*-methyl branched sugar lactones.

It was decided therefore to try an alternative Wittig reagent with which the Michael addition reaction would be disfavoured. Bromo methyldioxolane was stirred with triphenylphosphine to generate a bromo salt, which upon deprotonation with *n*butyl lithium gave ylid **158**. Treatment of **102** with ylid **158** was attempted in a range of solvents; toluene, dioxane, *N*,*N*-dimethylformamide and tetrahydrofuran and all were equally unsuccessful, with no reaction observed in any case after prolonged heating. Wadsworth-Eammons type reactions were also attempted,[107] using methyl diethylphosphonoacetate $(EtO)_2P(O)CH_2COMe$. Deprotonation of the phosphonate was attempted with *N*,*N*-diisopropylethylamine, followed by addition of the ketone in a solution of dichloromethane.[108] After several hours, no reaction was seen, and the solvent was exchanged to *N*,*N*-dimethylformamide to allow for prolonged heating, but even so, only starting material was recovered. A modified Wadsworth Eammons reaction was tried with lithium salts, without success.[109,110] Finally, a Reformatskii type reaction was attempted, using methyl bromoacetate, zinc powder and tributylphosphine in both toluene[111] and dioxane,[112] unfortunately to no avail.

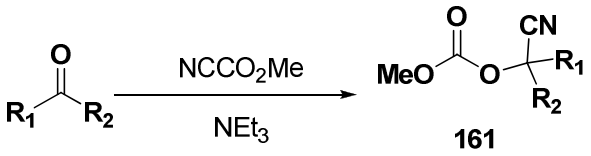
It is likely that the open chain form of the ketone is disfavoured, as all previous doubly branched systems show no evidence of their open chain form by ^{1}H NMR. This results in a sluggish reaction with the ylids, and if addition is achieved, the ring closing reaction is rapid giving only the tetrahydrofuran. Wittig reactions on branched susbtrates in other systems have been affected by rapid Michael addition.[113]

2.3.2 Alternative carbon chain extension methods

Given the success of the initial Kiliani reaction in the synthesis of 3,5-di-*C*-methyl-L-glucose **97**, which generated protected 2,4-di-*C*-methyl-L-arabinose **103** rapidly and in high yield, it was decided to investigate other ways in which to extend the carbon backbone by a single unit, to replace the second Kiliani reaction.

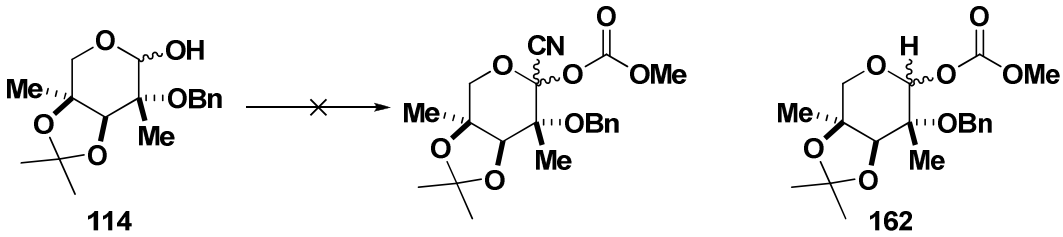
2.3.2a Methyl Cyanoformate

Elongation of a carbon chain by cyanide is a useful synthetic transformation, however in the previous synthesis of 3,5-di-*C*-methyl glucose, its utility was compromised by the poor steroselectivity exhibited in the Kiliani reaction, giving approximately a 50/50 mixture of the desired *gluco* configuration and the unsought *manno* configuration. Recent reports in the literature used methyl cyanoformate as an alternative source of cyanide.[114] This approach has the advantage of protecting the hydroxyl generated in the reaction as a methoxy carbonyl species **161** (S**cheme 2.38**), allowing for easier purification.



Scheme 2.38: Synthesis of *O*-methoxycarbonyl cyanohydrins

Protected 2,4-di-*C*-methy-L-arabinose **114** was treated with methyl cyanoformate and catalytic triethylamine in dichloromethane. The reaction progressed rapidly however analysis of the material generated revealed no addition of cyanide to the substrate, and instead compound **162** had been generated, demonstrated by the presence of an anomeric proton in the 5 ppm region of the ^{1}H NMR (S**cheme 2.39**).



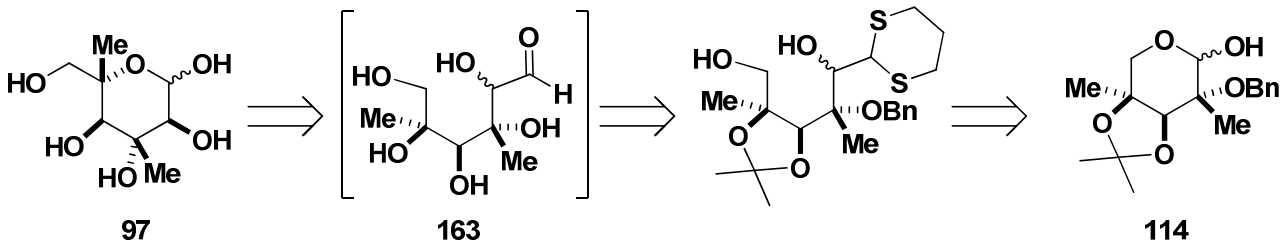
Reagents and Conditions: $CH_3OC(O)CN$**,** DCM, TEA, RT, 20 min, quant.

Scheme 2.39: Attempted cyanide addition.

The lactol had clearly remained shut, and the methyl cyanoformate had undergone nucleophilc attack by the hydroxyl to generate glycoside **162**.

2.3.2b Umpolung Approach

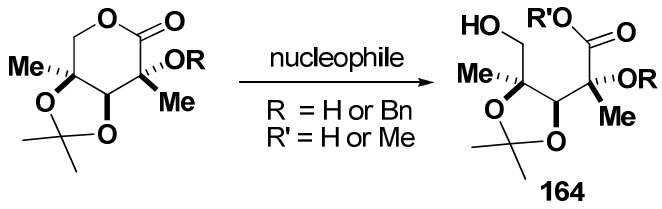
Alternative one carbon nucleophiles were considered; to minimise the number of steps in the sequence, the best choice of reagent would be an aldehyde synthon, as this would provide the correct oxidation level at C1, removing the need for a reduction step. The reagent chosen for initial investigations was 1,3-dithiane, which can be converted into an aldehyde group by oxidation or treatment with heavy metal salts. Deprotected sugar **163** should then close in situ to **97** (**Scheme 2.40**).[115,116,117]



Scheme 2.40: Proposed synthesis of 3,5-di-*C*-methyl-L-glucose **97** in 3 steps from protected arabinose **114.**

An excess of 1,3-dithiane was taken with *n*-butyl lithium to form the anion, to which was then added the lactol. Regrettably, after several hours only starting material was recovered, and test reactions with diphenylthiomethane $(PhS)_2CH_2$ were similarly unsuccessful. Investigations by a co-worker into this reaction found both lactol **114** and the corresponding lactone **113** to be resistant to nucleophilic attack by a wide range of related umpolung type reagents.[118] At this point, it was

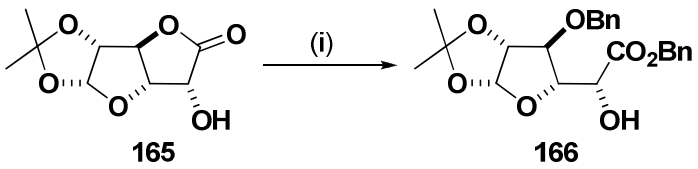
decided to try a different approach, and attempt to open the lactone to the ester, in the hope that the open chain ester **164** would be less hindered and therefore more reactive (**Scheme 2.41**).



Scheme 2.41: Trapping the lactone in the open chain ester form **164** to facilitate nucleophilic attack.

Initial investigations were carried out on the benzylated lactone, using acidic methanol, basic methanol, and saturated aqueous potassium hydroxide solution. In all three cases, LCMS analysis of the crude reaction mixture showed a possible product peak, and t.l.c. analysis also looked promising. In an attempt to avoid ring re-closure, the reaction mixtures were concentrated under reduced pressure without neutralising, but regrettably the removal of solvent favoured the ring closing reaction, and in each case only starting material was recovered.

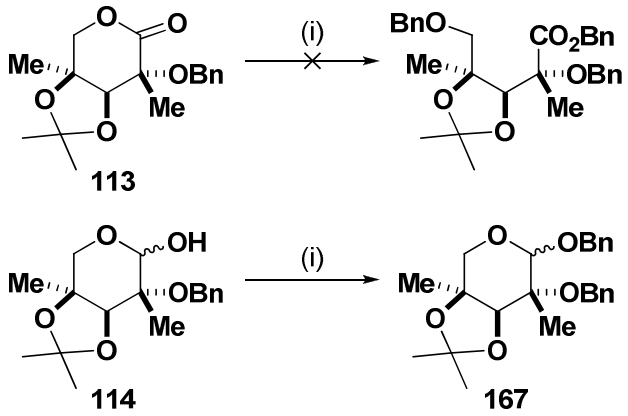
It had been reported in the literature that treatment of lactone **165** with benzyl bromide and a mixture of barium oxide and hydroxide gave the open chain benzylated ester **166** (**Scheme 2.42**).[119]



Reagents and Conditions: (i) DMF, BnBr, BaO, $Ba(OH)_2.8H_2O$, 5 h sonication then Amberlite IR 120 (H^+ resin), 4 h.

Scheme 2.42: Trapping a lactone in the open chain form.

Thus, benzylated lactone **113** was taken in a similar fashion, but no reaction was observed. Treatment of lactol **114** in an analogous way gave only the dibenzylated product **167** (**Scheme 2.43**).



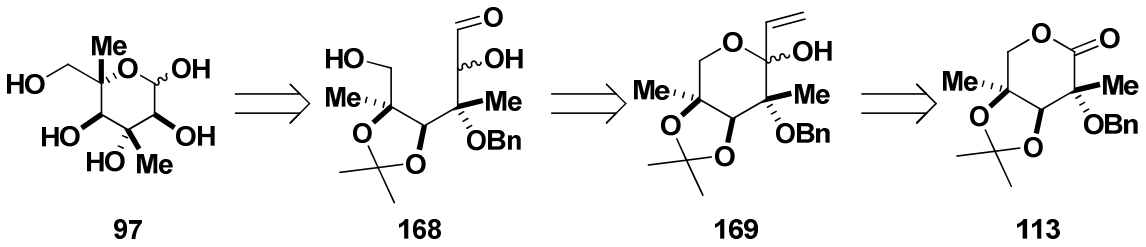
Reagents and Conditions: (i) DMF, BnBr, BaO, $Ba(OH)_2.8H_2O$, 1 h sonication.

Scheme 2.43: Attempted trapping of lactone **113** and lactol **114** in the open chain form.

At this point it became evident that a different approach was necessary, and, if it utilised the readily available arabinonolactone, needed also to use a highly reactive carbon based nucleophile.

2.3.4c Grignard Approach

Another possible synthesis was proposed using vinyl magnesium bromide as the nucleophile giving **169** (**Scheme 2.44**). Selective reduction of **169** to the open chain diol, followed by ozonolysis could afford aldehyde **168**, which would close on deprotection to the desired *gluco* compound **97**.

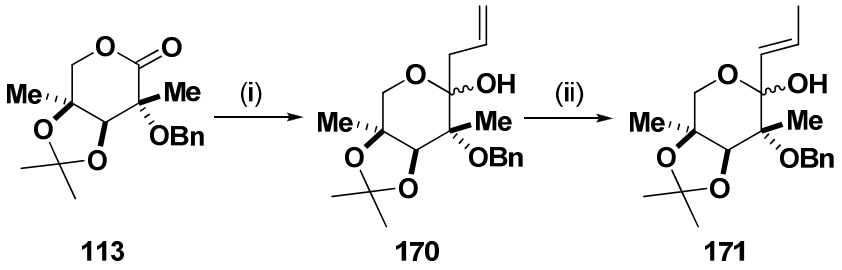


Scheme 2.44: Proposed retrosynthesis of **97** from **113** using vinyl MgBr.

Treatment of **113** with vinyl magnesium bromide proved to be rather more difficult than anticipated, and none of **169** could be isolated using standard conditions. The more reactive vinyl lithium reagent is difficult to obtain, and synthesis of this reagent is often plagued with low yields, it was therefore proposed to use the more reactive allyl magnesium bromide reagent, and isomerise the double bond.[120] Allyl magnesium bromide added in good yield to the protected lactone **113**, however, the product **170** was found to be highly unstable at room temperature and was used with

minimal purification (**Scheme 2.45**). Isomerisation of the 1,2-alkene to the 2,3-alkene was attempted several ways. It had been reported in the literature that when using Grubbs second generation catalyst to affect ring closing metathesis reactions, isomerisation of terminal alkenes was observed in certain substrates.[121] This had been noted as a common side reaction, and in recent years, work has been carried out to promote this isomerisation reaction.[122,123] Treatment of allyl compound **170** directly with Grubbs II in methanol afforded a complex mixture of products, from which no useful products could be identified, as demonstrated by the very dark colour of the reaction mixture. It is possible that ruthenium was contaminating the products, even after work up and column chromatography. Ruthenium is a spin active nucleus,[124] and as such even small traces cause interference in the NMR analyses. The problem of removing all traces of ruthenium is well known in the literature and a range of work up procedures have been proposed, for example the use of ligand exchange reactions to generate water soluble ruthenium complexes which can be easily removed.[125,126] Unfortunately, purification of the products continued to be a problem and, given the number of side products seen by t.l.c. analysis, it was decided to pursue an alternative mode of isomerisation.

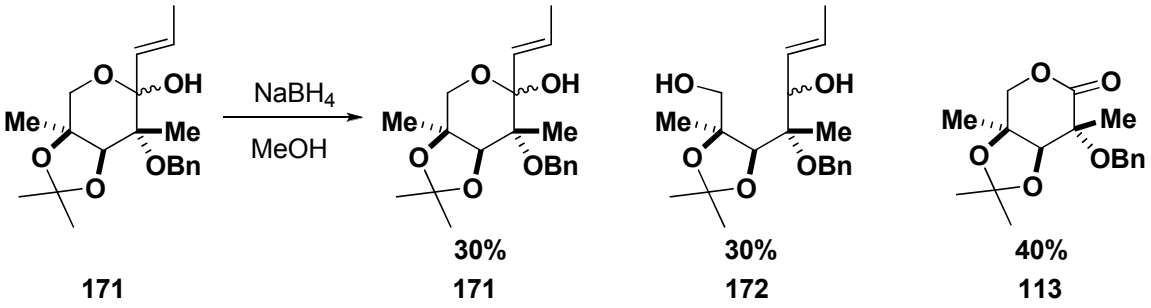
Since moving the double bond brings it into conjugation with a ketone (albeit masked as the lactol) it should be possible to affect the desired isomerisation by use of base. The base chosen was 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), due to its low nucleophilicity (**Scheme 2.45**). Initial results were disappointing as it was found that by prolonging the reaction and/or adding more DBU until the starting material was no longer visible on t.l.c., the desired product was beginning to decompose, and yields dropped accordingly. The optimal conditions were found to be halting the reaction after 2 hours, and recovering starting material. In this way, the yield of conjugated alkene **171** was consistently around 60-70%, with 30-40% of lactol **170** recovered.



Reagents and Conditions: (i) Allyl MgBr, Et_2O, -50°C, 3 h (used crude); (ii) DBU, THF, RT, 2 h (60% over two steps).

Scheme 2.45: Allyl magnesium bromide addition, followed by DBU isomerisation of the double bond circumvented the problem of the difficult vinyl addition reaction.

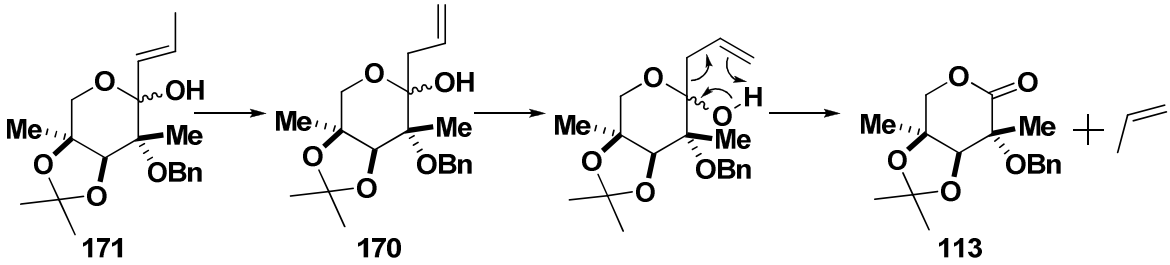
Reduction of the conjugated alkene to the diol would give the correct oxidation state at what would eventually become C2. Since C2 is the stereocentre which determines whether the *manno* or *gluco* stereochemistry is observed in the final product, it was important to find a stereoselective reducing agent, remembering that the previous double Kiliani synthesis gave approximately a 50/50 mixture of the two sugars. A straightforward reduction with sodium borohydride in methanol was attempted, to see if any selectivity could be imposed by the inherent bulk of the molecule. The results were surprising; t.l.c. analysis of the reaction mixture revealed three compounds, unreacted starting material (30%), a mixture of epimeric diols **172** (30%) and lactone **113** (40%) (**Scheme 2.46**). This seems to imply an oxidation reaction has occurred under reducing conditions!



Scheme 2.46: Reduction of **171** proved to be complex and poorly yielding.

A mechanism was proposed, which suggests that the alkene isomerises back to its original position. The electron rich alkene is then able, *via* a 6 membered ring transition state to abstract the lactol hydrogen in a concerted retro-ene type fashion, to generate the lactone (**Scheme 2.47**). No

mechanistic studies were carried out on this reaction, as the reaction was poorly yielding and alternative methods were found.

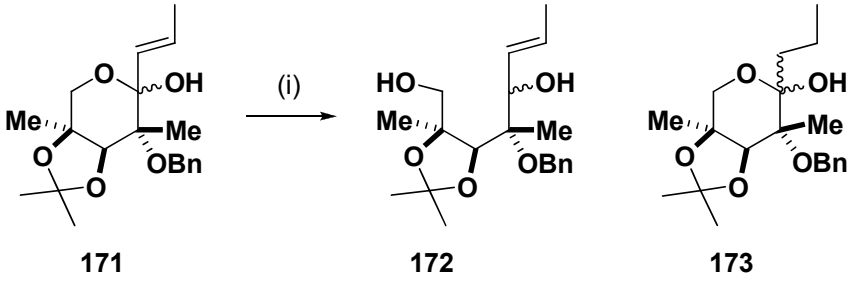


Scheme 2.47: Proposed retro-ene type mechanism.

It should be noted that the allylic compound **170** was known to be unstable, and was therefore used crude with DBU to effect the isomerisation to **171**. Attempted analysis of the decomposition products from leaving a sample of **170** exposed to air at room temperature was unsuccessful, and none of lactone **113** was observed, suggesting base catalysis is important. Indeed, traces of this lactone could be identified in ^{1}H NMR spectra from attempted DBU isomerisations which had been reacted with excess base or long reaction times.

Despite the unanticipated side product observed, a 30% yield of epimeric diols **172** were also found, but regrettably as an inseparable 50/50 mixture of diastereomers. Clearly, an alternative reagent was required, one which could be more selective and less basic, to minimise the undesirable lactone side product. In an attempt to optimise this reaction, a Luche type reduction was tried, using cerium trichloride with sodium borohydride.[127] This has been used successfully for selective reduction of a conjugated ketone, as cerium acts as a Lewis acid and its chelation directs the hydride towards the carbonyl group, instead of towards the alkene. Under these conditions, the lactone was not seen, but the reaction would not go to completion, and, as was the case with the previous isomerisation reaction, it was preferable to halt the reaction, rather than continue until no starting material remained, as this compromised yield. Again, the diols observed were in a 50/50 ratio. It was hoped that changing to a more reactive reagent, such as lithium aluminium hydride would improve yield, but unfortunately, instead of recovered starting material, a new product was observed, which was

shown to be the 'Michael addition' compound, with a saturated propane group at C1 **173** (**Scheme 2.48**).



Reagents and Conditions: (iii) $NaBH_4$, $CeCl_3$, MeOH, RT, 2h (48%) OR $LiAlH_4$, THF, 2 h (55%) OR Red-Al®, THF, 2h (51%). The yields given in brackets are for the epimeric diols **172**.

Scheme 2.48: Reduction of **171**.

Neither lithium aluminium hydride nor sodium bis(2-methoxyethoxy)aluminumhydride (Red-Al®) could change the ratio of diols observed, and in both cases, the side product **173** was seen, with only a roughly 50% yield of the desired compound. Whilst by no means and exhaustive list of conditions were tried, it was clear that introduction of stereoselectivity could not be achieved without use of a chiral ligand, and given the length of time a suitable screen could take, and the moderate yields so far in the synthesis, it was decided to first investigate a different approach.

2.4 From Fructose to Glucose?

Having looked at a wide variety of one and two carbon nucleophiles to complete the synthesis of 3,5-di-*C*-methyl glucose and mannose, a new approach was considered that would involve the biotechonological conversion of ketose and aldose sugars. The use of enzymes to sterospecifically interconvert monosaccharides was discussed briefly in **Section 2.2.7**, when looking at the availability of D-tagatose as a synthetic starting material. Izumori and co-workers have since expanded the range of enzymatic transformations beyond simple hexoses to include other classes of monosaccharides, such as 1- and 6- deoxy hexoses[128,129] azido hexoses[130] and in particular, branched sugars.[131,132] By careful choice of the aldose isomerase enzymes used, it was possible to

convert 5-*C*-methyl-D-fructose **174** selectively into either 5-*C*-methyl-D-mannose **175** or 5-*C*-methyl-D-glucose **145** (**Figure 2.17**).

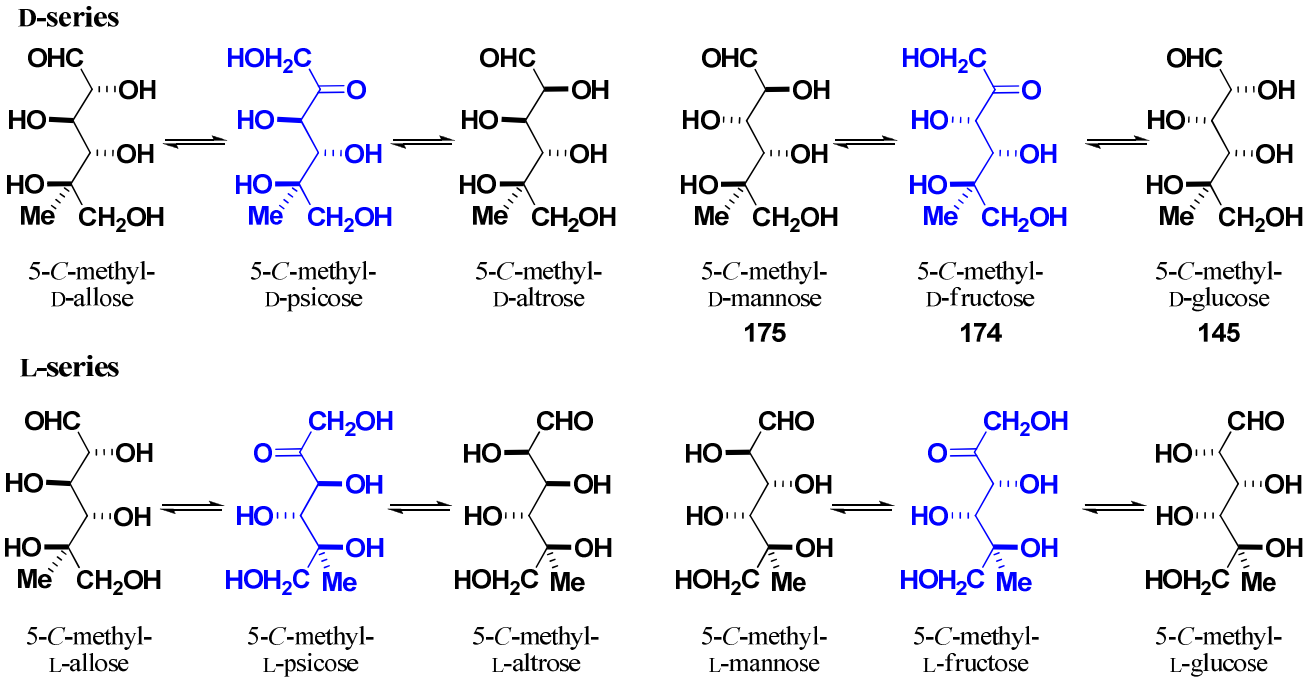
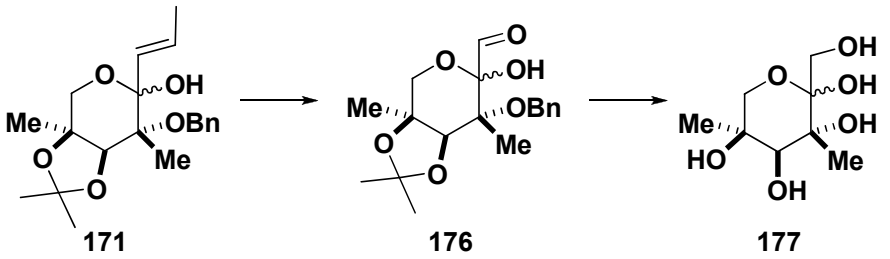


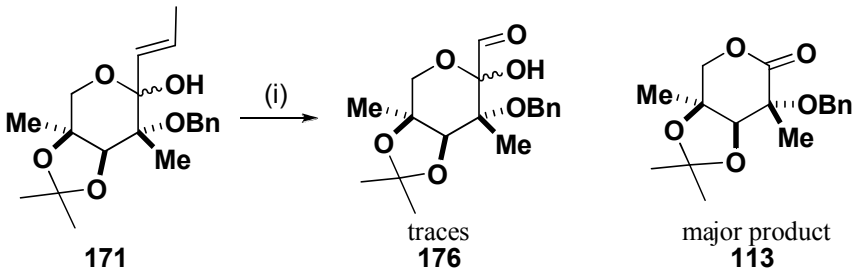
Figure 2.17: 5-*C*-Methyl aldoses by aldose isomerases on ketoses (shown in blue).

It was speculated that perhaps similar enzymes might be able to convert 3,5-di-*C*-methyl fructose into 3,5-di-*C*-methyl glucose **1** with total stereocontrol, something all previous syntheses have lacked. This chemistry could even be extended at this point, as an enzyme called D-tagatose-3-epimerase (DTE) can invert the stereochemistry at C3 of a ketose sugar. This would allow the conversion of, for example 5-*C*-methyl-D-fructose, into 5-*C*-methyl-D-psicose, which could in turn be turned into either the branched *allo* or *altro* sugars, depending on the aldose isomerise enzyme used. Thus, potentially a very large number of novel di-*C*-methyl branched sugars could be accessed quickly and stereospecifically in an environmentally friendly fashion. A synthesis of 3,5-di-*C*-methyl-L-fructose **177** was therefore proposed from **171** (**Scheme 2.49**).



Scheme 2.49: Proposed synthesis of dibranched L-fructose derivative **177**.

This route utilises the product following allyl magnesium bromide addition and DBU isomerisation to arabinonolactone **171**, synthesised in the previous approach to di-*C*-methyl glucose. It was hoped that ozonolysis of **171** followed by reduction and deprotection could give fructose **177** in 3 steps. Reductive ozonolysis of alkene **171** was attempted, by dissolving it in a mixture of dichloromethane and methanol and bubbling ozone gas through the reaction mixture, at -78°C. At the end of the reaction, sodium borohydride was added, and the solution allowed to warm to room temperature. Unfortunately, none of the desired alcohol was observed. A small quantity of the aldehyde **176** was seen, but the major product was found to be benzylated lactone **113**. It is possible that this came from the retro-ene reaction mechanism proposed earlier (**Scheme 2.50**).

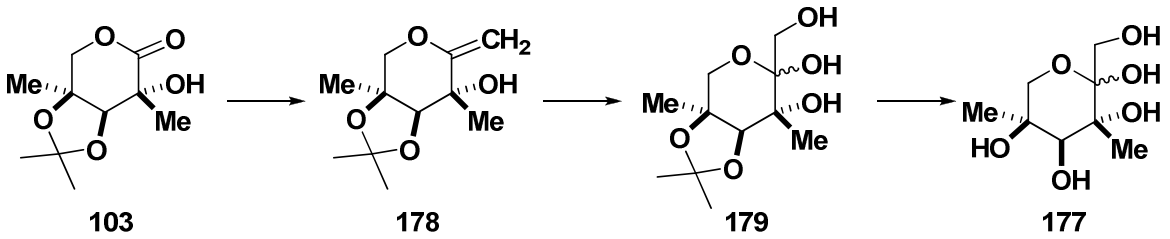


Reagents and Conditions: O_3, DCM, MeOH, -78°C, 30 min then $NaBH_4$.

Scheme 2.50: Attempted ozonolysis of **171**.

It was decided, therefore, to eliminate the possibility of this reaction occurring, by protecting the free hydroxyl at the anomeric position. As already seen, use of basic conditions increases the likelihood of an elimination pathway. Protection under acidic conditions, using benzyl trichloroacetimidate and triflic acid resulted in decomposition of the starting material.[133] Under neutral conditions, using diphenyl diazomethane,[134] no reaction was found, as it is likely that the hydroxyl group is too hindered to accommodate a bulky protecting group. Several other common

protecting groups were attempted but a satisfactory outcome could not be reached, therefore an alternative synthesis was proposed (**Scheme 2.51**).



Scheme 2.51: Proposed synthesis of dibranched L-fructose derivative **177**.

By replacing the lactone oxygen with a methylene group to give vinyl ether **178**, and dihydroxylation to **179**, we should have easy access to a protected dibranched fructose derivative. Acid catalysed isopropylidene removal should give fructose **177** in only three steps from the Kiliani product **103**. Introduction of the methylene group can be performed in several ways. Use of the appropriate Wittig reagent, of the type $P^{+}Ph_3CH_2^{-}$, is one possible choice; however the inherent instability of an unstabilised carbanion, coupled with the difficulties that can arise in removal of triphenyl phosphine oxide at the end of the reaction, means that alternatives are often sought. Use of a carbene 'CH_2' group has been exploited in recent years to great effect, where the carbon atom is stabilised by chelation to titanium. The Tebbe reagent,[135] an aluminium-titanium complex has been used in several syntheses to affect this transformation,[136,137] however, more recently, the Petasis reagent,[138] Cp_2TiMe_2, has been developed, and is more air stable than its predecessor. They both collapse during the reaction to the active species $Cp_2Ti{=}CH_2$ (F**igure 2.18**).

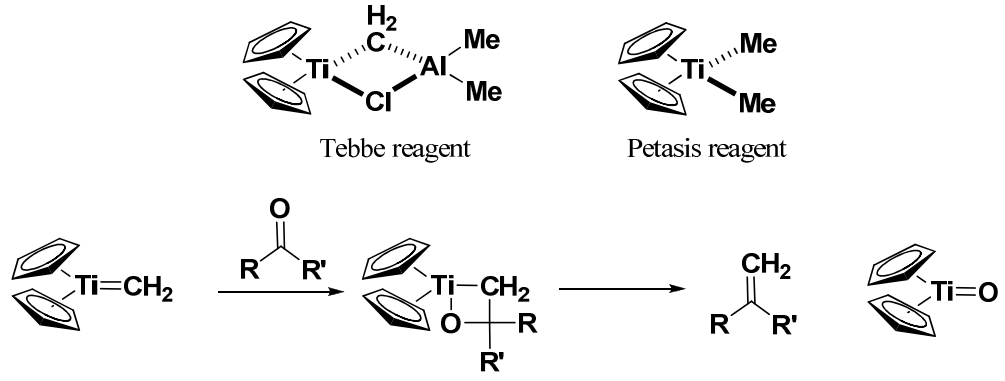
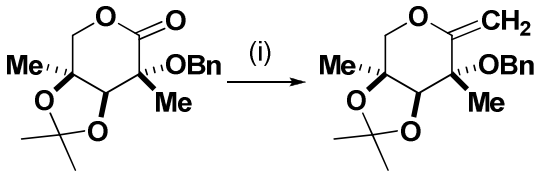


Figure 2.18: Reagents and mechanism for the transformation of C=O into $C{=}CH_2$.

The Petasis reagent can be prepared easily by treatment of Cp_2TiCl_2 with methyl lithium.[139] After an aqueous work up and recrystallisation, the reagent can be stored as a solution in toluene at -20°C for several months. Treatment of arabinonolactone **103** with this reagent, at reflux afforded a disappointing yield of the desired product (approximately 30%) and an unacceptably large amount of reagent, 6 equivalents, was needed to achieve this. Benzylation of the free hydroxyl however gave much better yields of around 75-85% of the desired product **180** (**Scheme 2.52**).

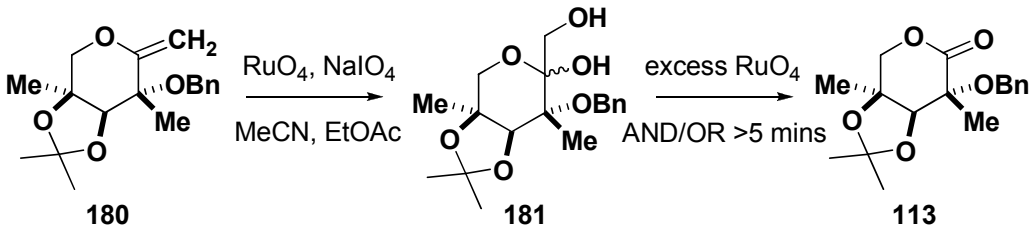


Reagents and Conditions: (i) Cp_2TiMe_2, toluene, reflux, 90 min, in the dark and under N_2.

Scheme 2.52: Performing the Petasis reaction on protected arabinose **103**.

Dihydroxylation to the corresponding diol was then attempted using potassium osmate and *N*-methylmorpholine *N*-oxide in aqueous methanol.[140] By t.l.c. analysis, only a trace of products could be seen, and attempts to push the reaction to completion with further reagent were unsatisfactory. Changing solvent to a mixture of aqueous *tert*-butanol in tetrahydrofuran gave approximately a 39% yield of the desired diol, but unfortunately, even after repeated column chromatography, the product could not be isolated cleanly, most likely due to residual NMO. Instead of the potassium salt, the dihydroxylation was attempted with osmium tetroxide using aqueous acetone as solvent.[141] This time, the reaction, although much cleaner, was sluggish; after forty hours, only 23% of the diol could be isolated, with 39% starting material recovered. It is plausible that steric hindrance α to the alkene, and its conjugation with the ring oxygen greatly reduced the alkene reactivity. A brief investigation of epoxidation using *meta*-chloroperbenzoic acid was carried out,[142] but only recovered starting material was observed. Dihydroxylation with tetrapropyl ammonium perruthenate (TPAP) was attempted,[143] but again, only starting material was recovered.

A review of the literature revealed the *cis* dihydroxylation of alkenes using ruthenium tetroxide.[144] Use of ruthenium tetroxide for dihydroxylation reactions is relatively unexploited due its tendency to cleave *cis* diols, but was successfully employed in the synthesis of the central segments of Zaragozic acids, controlling diol cleavage by the use of short reaction times and low temperatures.[145] Use of ruthenium rather than osmium tetroxide poses significant advantages due to its lower toxicity,[146] and lower cost.[147] Work by Plietker *et al* has developed this procedure further, illustrating its use on a wide range of substrates possessing a variety of functional groups.[148,149,150,151] Ruthenium tetroxide is generated *in situ*, from ruthenium trichloride and sodium periodate. These two reagents are combined in water, and added to the alkene dissolved in a solution of acetonitrile and ethyl acetate, stirring vigorously in an ice bath. The reaction was complete in only 5 minutes, and afforded, on test scale, a 77% yield of diol **181**, which could be isolated cleanly following aqueous work up and column chromatography. The very short reaction time lead to some problems in repetition of the procedure, and on several occasions the reaction yielded exclusively only unreacted starting material or the diol cleavage product, *i.e.* lactone **113** (**Scheme 2.53)**.



Scheme 2.53: Rapid dihydroxylation of vinyl ether **180** using ruthenium tetroxide.

Use of the biphasic system also lead to problems upon scale up, as poor mixing lead to uneven distribution of reagent and poor repeatability. However, both these problems were avoided by a slight modification to the original procedure – instead of preparing the ruthenium tetroxide and adding it to the alkene, preparing the ruthenium tetroxide in the reaction flask then adding the alkene to the reagent. Thus ruthenium trichloride and sodium periodate were dissolved in water and cooled in an ice bath to 0°C. This solution was stirred for a few minutes before adding the alkene in

an acetonitrile solution *via* syringe in one portion. By swapping the order of addition and removing the ethyl acetate, yields of up to 80% of **181** were now consistently achieved, independent of scale.

2.4.1 Deprotection of fructose 181

With a synthesis of 2-*O*-benzyl-3,5-di-*C*-methyl-4,5-*O*-isopropylidene-L-fructose **181** in hand, the final steps in the sequence were the removal of the benzyl and isopropylidene groups to afford the free sugar. De-benzylation was achieved in quantitative yields using palladium hydroxide, in a rapid hydrogenation reaction. Unfortunately, isopropylidene deprotection proved to be much less straightforward. Treatment of **179** with 1 M hydrochloric acid (aq) appeared to give a mixture of products. Repetition of this deprotection reaction was attempted several times, and each time, a different contaminant could be seen by ^{1}H NMR. A selection of the most interesting spectra is shown below, as prepared by Dr M. Wormald with anomalous peaks indicated by arrows (**Figure 2.19**).

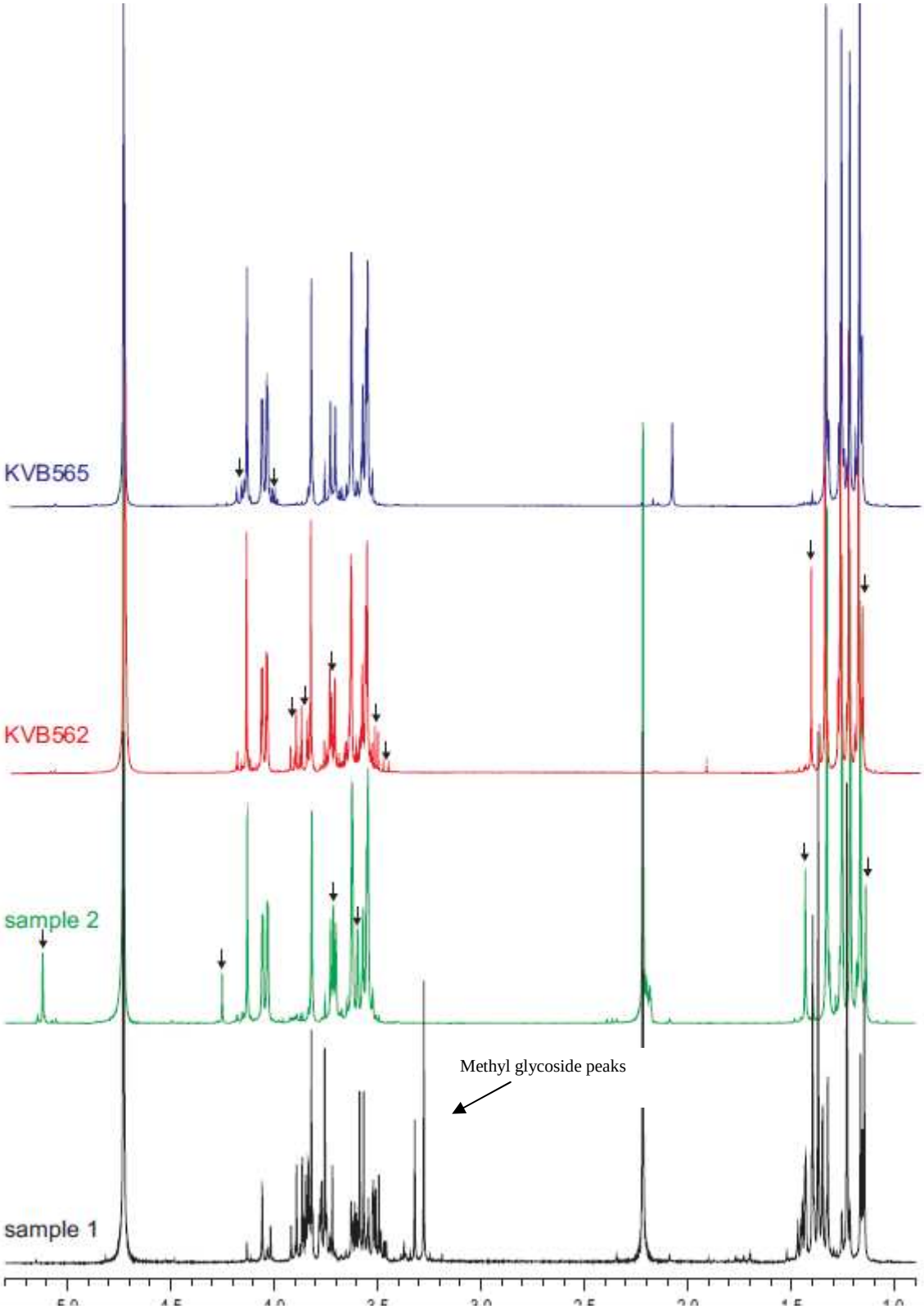
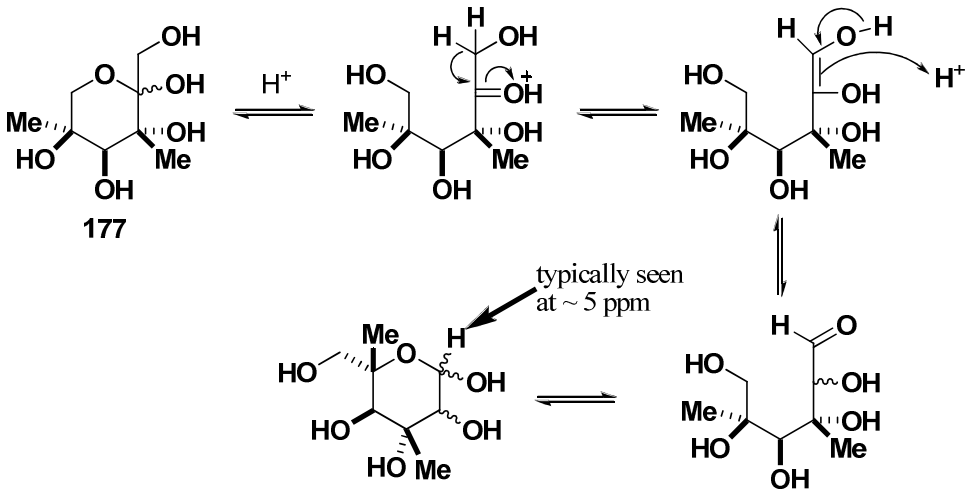


Figure 2.19: Repeated attempts at acid catalysed deprotection of isopropylidene protected di-*C*-methyl fructose derivative **179**. Anomalous peaks are indicated by arrows.

Sample one is included in this scheme to illustrate the ease with which formation of the methyl glycoside can occur; the sample had been initially submitted for ^{1}H NMR analysis in CD_3OD, as the

product was more soluble in this system than in D_2O. This has been a common feature of the deprotected di-*C*-methyl branched sugars generated in this work; D_2O cannot solubilise enough material for ^{13}C NMR analysis on a 400 MHz spectrometer so a less polar solvent was employed. CD_3OD has been used as a solvent for NMR analysis of the other free sugars generated thus far from acid catlaysed deprotection reactions without incident. However, in this instance methyl glycoside formation was rapid, as shown by the peaks in the 3.2-3.4 ppm region (**Figure 2.19**).

Sample two is the most surprising of the set, as this appears to show an *anomeric* proton at approximately 5.1 ppm (**Figure 2.19**). This peak and others associated with it are labelled with arrows. If this is an anomeric proton, it would suggest that under acid catalysis an aldose isomerisation reaction had occurred (**Scheme 2.54**).



Scheme 2.54: Suggested mechanism of acid catalysed ketose/aldose isomerisation.

The above mechanism may provide an explaination for the presence of a peak in the anomeric region, however this is contradicted by existing data for 3,5-di-*C*-methyl glucose and mannose. The singlet peak at around 4.2 ppm, which, assuming the product was 3,5-di-*C*-methyl glucose or mannose would be H4. By comparison with the ^1H NMR data given above for these compounds obtained *via* the Kiliani route, the H4 peak is expected to be found between 3.36 and 3.99 ppm, considerably lower than seen here. Also, the presence of a single anomeric proton is suspicious, as we know from the other NMR experiments that 3,5-di-*C*-methyl-L-glucose exists in a 2:1:1 ratio of

three major spin systems whilst the *manno* configured analogue is found as a 6:3:2:1 ratio of four spin systems. It is however plausible that aldose isomerisation occurred alongside epimerisation at one or more chiral centres.

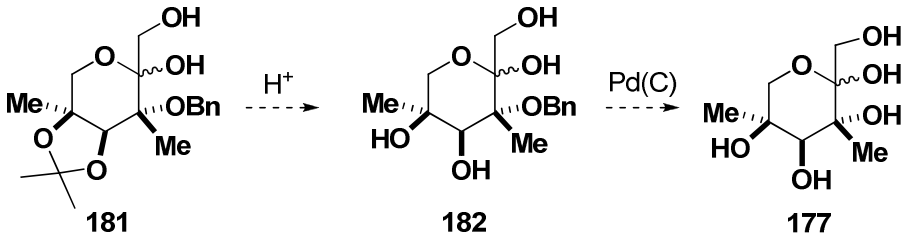
Samples KVB 562 and KVB 565 (**Figure 2.19**) were deprotections carried out on similar scales, for the same amount of time with 1 M hydrochloric acid (aq.). They only differ in their purification techniques; KVB 562 was purified by column chromatography once, whilst KVB 565 was passed through a silica column twice. It is clear that whilst a second purification on silica has resulted in a cleaner sample, the impurities in each case are different, as indicated by the labelled peaks. The impurity in KVB 562 possessess two methyl groups and five ring protons; it is therefore possible that it is an epimer of fructose. Full structural elucidation of the mixture is not possible, and attempts at separation by standard laboratory methods have been unsuccessful. The level of impurity in sample KVB 565 is low, and in one sense this may be viewed as a 'sucessful' deprotection, however, the yield obtained after repeated chromatography was poor, a meagre 22%.

Samples KVB 562 and KVB 565 have been sent to Prof. Izumori at Kagawa University, in the hope that advanced chromatographic techniques may be used to purify the material. This work is ongoing at the time of writing.

Other acids were used for this deprotection, including trifluoroacetic acid, acidic resin and the lewis acid erbium triflate,[152] but to no avail. It was hoped that by speeding up the reaction by use of stronger acids, fewer side products might be observed, however this was not found to be the case.

Finally, isopropylidene deprotection was attempted on the benzylated fructose moiety **181** (**Scheme 2.55**). It was hoped that if the isopropylidene could be removed with the benzyl group in place, the added lipophilicity of the product **182** would mean it could be purified more easily by column

chromatography. Subsequent removal of the benzyl group by hydrogenation may not necessarily require purification, eliminating the need to column a fully deprotected sugar.

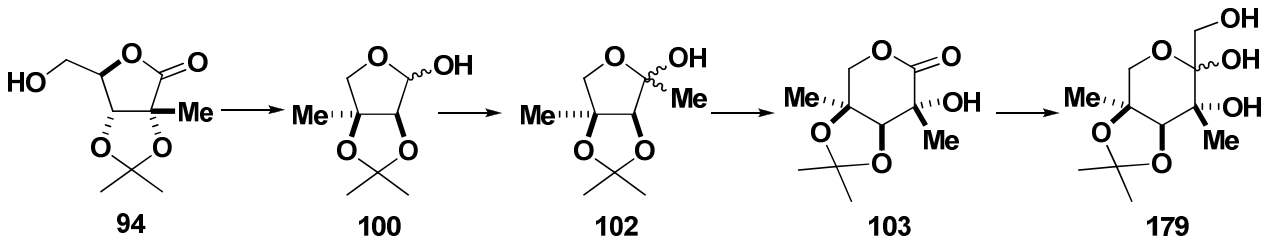


Scheme 2.55: Changing the order of deprotection.

Unfortunately successful removal of the isopropylidene group from protected fructose **181** could not be achieved and extensive decomposition was observed in every attempt. This may be linked to its reduced water solubility and the need, therefore, for mixed solvent systems and/or heating.

2.4.2 Alternative Protecting Groups

Clearly, isopropylidene removal from 3,5-di-*C*-methyl fructose was not a trivial procedure. This protecting group was introduced at the start of the synthesis of arabinonolactone **103**, by taking 2-*C*-methyl-D-ribono-1,4-lactone with acetone, copper(II) sulfate and acid. Key intermediates in the synthesis of fructose **179**, where isopropylidene removal could be achieved are shown in **Scheme 2.56**.

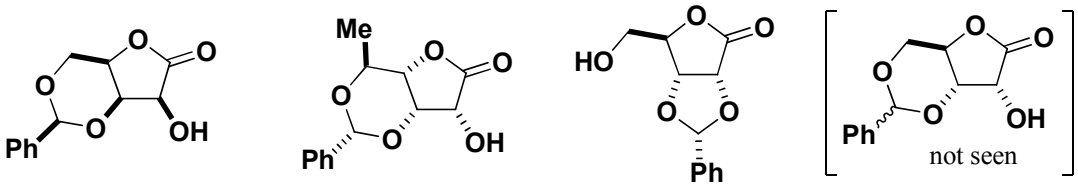


Scheme 2.56: Early introduction of the isopropylidene group.

Acidic deprotection of the isopropylidene group from lactol **100** was discussed when difficulties were encountered in the bromine oxidation. Whilst achievable, the reaction took an unacceptable length of time. Similarly, deprotection of ketone **102** was attempted prior to the Kiliani reaction and found to be low yielding. The success of the Kiliani reaction to form arabinonolactone **103** was

attributed to the stability of the isopropylidene group, preventing deprotection and therefore loss of material during the work up. Deprotection of lactone **103** will be discussed in more detail in **Section 2.5**, and was found to be more difficult than anticipated. Clearly, for the synthesis of fructose to be viable, an alternative protecting group for *cis* diols was required, to replace the isopropylidene group.

A suitable protecting group would need to be stable to mildly acidic (the synthesis uses a work up procedure with acidic resin on two occasions) and basic (sodium cyanide) conditions, as well as resistant to oxidising (sodium periodate and bromine water) and reducing (sodium/lithium borohydride) agents. It was proposed that a benzylidene acetal may be a suitable alternative to an isopropylidene group, however it is known to protect some pentono lactones in the 3,5 position, resulting in a six membered ring protecting group (**Figure 2.20**).[153,154] With ribonolactone, which is also 2,3 *cis* like the *lyxono* and *rhamnono* lactones, the product is not 3,5 protected, but 2,3 (**Figure 2.20**).[155,156,157,158] It was therefore hoped that 2-*C*-methyl-D-ribonolactone **88** could react in an analogous fashion.



lyxono *rhamnono* *ribono*

Figure 2.20: Benzylidene protection of sugar lactones.

2-*C*-Methyl-D-ribonolactone **88** was suspended in a mixture of benzaldehyde and stirred with concentrated hydrochloric acid.[159] This resulted in the formation of two products by t.l.c. analysis. The more polar component of the reaction mixture accounted for 71% of the material, and was shown to be a mixture of two co-eluting compounds by NMR analysis, in an aproximately 2:1 ratio. These were found to be a mixture of the desired 2,3 protected lactones **183** and **184** (**Figure 2.21**).

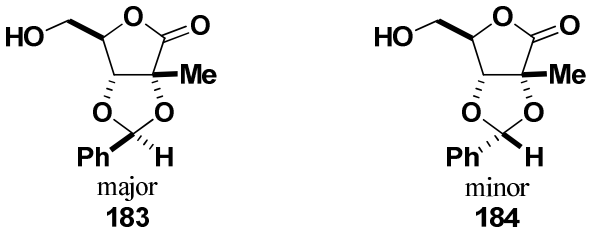


Figure 2.21: Major products from the benzylidene protection of 2-*C*-methyl-D-ribono lactone

Recrystallisation *via* slow evaporation resulted in large plate-like crystals and X-ray crystallography clearly showed the structure of the major compound to be **183** (**Figure 2.22**).

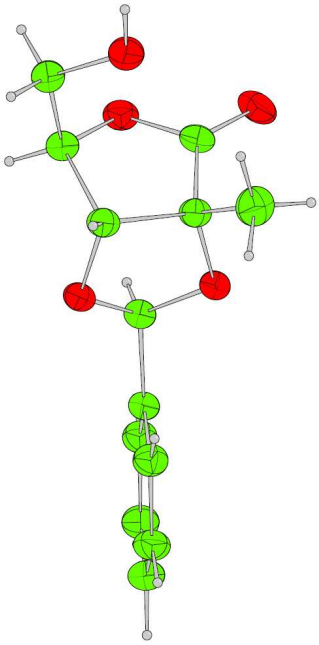
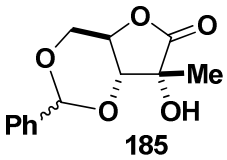


Figure 2.22: 2,3-*O*-(*S*)-Benzylidene-2-*C*-methyl-D-ribono-1,4-lactone with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

The second product visible by t.l.c. analysis of the reaction mixture accounted for the remainer of the material. Unfortunately, NMR analysis revealed this to be not one, but four compounds, all in approximately the same concentrations, which co-eluted in the solvent systems used for chromatography. HMBC analysis showed all four compounds to be 5 ring lactones, a finding supported by the existence of an IR stretch at 1785 cm^{-1}. It is possible that two of these compounds might be the 3,5 protected lactones **185**, however it has not been possible to confirm this or to identify the other two components (**Figure 2.23**).



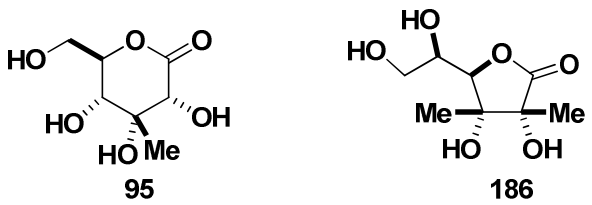
185

Figure 2.23: Proposed structures of two of the four side products formed in the benzylidene protection of 2-*C*-methyl-D-ribono lactone.

Given the large amount of novel chemistry that would be involved in taking this molecule through to 3,5-di-*C*-methyl-L-fructose **177**, which would then have to be repeated to generate the D-enantiomer, it was felt this was a rather larger project than initially intended. It was also anticipated that there may be problems with the Kiliani reaction due to the reduced hydrophilicity of the benzylidene protected lactol compared to the isopropylidene protected lactol, as it is known from previous investigations that mixed aqueous solvent systems can result in reduced yields.[160] For these reasons, this synthesis was not persued any further at this time.

2.5 Di-branched Sugar Lactones: Pyranose or Furanose?

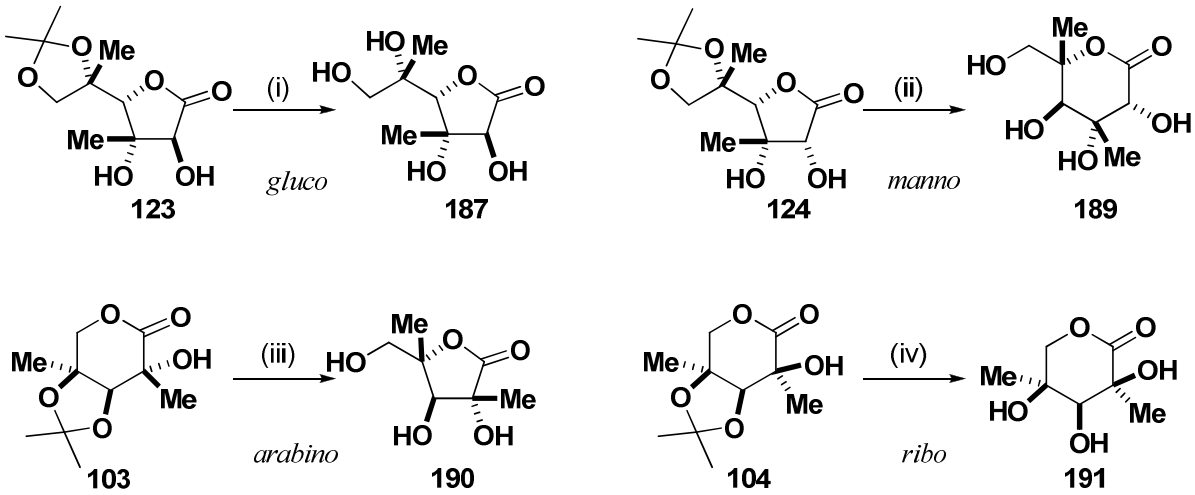
In the couse of this work, detailed NMR analysis of the di-*C*-methyl branched sugars generated has been undertaken to establish the preferred ring size of these unusual sugars in aqueous systems. It was also of interest to investigate the favoured ring size of the corresponding di-*C*-methyl sugar lactones. Even less is known about the conformational preferences of branched sugars lactones, as these are scarce in the natural world. It is known however that in general, the 5-ring form of a cyclic ester is favoured, because it more easily accommodates the bond length requirements and co-planarity of the atoms than the 6-ring geometry.[161] In the literature it had been reported that 3-*C*-methyl-D-allono lactone **95** adopts the unusual six ring structure in the solid state.[162] However, the di-*C*-methyl branched analogue, 2,3-di-*C*-methyl allono lactone **186**, exists in the 5 ring form in the solid state (**Figure 2.24**).[163] Both these compounds were synthesised *via* an isopropylidene deprotection from the 6 ring lactone; does this imply that equilibration to the 5 ring form is favoured upon crystallisation for di-*C*-methyl branched systems?



Scheme 2.24: Ring size of known branched sugar lactones

Thus, the di-*C*-methyl branched lactones generated in this work were treated with acid to remove the isopropylidene groups. Unhappily, recrystallisation of the deprotected di-*C*-methyl sugars repeatedly afforded poor quality crystals which were unsuitable for X-ray crystallographic analysis, and therfore no conclusions regarding solid state conformation can be drawn. The preferred ring size of the lactones generated in this work were investigated in the solution phase, using MeOD as the solvent for HMBC analysis, unless otherwise stated, as they were only sparingly soluble in D_2O.

Isopropylidene removal from 3,5-di-*C*-methyl-L-glucono **123** and mannono **124** lactones was effected with Dowex® acidic resin (**Scheme 2.57**). Interestingly, the ease of deprotection was the opposite of that observed for the lactols, namely that now the *gluco* lactone required a long reaction time of 96 hours, whilst the *manno* lactone deprotected in 16 hours. Deprotection of arabinonolactone **103** with acidic resin in water could not be achieved, and attempts to drive the reaction through heating resulted in complex mixtures of decomposition products. Trifluoroacetic acid was able to remove the isopropylidene group, but this also required a relatively long reaction time (30 hours). The analogous *ribo* lactone was deprotected with acidic resin in water, but high temperature was needed to achieve full deprotection. The difficulty in effecting deprotection of the arabinonolactone with acidic resin may be related to its relatively low polarity compared to similar molecules; its R_f is 0.54 (ethyl acetate/cyclohexane 1:1) compared with the *ribo* lactone **104** with an R_f of 0.21.



Reagents and Conditions: (i) Dowex® (50W X8 H^+ form), H_2O, RT, 96 h; (ii) Dowex® (50W X8 H^+ form), H_2O, RT, 16 h; (iii) $TFA_{(aq)}$, RT, 30 h; (iv) Dowex® (50W X8 H^+ form), H_2O, 50°C 3 h then 90°C 4 h.

Scheme 2.57: Deprotection of di-*C*-methyl branched sugar lactones.

The results shown by **Scheme 2.57** seem to suggest that if the C2 and C3 hydroxyls are on the same face of the molecule, the 6-ring conformation is preferred in solution. The possible conformations of the *ribo* and *arabino* stereochemistries are shown by **Fig 2.25**. In the *ribo* case, the C2 and C3 hydroxyls are both on the top face of the ring. Lack of protons around the ring means that nOe enhancements cannot be used to confirm whether the molecule adopts a chair or boat conformation. If the *ribo* molecule was to adopt a boat conformation, the flagpole positions are occupied by hydrogen and a methyl group. If it was to adopt a chair conformation, then steric clashes are avoided, both methyl groups are equatorial, and there is a possibility in certain solvents of forming a stabilising hydrogen bond between the 2,4-diaxial hydroxyl groups.

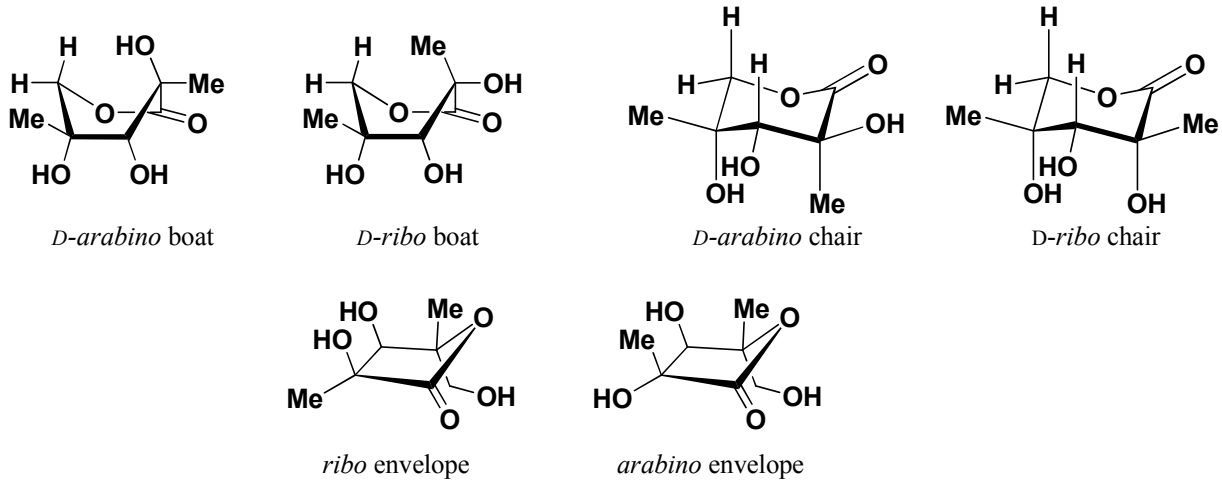
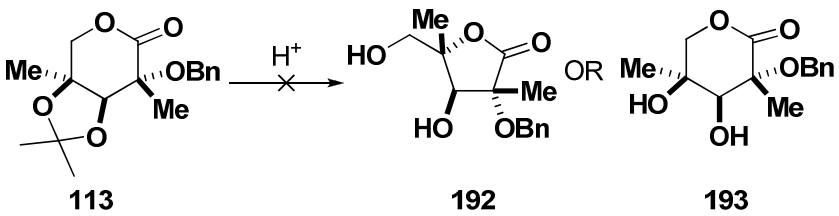


Fig 2.25: Conformational analysis of the 5 *vs.* 6 ring lactones. The D-enatiomers are drawn for clarity.

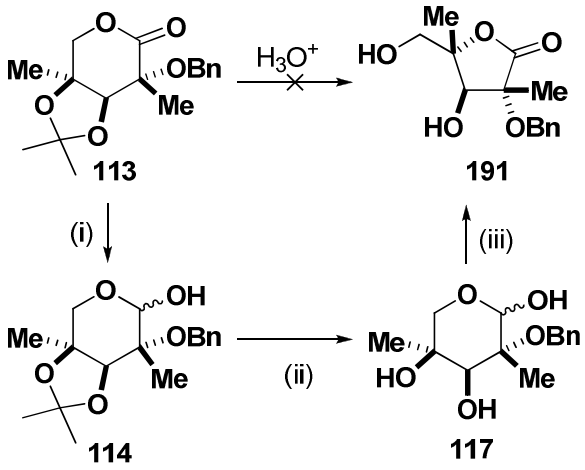
In the 5-ring conformation, the bottom face of the *ribo* molecule contains a methyl group and the hydroxyl methyl group. The possible steric clash here leads to the tentative conclusion that the *ribo* lactone adopts the 6-ring form because it can form a chair conformation within which there are no obvious unfavourable steric clashes. Comparing it with the *arabino* stereochemistry, the flagpole position in the boat conformation would now contain the hydroxymethyl group; in a chair form, there would be an axial methyl group, which is disfavoured over the equatorial position. These together support the discovery that the *arabino* form favours the 5-ring conformation.

A similar argument may also rationalise the preferred ring size of the *gluco* and *manno* lactones in solution. As a test of this theory, it would be of interest to discover whether, by increasing bulk at the 2-position, the 6-ring *arabino* lactone **193** would now be preferred. With the benzylated lactone **113** in hand, it was decided to remove the isopropylidene group and look again at the solution phase conformation (**Scheme 2.58**).



Scheme 2.58: It was not possible to directly deprotect lactone **113** with acid.

Lactone **113** was taken with a variety of different acids; deprotection was equally unsuccessful whether mineral acids, organic acids or acidic resins were employed. At low temperatures, no reaction was seen, and upon raising the temperature of these reactions, extensive decomposition of starting material was observed. Given that the benzylated lactol deprotects with relative ease (**Section 2.2**) and the lactol and lactone in this situation have almost identical R_f values, and therefore similar solubilities, the difficulty of deprotection was hard to understand. Throughout this project, it has been found that the isopropylidene group of a system can be removed more easily in the lactol than in a lactone (e.g. 3,4-*O*-isopropylidene-2,4-di-*C*-methyl-L-arabinose *vs.* 2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone). Given that in both these cases, the lactol and lactone have similar solubilities, and the difference in stereoelectronics in the lactol *vs.* lactone will be small, as the relevant functional group is β to the carbonyl, the difference in reactivity is thought to lie in the relative percentages of open chain form when in an acidic medium. Since deprotection of **113** could not be readily achieved under any of the conditions attempted, the deprotected lactol **117** was oxidised to the desired lactone using *N*-iodosuccinamide (**Scheme 2.59**). HMBC analysis of the product was able to confirm it to be the 5, rather than 6 ring form. This result appears to suggest that bulk at C2 does not determine conformation, however, the low solubility of **191** meant that NMR analyses, and therefore HMBC analysis of the ring size had to be performed in DMSO-d_6, rather than CD_3OD which had been used as a solvent for all previous lactones, thus solvents effects may skew the validity of this result.



Reagents and Conditions: (i) DIBAL, DCM, -78°C, 100 min, 89%; (ii) Dowex® (50W X8 H^+ form), H_2O, 70°C, 2 h quant; (iii) NIS, TBAI, DCM, RT, 2 h, 57%.

Scheme 2.59: Reduction/oxidation pathway to benzylated lactone **192**.

Given the size of this investigation, it would be insensible to draw any firm conclusions regarding the preferred conformation of di-*C*-methyl branched lactones in solution. However, it is clear that there can be a preference for the less common 6 ring form, although the circumstances that lead to this preference are not yet apparent. Also, for future investigations, it is worth noting that isopropylidene removal can be far from trivial in such cases, and an alternative protecting group should be considered for heavily branched systems.

2.6 Conclusions and Future Work

The work in this project on the synthesis of di-*C*-methyl branched sugars has resulted in the synthesis of several novel branched sugars and sugar lactones. The synthesis of 2-*C*-methyl-D-ribono lactone **88** from D-glucose has been shown to be readily scaleable, making it a valuable branched carbohydrate starting material. A short and expedient synthesis of both enantiomers of 2,4-di-*C*-methyl arabinose *via* the corresponding arabinonolactone (the synthetic utility of which as a building block will be explored in **Chapter 3**) has been established. Several possible routes from 2,4-di-*C*-methyl-L-arabinono-1,5-lactone **103** to 3,5-di-*C*-methyl-L-glucose **97** have been explored, the most successful of which utilises the Kiliani reaction, and has resulted in the synthesis of both

enantiomers of 3,5-di-*C*-glucose and mannose. The synthesis of 3,5-di-*C*-methyl-L-fructose was investigated.

GP inhibition studies have shown that 3,5-di-*C*-methyl-D-glucose **1** is a weak glycogen phosphorylase inhibitor, providing support to the initial assumption based on molecular modelling that the added lipophilicity could increase inhibition. With imino sugars it has been found that the corresponding L-enantiomers also act as inhibitors, albeit in a non-competitive fashion. Thus testing of 3,5-di-*C*-methyl-L-glucose for GP inhibition would be of interest.

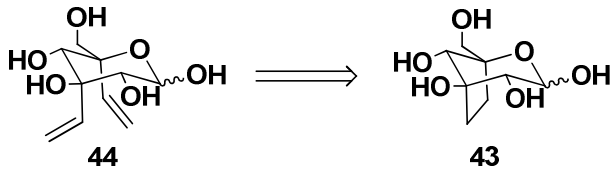
Detailed NMR analyses of the di-*C*-methyl sugars obtained in this work have shown that the presence of methyl branch points appears to favour the furanose configuration and in some cases this effect can be quite pronounced. More work will be needed before any similar conclusions can be reached for the corresponding di-*C*-methyl sugar lactones.

Given the difficulties the use of an isopropylidene protecting group presented in the final stages of many of these investigations, use of alternative protecting group could be explored. In the 3,5-di-*C*-methyl fructose synthesis, a benzylidene group was put forward as a viable alternative. Other potential protecting groups for the *cis* 2,3 diol in 2-*C*-methyl ribono lactone **88** include a cyclopentylidene ketal, methylene, ethylidene and 2,2,2-trichloroethylidene acetals. Orthoesters may also present a suitable alternative, but the acid lability of each case would have to be carefully assessed.

Contaminated samples of 3,5-di-*C*-methyl-L-fructose **177** have been sent to Prof. Izumori at Kagawa University, in the hope that advanced chromatographic techniques may be used to efficiently clean the material, allowing for initial investigations into the biotechnological aldose isomerase process, which would hopefully generate 3,5-di-*C*-methyl-L-glucose **97** sterospecifically.

Unfortunately, it was not possible to produce 3,5-di-*C*-methyl fructose or sufficient quantities of the 3,5-di-*C*-methyl glucose or mannose to test the taste characteristics of these compounds. However, with the methodology in place, and a revised synthesis of the fructose proposed, it may be possible to explore these new foodstuffs in the future.

3,5-Di-*C*-methyl-D-glucose was synthesised as a test system for the methodology of introducing two branch points into a sugar, with a view to extending this idea to the introduction of vinyl groups which could be linked to form an ethyl bridge (**Scheme 2.60**).



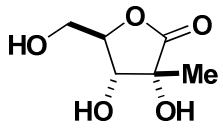
Scheme 2.60: Future work could look at the synthesis of **43**, to see if the added rigidity enhances GP inhibition.

2.7 General Experimental

Tetrahydrofuran was purchased dry from the Aldrich chemical company in sure-seal™ bottles or distilled over calcium hydride. Pyridine and *N*,*N*-dimethylformamide were purchased dry from the Aldrich chemical company in sure-seal™ bottles. Water was distilled. All other solvents were used as supplied (analytical or HPLC grade) without further purification, unless stated otherwise. Reactions performed under an atmosphere of argon or hydrogen were maintained by an inflated balloon. Reagents were used as supplied, without prior purification. Thin-layer chromatography (t.l.c.) was carried out on aluminium sheets coated with $60F_{254}$ silica from Merck. Sheets were visualised using a spray of 0.2% *w/v* cerium (IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid or basic potassium permanganate solution and subsequent heating. Flash column chromatography was carried out using Sorbasil 40/60 silica from VWR. Melting points (m.p.) were measured on a Kofler hot-block apparatus and are uncorrected. Proton nuclear magnetic resonance

spectra (δ_H) were recorded on a Bruker AV400 (400 MHz) or a Bruker AV500 (500 MHz) spectrometer. ^{13}C NMR spectra (δ_C) were recorded on a Bruker AV400 (100 MHz) or a Bruker AV500 (125 MHz) spectrometer in the deuterated solvent stated. D_2O spectra are referenced to either acetone or acetonitrile. Chemical shifts (δ) are denoted in ppm and coupling constants (*J*) in Hz. Residual signals from the solvents were used as an internal reference. ^1H resonances were assigned using COSY and HMQC spectra. ^{13}C resonances were assigned using DEPT, HSQC, HMQC and HMBC spectra as appropriate. The following abbreviations are used to denote multiplicities: s, singlet; d, doublet; dd, double doublet; ddd, double-double-doublet; t, triplet; q, quartet; dt, double triplet; m, multiplet; br, broad; a, apparent. Where a compound exists as a mixture of anomers, **A** is used to denote the major component, **B** the minor, unless otherwise stated. Infrared spectra were recorded on a Bruker Tensor 27 FT IR spectrophotometer using thin films on NaCl or Ge plates and peaks are given in cm^{-1}. Only characteristic peaks are quoted. Low resolution mass spectra (LRMS) were recorded on a Fisons Platform (ESI) spectrometer or a Micromass VG Autospec 500 OAT (CI(NH_3)) spectrometer. High resolution mass spectra (HRMS) were recorded on a Micromass LCT (ESI) spectrometer, a Micromass VG Autospec 500 OAT (CI(NH_3)) spectrometer or a Micromass GCT (FI) spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm; concentrations (*c*) are quoted in g/100 mL. Elemental analyses were performed by the microanalysis service of the Inorganic Chemistry Laboratory (Oxford).

2-*C*-Methyl-D-ribono-1,4-lactone 88



Dimethylamine (33% in absolute ethanol, 159 mL, 0.90 mol) was added dropwise over 30 minutes to a solution of D-glucose (157 g, 0.87 mol) in industrial methylated spirits (235 mL) and acetic acid (50.3 mL, 0.87 mol) in a 2 L jacketed reactor with JT* 17°C [this step was exothermic; JT was set to 17°C to ensure the internal temperature remained at 20°C]. The reaction mixture was then heated (JT 75°C) over 0.5 h, cooled to 55°C over 30 minutes and stirred at 55°C for 90 minutes. T.l.c. analysis (14:3:1:1:1, EtOH:H_2O:Py:AcOH:^{n}BuOH) showed the formation of a single major product (R_f 0.39) **2**; no glucose (R_f 0.79) remained. The solution was concentrated at 50 mbar, JT 50°C, until bubbling ceased (90 minutes) and the brown oil was diluted with distilled water (330 mL). The reactor was evacuated and filled with nitrogen and JT was adjusted to 0°C. Calcium oxide (73.3 g, 1.31 mol) was added in 4 portions at 10 minute intervals [the reaction was exothermic; the internal temperature rose by approximately 5°C after each addition]. Under a nitrogen atmosphere (maintained by inflated balloon), the reaction mixture was heated to JT 25°C over 20 minutes and then heated to JT 40°C over 18 hours and stirred at JT 40°C for a further 4 hours. T.l.c. analysis (14:3:1:1:1, EtOH:H_2O:Py:AcOH:^{n}BuOH) after this time indicated the absence of the Amadori ketose, and the formation of a product (R_f 0.00) **88**. JT was lowered to 0°C, and 96% sulfuric acid (88 mL, 1.65 mol) was added over 90 minutes, keeping JT at 0°C. The pH was checked at this point to ensure it was in the range 2.4 - 2.7. Addition of extra sulfuric acid was occasionally necessary. Once addition was complete, JT was set to rise to 45°C over 30 minutes and the reaction mixture stirred at JT 45°C for 12 h. The mixture was then allowed to cool to room temperature before filtration; the reactor and filter cake were rinsed with distilled water (500 mL). T.l.c. analysis

* JT refers to jacket temperature of reactor vessels, controlled by Julabo heater/chiller units.

(14:3:1:1:1, EtOH:H_2O:Py:AcOH:^{n}BuOH) of the filtrate revealed a major product (R_f 0.92). The filtrate (approx 1 L) was concentrated at 50°C to give a brown residue which was re-dissolved in a mixture of acetone (800 mL) and water (80 mL) and Fullers Earth (90 g) was added. The reaction mixture was refluxed for 0.5 h and the organic phase decanted and filtered. The solid remaining in the flask was then extracted a further 3 times with 10% aqueous acetone (275 mL x 3), refluxing for 5 minutes and decanting the acetone each time. The combined filtrates were concentrated under reduced pressure at 40°C to give 109 g of solid brown crude. Recrystallisation from hot acetone gave the lactone **88** as a cream coloured solid (37.5 g, 27%).

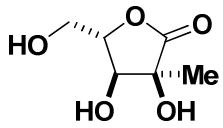
m.p. 157-158°C; $[\alpha]_D^{20}$ +87.6 (*c*, 0.86 water);{Lit.[164] m.p. 158-159°C; $[\alpha]_D^{13.5}$ +87.5 (*c*, 0.76 water)}; δ_H (CD_3OD, 400 MHz): 1.43 (3H, s, CH_3-2), 3.73 (1H, dd, H-5 $J_{5,4}$ 4.5, $J_{5,5'}$ 12.8), 3.93 (1H, d, H-3 $J_{3,4}$ 7.8), 3.97 (1H, dd, H-5', $J_{5',4}$ 2.4, $J_{5',5}$ 12.8), 4.32 (1H, ddd, H-4 $J_{4,5'}$ 2.4, $J_{4,5}$ 4.5, $J_{4,3}$ 7.8) δ_C (CD_3OD, 100 MHz): 20.0 (CH_3-2), 60.0 (C-5), 72.6 (C-2), 72.6 (C-3), 83.4 (C4), 177.0 (C-1).

*'JT' refers to oil jacket temperature of reactor vessels, as controlled by Julabo heater/chiller units.

Scale up procedure - 6 L jacketed reactor for 1.25 kg glucose:

D-Glucose (1.26 Kg, 7.00 mol); dimethylamine (33% in absolute ethanol, 1.28 L, 7.21 mol); industrial methylated spirits (1.89 L); acetic acid (405 mL, 7.01 mol.); water (2.66 L); calcium oxide (589 g, 10.5 mol); 96% sulfuric acid (710 mL, 13.3 mol). The procedure was carried out as above; however additional purification, in the form of exhaustive treatment with aqueous acetone/Fuller's Earth and a subsequent reflux with Norit activated charcoal in acetone, was required before crystallization was possible. The total mass of 2-*C*-methyl-D-ribonolactone **88** obtained was 229 g (20%).

2-*C*-Methyl-L-ribono-1,4-lactone 132



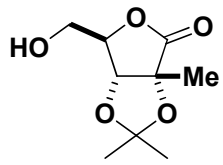
The procedure was carried out as previously described above for the preparation of 2-*C*-methyl-L-ribonolactone **132** using the following amounts:

L-Glucose (42.4 g, 0.235 mol); dimethylamine (33% in absolute ethanol, 43.2 mL, 0.243 mol); industrial methylated spirits (64 mL); acetic acid (13.6 mL, 0.235 mol); water (89 mL); calcium oxide (19.8 g, 0.353 mol); 96% sulfuric acid (23.9 mL, 0.424 mol).

After direct crystallization of lactone 3 (5.37 g), the mother liquor was concentrated, and the residue purified by column chromatography (40-60 petrol/acetone 4:1→1:1) and crystallization to give further product (4.20 g). The total yield of 2-*C*-methyl-L-ribono-1,4-lactone **132** was 9.57 g (25%).

m.p. 158-159°C; $[\alpha]_D^{21}$ -87.6 (*c*, 0.8 water); {lit. m.p. 160-162°C; $[\alpha]_D^{21.5}$ -87.7 (*c*, 0.6 water)}; the ^1H and ^{13}C NMR spectra were identical to those of the enantiomer **88** above.

2,3-*O*-Isopropylidene-2-*C*-methyl-D-ribono-1,4-lactone 94

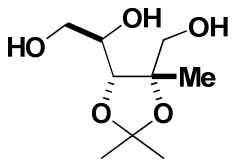


2-*C*-Methyl-D-ribono-1,4-lactone **88** (10.8 g, 66.5 mmol) was dissolved in acetone (200 mL) and stirred at room temperature with anhydrous copper(II) sulfate (21.0 g, 131.3 mmol) and concentrated sulfuric acid (0.4 mL) for 96 hours. T.l.c. analysis (ethyl acetate) after this time showed the formation of a single product (R_f 0.78) and complete consumption of starting material (R_f 0.35). The reaction mixture was neutralised with sodium carbonate(s), filtered through a pad of Celite® and concentrated under reduced pressure. The crude was purified by flash column

chromatography (ethyl acetate/cyclohexane, 1:1) to yield 2,3-*O*-isopropylidene-2-*C*-methyl-D-ribono-1,4-lactone **94** (13.3 g, quant.) as a pale yellow solid.

HRMS (ESI+ve): found 225.0733 $[M+Na]^+$; $C_9H_{14}Na_1O_5$ requires 225.0733; m.p. 54-56°C; $[\alpha]_D^{22}$ -36.3 (*c*, 1.79 acetone) {Lit.[165] m.p. 52-54°C; $[\alpha]_D^{19}$ -35.6 (*c*, 1.4 acetone)}; ν_{max} (thin film): 3442 (s, br, OH), 1774 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.43 (3H, s, $C(CH_3)_2$), 1.46 (3H, s, $C(CH_3)_2$), 1.66 (3H, s, CH_3-2), 2.05 (1H, s, OH), 3.84 (1H, d, H5, $J_{5,5'}$ 12.4), 4.00 (1H, d, H5', $J_{5',5}$ 12.4), 4.53-4.56 (2H, m, H3, H4); δ_C ($CDCl_3$, 100.6 MHz): 19.9 (CH_3-2), 26.7 ($C(\mathbf{C}H_3)_2$), 26.8 ($C(\mathbf{C}H_3)_2$), 62.3 (C5), 82.6, 83.2 (C3, C4), 82.8 (C2), 113.0 ($\mathbf{C}(CH_3)_2$), 177.8 (C=O); LRMS (ESI-ve): 261 ($[M+CH_3CO_2]^-$, 100%).

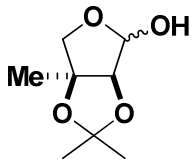
2,3-*O*-Isopropylidene-2-*C*-methyl-D-ribitol 99



2,3-*O*-Isopropylidene-2-*C*-methyl-D-ribono-1,4-lactone **94** (1.12 g, 5.71 mmol) was dissolved in tetrahydrofuran (10 mL) and lithium borohydride solution (2 M in tetrahydrofuran, 1.4 mL) was added dropwise whilst stirring. [For reactions performed on over 1.5 g of starting material, the reaction mixture was cooled to 0°C before addition of reagent.] T.l.c. analysis (ethyl acetate) after 1 hour showed the formation of a single product (R_f 0.43) and starting material (R_f 0.75); a further 0.3 mL of lithium borohydride was added. T.l.c. analysis (ethyl acetate) after a further 30 minutes showed complete consumption of starting material. The reaction mixture was neutralised with Dowex® (50W X8 H^+ form), filtered, and concentrated under reduced pressure to afford 2,3-*O*-isopropylidene-2-*C*-methyl-D-ribitol **99** (1.32 g, quant.) as a colourless oil, which was used without further purification. A small sample was purified by flash column chromatography (ethyl acetate/cyclohexane, 2:1) for the purpose of characterisation.

HRMS (ESI+ve): found 243.1046 $[M+Na]^+$; $C_9H_{18}Na_1O_5$ requires 243.1048; $[\alpha]_D^{21}$ -11.4 (*c*, 2.33 ethanol); ν_{max} (thin film): 3385 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.34 (3H, s, C(**C**$H_3)_{2)}$, 1.36 (3H, s, C(**C**$H_3)_2$), 1.42 (3H, s, CH_3-2), 2.47 (1H, s, OH-1), 3.42 (1H, a-d, H1, *J* 9.6), 3.61-3.68 (1H, m, H5), 3.74 (2H, a-d, H1', H3, *J* 9.0), 3.84-3.91 (2H, m, H4, H5'), 4.36 (1H, d, OH-4, $J_{OH,4}$ 3.3); δ_C ($CDCl_3$, 100 MHz): 23.9 (C(**C**$H_3)_2$), 26.6 (CH_3-2), 28.4 (C(**C**$H_3)_2$), 64.9, 65.1 (C1, C5), 70.5 (C4), 81.8 (C3), 82.2 (C2) 108.9 (**C**$(CH_3)_2$); LRMS (ESI-ve): 205 ($[M-H]^-$, 100%).

2,3-*O*-Isopropylidene-3-*C*-methyl-L-erythrose 100

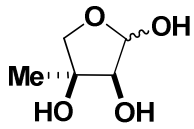


2,3-*O*-Isopropylidene-2-*C*-methyl-D-ribitol, **99** (1.78 g, 8.64 mmol) was dissolved in a mixture of water (10 mL) and methanol (30 mL) and sodium periodate (2.22 g, 10.37 mmol) was added whilst stirring. A thick, white precipitate formed, and t.l.c. analysis (ethyl acetate) after 2 hours showed complete consumption of the starting material (R_f 0.43) and the formation of a single product (R_f 0.78). The reaction mixture was concentrated under reduced pressure, preadsorbed on silica and purified by silica plug (ethyl acetate), to yield 2,3-*O*-isopropylidene-3-*C*-methyl-L-erythrose **100** (1.57 g, quant.) as a colourless oil, in a 5:1 ratio of anomers.

HRMS (ESI+ve): found 173.0808 $[M+Na]^+$; $C_8H_{13}Na_1O_4$ requires 173.0813; $[\alpha]_D^{20}$ +50.5 (*c*, 1.43 chloroform); ν_{max} (thin film): 3423 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.38, (3H, s, C(**C**$H_3)_2^{\mathbf{A}}$), 1.44 (6H, s, C(**C**$H_3)_2^{\mathbf{A}}$, C(**C**$H_3)_2^{\mathbf{B}}$), 1.47 (3H, s, CH_3-$3^{\mathbf{B}}$), 1.52 (3H, s, C(**C**$H_3)_2^{\mathbf{B}}$), 1.54 (3H, s, CH_3-$3^{\mathbf{A}}$), 2.93 (1H, d, $OH^{\mathbf{A}}$, $J_{OH,1}$ 2.4), 3.32 (1H, d, $H4^{\mathbf{B}}$, $J_{4,4'}$ 10.3), 3.88 (1H, d, $H4^{\mathbf{A}}$, $J_{4,4'}$ 9.7), 3.89 (1H, d, $H4'^{\mathbf{B}}$, $J_{4',4}$ 10.3), 3.99 (1H, d, $H4'^{\mathbf{A}}$, $J_{4',4}$ 9.7), 3.99 (1H, d, $OH^{\mathbf{B}}$, $J_{OH,1}$ 11.3), 4.14 (1H, d, $H2^{\mathbf{B}}$, $J_{2,1}$ 3.3), 4.21 (1H, s, $H2^{\mathbf{A}}$), 5.03 (1H, dd, $H1^{\mathbf{B}}$, $J_{1,2}$ 3.3, $J_{1,OH}$ 11.8), 5.41 (1H, d, $H1^{\mathbf{A}}$, $J_{1,OH}$ 2.3); δ_C ($CDCl_3$, 100 MHz): 22.9 (CH_3-$3^{\mathbf{B}}$), 23.3 (CH_3-$3^{\mathbf{A}}$), 27.5 (C(**C**$H_3)_2^{\mathbf{A}}$), 27.5 (C(**C**$H_3)_2^{\mathbf{B}}$), 27.6

($C(\mathbf{C}H_3)_2^{\mathbf{A}}$), 27.8 ($C(\mathbf{C}H_3)_2^{\mathbf{B}}$), 73.8 ($C4^{\mathbf{B}}$), 77.8 ($C4^{\mathbf{A}}$), 84.4 ($C2^{\mathbf{B}}$), 88.3 ($C3^{\mathbf{B}}$), 88.8 ($C3^{\mathbf{A}}$) 90.2 ($C2^{\mathbf{A}}$), 97.9 ($C1^{\mathbf{B}}$), 102.6 ($C1^{\mathbf{A}}$), 112.6 ($\mathbf{C}(CH_3)_2^{\mathbf{A}}$), 114.0 ($\mathbf{C}(CH_3)_2^{\mathbf{B}}$). LRMS (ESI-ve): 203 ($[M+MeO]^-$, 100%).

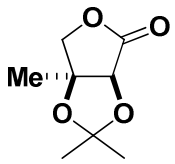
3-*C*-Methyl-L-erythrose 98



2,3-*O*-Isopropylidene-3-*C*-methyl-L-erythrose **100** (1.60 g, 9.20 mmol) was dissolved in water (20 mL) and stirred with Dowex® (50W X8 H^+ form, 1 g) at room temperature. T.l.c. analysis (ethyl acetate) after 24 hours revealed the presence of starting material (R_f 0.81) and the formation of a single product (R_f 0.27); additional resin (500 mg) added and the mixture heated to 40°C for 48 hours, then cooled to room temperature and stired for a further 96 hours, after which time t.l.c. analysis showed the complete consumption of the starting material. The resin was filtered away and the solvent concentrated under reduced pressure to yield 3-*C*-methyl-L-erythrose **98** (1.23 g, quant.) as a single anomer, as a colourless oil.

HRMS (ESI+ve): found 157.0471 $[M+Na]^+$; $C_5H_{10}Na_1O_4$ requires 157.0473; $[\alpha]_D^{19}$ +11.4 (*c*, 2.41 ethanol); ν_{max} (thin film): 3386 (s, br, OH); δ_H (CH_3OD, 400 MHz): 1.29, (3H, s, CH_3-3), 3.57 (1H, d, H2 *J* 4.1), 3.68 (1H, d, H4 $J_{4,4'}$ 9.1), 3.74 (1H, d, H4' $J_{4',4}$ 9.4), 5.14-5.16 (1H, m, H1); δ_C (CH_3OD, 100 MHz): 22.3 (CH_3-3), 77.2 (C4), 82.7 (C2), 98.0 (C3), 103.8 (C1); LRMS (ESI-ve): 193 ($[M+CH_3CO_2]^-$, 100%).

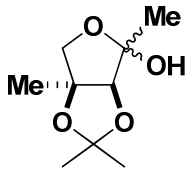
2,3-*O*-Isopropylidene-3-*C*-methyl-L-erythrono-1,4-lactone 101



2,3-*O*-Isopropylidene-3-*C*-methyl-L-erythrose **100** (3.34 g, 19.17 mmol) was dissolved in water (70 mL) and cooled to 0°C. The flask was wrapped in foil and barium carbonate (5.67 g, 28.8 mmol) was added, followed by dropwise addition of bromine (1.48 mL, 28.8 mmol). The solution was allowed to warm to room temperature, and stirred for 18 hours, after which time, t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) revealed the formation of a single product (R_f 0.60) and the complete consumption of starting material (R_f 0.51). The reaction mixture was quenched with saturated sodium thiosulfate solution (5 mL), and the product extracted with ethyl acetate (200 mL). The solvent was concentrated under reduced pressure to afford 2,3-*O*-isopropylidene-3-*C*-methyl-L-erythrono-1,4-lactone **101** (3.05 g, 93%) as a white crystalline solid, which was used without further purification.

Found C 55.78; H 6.99; $C_8H_{12}O_4$ requires C 55.81; H 7.02; HRMS (ESI+ve): found 195.0628 $[M+Na]^+$; $C_8H_{12}Na_1O_4$ requires 195.0624; $[\alpha]_D^{19}$ +103.6 (*c*, 1.26 chloroform); m.p. 82-83°C; ν_{max} (thin film): 1775 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.42 (3H, s, $C(CH_3)_2$), 1.51 (3H, s, $C(CH_3)_2$), 1.55 (3H, s, CH_3-3), 4.14 (1H, d, H4 *J* 10.4 Hz), 4.44-4.46 (2H, m, H2 & H4'); δ_C ($CDCl_3$, 100 MHz): 22.1 (CH_3-3), 27.9 ($C(\mathbf{C}H_3)_2$), 28.7 ($C(\mathbf{C}H_3)_2$), 75.8 (C4), 79.6 (C2), 83.7 (C3), 114.2 ($\mathbf{C}(CH_3)_2$), 174.8 (C1). LRMS (ESI-ve): 189 ($[M+OH]^-$, 100%).

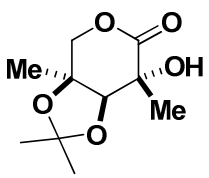
1-Deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribulose 102



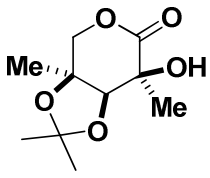
2,3-*O*-Isopropylidene-3-*C*-methyl-L-erythrono-1,4-lactone **101** (3.29 g, 19.10 mmol) was dissolved in tetrahydrofuran (33 mL) and the solution cooled to -78°C. Methylmagnesium bromide solution (3 M in ether, 7.0 mL, 21.0 mmol) was added dropwise to the lactone and the mixture stirred under argon. IR analysis after 45 minutes revealed the complete consumption of starting material by the absence of a peak at 1775 cm^{-1}. The reaction mixture was quenched with water (15 mL) and the aqueous extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried (magnesium sulfate), filtered, and concentrated under reduced pressure to afford 1-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribulose **102** (3.14 g, 87%) as a cream coloured solid in a 3:1 ratio of anomers; this was used without further purification.

HRMS (ESI-ve): found 187.0965 $[M-H]^-$; $C_9H_{15}O_4$ requires 187.0966; m.p. 54-56°C; $[\alpha]_D^{25}$ +55.2 (*c*, 1.60 chloroform); ν_{max} (thin film): 3412 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.40 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.44 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.46 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.52 (3H, s, CH_3-$4^{\mathbf{B}}$), 1.54 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.56 (3H, s, CH_3-$4^{\mathbf{A}}$), 2.29 (1H, s, $OH^{\mathbf{A}}$), 3.40 (1H, d, $H5^{\mathbf{B}}$, $J_{5,5'}$ 10.2), 3.82 (1H, d, $H5^{\mathbf{A}}$, $J_{5,5'}$ 9.6), 3.89 (1H, d, $H5'^{\mathbf{B}}$, $J_{5',5}$ 10.2), 3.91 (1H, d, $H5'^{\mathbf{A}}$, $J_{5',5}$ 9.6), 3.92 (1H, s, $H3^{\mathbf{B}}$), 4.04 (1H, s, $H3^{\mathbf{A}}$), 4.17 (1H, s, $OH^{\mathbf{B}}$); δ_C ($CDCl_3$, 100 MHz): 21.9 ($C1^{\mathbf{B}}$), 22.5 ($C1^{\mathbf{A}}$), 23.6 (CH_3-$4^{\mathbf{A}}$), 23.8 (CH_3-$4^{\mathbf{B}}$), 27.4 ($C(\mathbf{C}H_3)_2^{\mathbf{A}}$), 27.5 ($C(\mathbf{C}H_3)_2^{\mathbf{B}}$), 74.8 ($C5^{\mathbf{B}}$), 77.2 ($C5^{\mathbf{A}}$), 87.9 ($C3^{\mathbf{B}}$), 88.5 ($C4^{\mathbf{B}}$), 89.6 ($C4^{\mathbf{A}}$), 90.2 ($C3^{\mathbf{A}}$), 103.4 ($C2^{\mathbf{B}}$), 106.6 ($C2^{\mathbf{A}}$), 112.4 ($\mathbf{C}(CH_3)_2^{\mathbf{A}}$), 113.5 ($\mathbf{C}(CH_3)_2^{\mathbf{B}}$) LRMS (ESI-ve): 187 ($[M-H]^-$, 100%).

2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone 103, 2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone 104



arabino



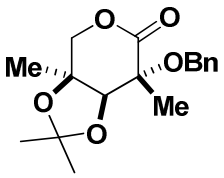
ribo

1-Deoxy-3,-4-*O*-isopropylidene-4-*C*-methyl-L-ribulose **102** (5.00 g, 26.6 mmol) was dissolved in water (60 mL) and stirred with sodium cyanide (1.70 g, 34.9 mmol) at room temperature for 24 hours, followed by heating to 85°C for 48 hours. The solution was then allowed to cool to room temperature before being passed through a column of Amberlite®-IR120 (H^+ resin) eluted with water (1 L). T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) of this solution revealed the presence of one major product (R_f 0.54) and a minor product (R_f 0.21). Evaporation of the solvents under reduced pressure afforded a cream solid (5.07 g). Recrystallisation from ethyl acetate (50 mL) gave 1.88 g of the arabinonolactone **103** as colourless needles. Concentration of the mother liquor and repeated recrystallisation afforded a further 0.993 g of **103**. The remaining liquor as concentrated under reduced pressure and purified by flash column chromatography (ethyl acetate/cyclohexane, 1:6), to give arabinonolactone **103** (combined mass 4.42 g, 77%; R_f 0.54), and ribono lactone **104** (201 mg, 4%; R_f 0.21) as pale yellow oil.

*Data for **103**:* Found: C, 55.84; H, 7.54; $C_{10}H_{16}O_5$ requires: C, 55.55; H, 7.46; HRMS (ESI+ve): found 239.0890 $[M+Na]^+$; $C_{10}H_{16}O_5Na$ requires 239.0889; m.p. 112-118°C; $[\alpha]_D^{23}$ +131.8 (*c*, 1.50 chloroform); ν_{max} (thin film): 3456 (s, br, OH), 1731 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.38, 1.40 (2 x 3H, s, $C(CH_3)_2$), 1.43 (3H, s, CH_3-4), 1.58 (3H, s, CH_3-2), 2.81 (1H, s, OH), 4.01 (1H, s, H3), 4.13 (1H, d, H5, $J_{5,5'}$ 11.4), 4.62 (1H, d, H5', $J_{5',5}$ 11.4); δ_C ($CDCl_3$, 100 MHz): 23.0 (CH_3-2), 24.7 (CH_3-4), 26.7 ($C(\mathbf{C}H_3)_2$), 27.4 ($C(\mathbf{C}H_3)_2$), 72.7 (C2), 74.1 (C5), 79.3 (C4), 84.7 (C3), 110.1 ($\mathbf{C}(CH_3)_2$), 171.5 (C1); LRMS (ESI-ve): 187 (100%), 215 ($[M-H]^-$, 85%).

*Data for **104**:* HRMS (ESI+ve): found 239.0890 $[M+Na]^+$; $C_{10}H_{16}Na_1O_5$ requires 239.0889; $[\alpha]_D^{22}$ +127.3 (*c*, 0.90 chloroform); ν_{max} (thin film): 3463 (s, br, OH), 1753 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.43 (6H, s, $C(CH_3)_2$), 1.46 (3H, s, CH_3-4), 1.49 (3H, s, CH_3-4), 3.54 (1H, s, OH), 4.15 (1H, s, H3), 4.18-4.31 (2H, a-dd, H6, H6', *J* 0.7, *J* 13.0); δ_C ($CDCl_3$, 100 MHz): 19.8 (CH_3-2), 24.5 (CH_3-4), 26.5 ($C(\mathbf{C}H_3)_2$), 27.3 ($C(\mathbf{C}H_3)_2$), 71.8 (C2), 73.9 (C5), 77.5 (C4), 85.7 (C3), 110.0 ($\mathbf{C}(CH_3)_2$), 174.1 (C1).

2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone 113

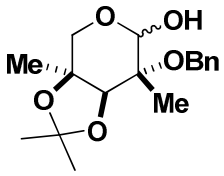


2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** (406 mg, 1.88 mmol) was dissolved in *N*,*N*-dimethylformamide (6 mL) with 4Å molecular sieves (10 mg). Benzyl bromide (0.27 mL, 2.26 mmol) and sodium hydride (60% suspension in mineral oil; 90 mg, 2.26 mmol) were added and the solution stirred at room temperature for 2 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 2:1) after this time revealed complete consumption of starting material (R_f 0.29) and the formation of a single product (R_f 0.63). The reaction mixture was filtered through Celite®, eluted with dichloromethane, and the solvents concentrated under reduced pressure. The crude was dissolved in dichloromethane (15 mL) and washed with water (3 x 15 mL). The organic phase was dried (magnesium sulfate), filtered and the solvents concentrated under reduced pressure to give the crude lactone. This was purified by elution through a silica plug (ethyl acetate/cyclohexane, 1:10) to afford 2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone, **113** (512 mg, 89%) as a white solid.

Found: C, 66.51; H, 7.22; $C_{17}H_{22}O_5$ requires: C, 66.65; H, 7.24; HRMS (ESI+ve): found 329.1357 $[M+Na]^+$; $C_{17}H_{22}Na_1O_5$ requires 329.1359; m.p. 61-64°C; $[\alpha]_D^{23}$ +58.8 (*c*, 0.82 chloroform); ν_{max}

(thin film): 1750 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.39 (3H, s, CH_3-4), 1.42 (6H, s, $C(CH_3)_2$), 1.65 (3H, s, CH_3-2), 4.11 (1H, s, H3), 4.16 (1H, d, H5, $J_{5,5'}$ 11.7), 4.32 (1H, d, OCH_2Ph, J_{gem} 11.0), 4.48 (1H, d, H5', $J_{5',5}$ 11.7), 4.68 (1H, d, OCH_2Ph, J_{gem} 11.0), 7.26-7.36 (5H, m, Ar-H); δ_C ($CDCl_3$, 100.6 MHz): 17.9 (CH_3-2), 24.9 (CH_3-4), 26.7 ($C(\mathbf{C}H_3)_2$), 27.4 ($C(\mathbf{C}H_3)_2$), 67.2 ($\mathbf{C}H_2Ph$), 74.3 (C5), 78.6 (C2), 79.4 (C4), 84.5 (C3), 110.0 ($\mathbf{C}(CH_3)_2$), 127.7 (Ar-C), 128.2 (Ar-C), 128.7 (Ar-C), 137.0 (Ar-C_{quat}), 169.6 (C1); LRMS (ESI+ve): 329 ($[M+Na]^+$, 100%).

2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose 114

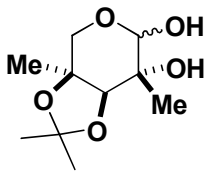


2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **113** (463 mg, 1.51 mmol) was dissolved in dichloromethane (5 mL) and cooled to -78°C. Di*iso*butylaluminium hydride solution (1 M in toluene; 1.6 mL, 1.59 mmol) was added and the reaction mixture stirred under argon. T.l.c. analysis (ethyl acetate/cyclohexane, 1:6) after 90 minutes revealed complete consumption of starting material (R_f 0.32) and the formation of a single product (R_f 0.15). The reaction was quenched with methanol until the evolution of gas had ceased and stirred with sodium potassium tartrate solution (10 mL) for 45 minutes. The mixture was partitioned between dichloromethane (20 mL) and water (20 mL), and the aqueous phase extracted with dichloromethane (2 x 10 mL). The combined organic phases were dried (magnesium sulfate), filtered and concentrated under reduced pressure to afford 2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose, **114** (485 mg, quant.) as a colourless oil, in a 7:1 ratio of anomers.

Found: C, 66.15; H, 7.85; $C_{17}H_{24}O_5$ requires: C, 66.21; H, 7.84; HRMS (ESI+ve): found 331.1513 $[M+Na]^+$; $C_{17}H_{24}Na_1O_5$ requires 331.1516; m.p. 70-73°C; $[\alpha]_D^{18}$ +20.0 (*c*, 1.18 chloroform); ν_{max} (thin film): 3425 (br, OH); δ_H ($CDCl_3$, 400 MHz): 1.39 (6H, s, $C(CH_3)_2^{\mathbf{B}}$, CH_3-$4^{\mathbf{A}}$), 1.40 (3H, s,

$C(CH_3)_2^{\mathbf{A}}$), 1.45 (3H, s, CH_3-4$^{\mathbf{B}}$), 1.48 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.50 (3H, s, CH_3-2$^{\mathbf{A}}$), 1.53 (3H, s, CH_3-2$^{\mathbf{B}}$), 1.55 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 3.52 (1H, d, H5$^{\mathbf{B}}$, $J_{5,5'}$ 12.1), 3.57 (1H, d, H5$^{\mathbf{A}}$, $J_{5,5'}$ 11.8), 3.67 (1H, d, H5'$^{\mathbf{A}}$, $J_{5',5}$ 11.8), 3.90-3.91 (2H, m, H3$^{\mathbf{B}}$, H5'$^{\mathbf{B}}$), 3.93 (1H, d, OH$^{\mathbf{A}}$, $J_{OH,1}$ 11.9), 4.11 (1H, s, H3$^{\mathbf{A}}$), 4.17 (1H, d, OH$^{\mathbf{B}}$, $J_{OH,1}$ 11.0), 4.51 (1H, d, $OCH_2Ph^{\mathbf{B}}$, J_{gem} 10.9), 4.57 (1H, d, $OCH_2Ph^{\mathbf{B}}$, J_{gem} 10.9), 4.59 (1H, d, $OCH_2Ph^{\mathbf{A}}$, J_{gem} 10.8) 4.66 (1H, d, $OCH_2Ph^{\mathbf{A}}$, J_{gem} 10.9), 4.77 (1H, d, H1$^{\mathbf{A}}$, $J_{1,OH}$ 11.9), 5.12 (1H, a-dd, H1$^{\mathbf{B}}$, *J* 1.5, 10.9), 7.15-7.39 (10H, m, Ar-H$^{\mathbf{A\&B}}$); δ_C ($CDCl_3$, 100 MHz): 18.6 (CH_3-2$^{\mathbf{A}}$), 18.7 (CH_3-2$^{\mathbf{B}}$), 23.5 (CH_3-4$^{\mathbf{A}}$), 24.1 (CH_3-4$^{\mathbf{B}}$), 26.8 ($C(\mathbf{C}H_3)_2^{\mathbf{B}}$), 26.9 ($C(\mathbf{C}H_3)_2^{\mathbf{A}}$), 28.0 ($C(\mathbf{C}H_3)_2^{\mathbf{A}}$), 28.3 ($C(\mathbf{C}H_3)_2^{\mathbf{B}}$), 64.3 (C5$^{\mathbf{B}}$) 64.4 ($CH_2Ph^{\mathbf{B}}$), 65.5 ($CH_2Ph^{\mathbf{A}}$), 69.3 (C5$^{\mathbf{A}}$), 73.6 (C2$^{\mathbf{B}}$), 74.9 (C2$^{\mathbf{A}}$), 76.9 (C4$^{\mathbf{B}}$), 77.6 (C4$^{\mathbf{A}}$), 80.0 (C3$^{\mathbf{A}}$), 82.3 (C3$^{\mathbf{B}}$), 93.5 (C1$^{\mathbf{B}}$), 95.8 (C1$^{\mathbf{A}}$), 108.5 ($\mathbf{C}(CH_3)_2^{\mathbf{B}}$), 108.7 ($\mathbf{C}(CH_3)_2^{\mathbf{A}}$), 127.5, 127.6, 127.7, 128.0, 128.4, 128.5, 128.7, 129.2 (8 x Ar-C$^{\mathbf{A\&B}}$), 137.7 (Ar-$C_{quat}^{\mathbf{A}}$), 138.3 (Ar-$C_{quat}^{\mathbf{B}}$); LRMS (ESI-ve): 307 ($[M-H]^-$, 100%).

2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose 110



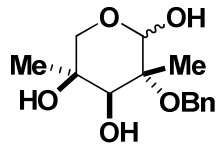
M**ethod 1:** 2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** (1.03 g, 4.75 mmol) was dissolved in dichloromethane and cooled to -78°C, under argon. 1 M di*iso*butylaluminium hydride solution in toluene (10 mL, 9.97 mmol) was added dropwise whilst stirring. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 1 hour showed the presence of unreacted starting material (R_f 0.54) and a single product (R_f 0.34). A further 0.5 mL of reagent was added and the solution stirred for a further 40 min. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) showed remaining starting material (R_f 0.54), and two products (R_f 0.34 & R_f 0.00). The reaction was quenched with methanol until the evolution of gas had ceased (approx. 5 mL). Ethyl acetate (10 mL), sat. sodium hydrogen carbonate solution (5 mL) and potassium hydrogen orthophosphate (500

mg) were added. This mixture was allowed to warm to room temperature before the addition of magnesium sulfate; the resulting gel was filtered through a pad of Celite® and the solvents concentrated under reduced pressure to afford crude lactol (1.20 g) as a white crystalline solid. Purification by column chromatography (ethyl acetate/cyclohexane, 1:4) gave 2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose, **110** in a 10:1 ratio of anomers (0.69 g, 67%) and unreacted starting material (227 mg, 22%), both as white crystalline solids.

Method 2: 2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose **114** (87 mg, 0.28 mmol) was dissolved in ethanol (3 mL). Palladium hydroxide (20% on carbon; 17 mg) was added to the solution whilst stirring. The reaction mixture was degassed and stirred under hydrogen; T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 2 hours revealed complete consumption of starting material (R_f 0.85) and the formation of a single product (R_f 0.34). The reaction mixture was degassed and filtered through a pad of Celite®, eluting with methanol. The solvents were concentrated under reduced pressure to afford 2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose, **110** (62 mg, quant.) as a white crystalline solid, which was used without further purification.

HRMS (ESI+ve): found 241.1046 $[M+Na]^+$; $C_{10}H_{18}Na_1O_5$ requires 241.1196; m.p. 84-86°C; $[\alpha]_D^{17}$ +26.6 (*c*, 0.60 chloroform); ν_{max} (thin film): 3417 (s, br, OH); δ_H ($CDCl_3$, 400 MHz): (*NMR data for major anomer only*) 1.35 (3H, s, CH_3-2), 1.37 (3H, s, $C(CH_3)_2$), 1.39 (3H, s, CH_3-4), 1.44 (3H, s, $C(CH_3)_2$), 2.50 (1H, s, OH), 3.52 (1H, d, H5, $J_{5,5'}$ 11.8), 3.52 (1H, br, s, OH), 3.72 (1H, d, H5', $J_{5',5}$ 11.7), 3.83 (1H, s, H3), 4.82 (1H, s, H1); δ_C ($CDCl_3$, 100 MHz): 23.5 (CH_3-2), 23.9 (CH_3-4), 26.5 ($C(\mathbf{C}H_3)_2$), 27.7 ($C(\mathbf{C}H_3)_2$), 69.2 (C5), 70.2 (C2), 77.8 (C4), 94.3 (C1), 108.5 ($\mathbf{C}(CH_3)_2$); LRMS (ESI-ve): 217 ($[M-H]^-$, 100%).

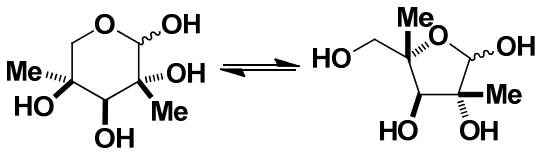
2-*O*-Benzyl-2,4-di-*C*-methyl-L-arabinose 117



2-*O*-Benzyl-3,4-*O*-isopropylidene-2,4-di-*C*-methyl-L-arabinose **114** (178 mg, 0.58 mmol) was suspended in water (3 mL) and heated to 70°C. Dowex® (50W X8 H^+ form, 250 mg) was added and the reaction mixture stirred for 2 hours during which time a white film formed on top of the reaction mixture. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed complete consumption on starting material (R_f 0.49) and the formation of a single product (R_f 0.07). The mixture was filtered using hot acetone and the solvents concentrated under reduced pressure to afford a white solid, 2-*O*-benzyl-2,4-di-*C*-methyl-L-arabinose, **117** (167 mg, quant.) as a single anomer, which was used further purification.

HRMS (ESI+ve): found 291.1208 $[M+Na]^+$; $C_{14}H_{20}Na_1O_5$ requires 291.1213; m.p. 148-150°C; δ_H (DMSO-d_6, 500 MHz): 1.01 (3H, s, CH_3-4), 1.27 (3H, s, CH_3-2), 3.27 (1H, a-d, H5', *J* 12.9), 3.32 (1H, d, H3, $J_{3,OH}$ 7.3), 3.50 (1H, a-d, H5, *J* 12.0), 4.05 (1H, s, OH-2), 4.44 (1H, d, H1, $J_{1,OH}$ 5.4), 4.63 (1H, d, OCH_2Ph, J_{gem} 11.8), 4.64 (1H, d, OH-3, $J_{OH,3}$ 7.4), 4.67 (1H, d, OCH_2Ph, J_{gem} 11.8 Hz), 6.60 (1H, d, OH-1, $J_{OH,1}$ 5.6), 7.22-7.34 (5H, m, Ar-H). δ_C (DMSO-d_6, 125 MHz): 10.6 (CH_3-2), 23.5 (CH_3-4), 64.7 (O**C**H_2Ph), 70.7 (C5), 77.2 (C3), 78.8 (C2 & C4), 98.6 (C1), 126.7, 172.1, 127.3, 127.9, 128.1 (Ar-C), 140.5 (Ar-C_{quat}). LRMS (ESI-ve): 205 ($[M-H]^-$,100%).

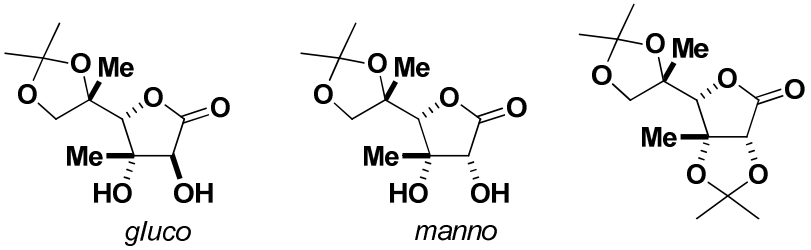
2,4-Di-*C*-methyl-L-arabinose 119



2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose, **110** (480 mg, 2.2 mmol) was dissolved in water (6 mL) and Dowex® (50W X8 H^+ form, 250 mg) was added. The reaction mixture was heated to 90°C for 90 minutes. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time showed complete consumption of starting material (R_f 0.35) and the formation of a single product (R_f 0.05). The mixture was filtered and the solvents removed under reduced pressure to afford 2,4-di-*C*-methyl-L-arabinose **119** (400 mg, quant.) as a cream coloured crystalline solid.

HRMS (ESI+ve): found 201.0732 $[M+Na]^+$; $C_7H_{14}Na_1O_5$ requires 201.0733; m.p. 132-134°C; $[\alpha]_D^{17}$ +13.0 (*c*, 0.90 methanol); ν_{max} (thin film): 3385 (s, br, OH); See appendix for NMR data; LRMS (ESI-ve): 177 ($[M-H]^-$, 100%).

3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-glucono-1,4-lactone 123, 3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-mannono-1,4-lactone 124 and 2,3:5,6-Di-*O*-isopropylidene-3,5-di-*C*-methyl-L-mannono-1,4-lactone 125



2,4-Di-*C*-methyl-L-arabinose **119** (390 mg, 2.19 mmol) was dissolved in water (4 mL) and stirred with sodium cyanide (140 mg, 2.85 mmol) at room temperature for 24 hours, then heated to 90°C for 24 hours. The solution was then allowed to cool to room temperature before being passed down a column of Amberlite®-IR120 (H^+ resin), eluting with water (1 L). T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) of this solution revealed a complex mixture of products. Evaporation of

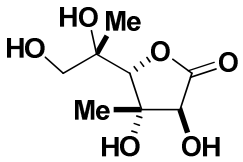
the solvents under reduced pressure afforded a creamy solid (491 mg) which was dissolved in acetone (6 mL) and stirred at room temperature overnight with anhydrous copper(II) sulfate (761 g, 4.77 mmol) and catalytic concentrated sulfuric acid (0.1 mL). T.l.c. analysis (ethyl acetate) after this time revealed the presence of three major products with R_f 0.88, 0.68 and 0.60 respectively. The mixture was neutralised with sodium carbonate and the salts filtered through Celite®. Evaporation of solvent afforded 540 mg of dark brown crude, which was preadsorbed on silica and purified by flash column chromatography (ethyl acetate/cyclohexane, 10:1 → 1:1) to yield three white crystalline solids; 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-L-glucono-1,4-lactone **123** (120 mg, 22%); 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-L-mannono-1,4-lactone **124** (130 mg, 24%) and 2,3:5,6-di-*O*-isopropylidene-3,5-di-*C*-methyl-L-mannono-1,4-lactone **125** (26 mg, 8%). All three were recrystallised from ethyl acetate and cyclohexane to form long colourless crystals.

*Data for **123**:* HRMS (ESI+ve): found 269.0993 $[M+Na]^+$; $C_{11}H_{18}Na_1O_6$ requires 269.0996; $[\alpha]_D^{18}$ -40.4 (*c*, 1.04 chloroform); m.p. 97-102°C; ν_{max} (thin film): 3418 (s, br, OH), 1772 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.42 (3H, s, $C(CH_3)_2$), 1.45 (3H, s,CH_3-3), 1.47 (3H, s, CH_3-5), 1.50 (3H, s, $C(CH_3)_2$), 3.59 (1H, d, OH-2, $J_{OH\text{-}2,2}$ 4.3), 3.78 (1H, d, H6, $J_{6,6'}$ 9.5), 3.92 (1H, s, OH-3), 4.14 (1H, s, H4), 4.18 (1H, d, H6', $J_{6',6}$ 9.5), 4.58 (1H, d, H2, $J_{2,OH\text{-}2}$ 4.2); δ_C ($CDCl_3$, 100 MHz): 20.4 (CH_3-5), 24.1 (CH_3-3), 26.1 ($C(\mathbf{C}H_3)_2$), 27.4 ($C(\mathbf{C}H_3)_2$), 73.6 (C6), 75.3 (C2), 77.5 (C3), 81.7 (C5), 85.7 (C4), 111.0 ($\mathbf{C}(CH_3)_2$), 174.3 (C1); LRMS (ESI-ve): 305 ($[M+CH_3COO]^-$, 100%).

*Data for **124**:* Found C 53.75; H 7.38; $C_{11}H_{18}O_6$ requires C 53.65; H 7.37; HRMS (ESI+ve): found 269.0994 $[M+Na]^+$; $C_{11}H_{18}NaO_6$ requires 269.0996; $[\alpha]_D^{17}$ -24.7 (*c*, 1.28 acetone); m.p. 160-164°C; ν_{max} (thin film): 3442 (s, br, OH), 1776 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.41 (3H, s, $C(CH_3)_2$), 1.43 (3H, s, $C(CH_3)_2$), 1.46 (3H, s, CH_3-5), 1.54 (3H, s, CH_3-3), 3.01 (1H, s, OH), 3.77 (1H, a-d, H6, *J* 9.5), 4.10 (1H, a-d, H6', *J* 10.1), 4.23 (1H, s, H4), 4.29 (1H, s, H2); δ_C ($CDCl_3$, 100 MHz): 22.9 (CH_3-5), 23.9 (CH_3-3), 26.5, 27.4 ($C(\mathbf{C}H_3)_2$), 71.9 (C6), 74.8 (C2), 76.0 (C3), 81.8 (C5), 85.8 (C4), 109.6 ($\mathbf{C}(CH_3)_2$), 175.4 (C1) . LRMS (ESI-ve): 245 ($[M-H]^-$, 100%).

*Data for **125**:* HRMS (ESI+ve): found 309.1309 $[M+Na]^+$; $C_{14}H_{22}Na_1O_6$ requires 309.1309; $[\alpha]_D^{17}$ -48.2 (*c*, 0.88 chloroform); m.p. 92-96°C; ν_{max} (thin film): 1789 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.40 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.48 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.62 (3H, s, CH_3), 3.75 (1H, d, H6, $J_{6,6'}$ 9.1), 4.07 (1H, d, H6', $J_{6',6}$ 9.1), 4.31 (1H, s, H4), 4.52 (1H, s, H2); δ_C ($CDCl_3$, 100 MHz): 22.3 (CH_3), 23.9 (CH_3), 26.6 (CH_3), 27.3 (CH_3), 27.8 (CH_3), 29.1 (CH_3), 72.2 (C6), 81.6 & 84.7 (C3 & C5), 82.0 (C2), 85.1 (C4), 109.8 (**C**$(CH_3)_2$ [5,6]), 115.0 (**C**$(CH_3)_2$ [2,3]), 173.6 (C1).

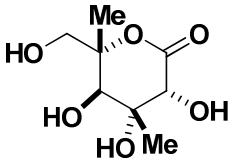
3,5-Di-*C*-methyl-L-glucono-1,4-lactone 187



3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-glucono lactone **123** (25 mg, 0.10 mmol) was dissolved in water (0.5 mL) and Dowex® (50W X8 H^+ form, 25 mg) was added whilst stirring at room temperature. T.l.c. analysis (ethyl acetate) after 4 days showed complete consumption of starting material (R_f 0.68) and the formation of a single product (R_f 0.13). The resin was removed by filtration and the solvents concentrated under reduced pressure to afford 3,5-di-*C*-methyl-L-glucono-1,4-lactone **187** as a colourless oil (21 mg, quant.). The five ring lactone was shown by HMBC analysis.

HRMS (ESI+ve): found 229.0680 $[M+Na]^+$; $C_8H_{14}Na_1O_6$ requires 229.0683; $[\alpha]_D^{22}$ -47.3 (*c*, 1.06 methanol); ν_{max} (thin film): 3384 (br, OH), 1773 (s, C=O). δ_H (CD_3OD, 500 MHz): 1.32 (3H, s, CH_3-5), 1.42 (3H, s, CH_3-3), 3.57 (1H, d, H6, $J_{6,6'}$ 11.2), 3.72 (1H, d, H6', $J_{6',6}$ 11.0), 4.27 (1H, s, H4), 4.35 (1H, s, H2); δ_C (CD_3OD, 125 MHz): 21.8 (CH_3-3), 21.9 (CH_3-5), 67.8 (C6), 75.8 (C5), 79.1 (C3), 76.8 (C2), 87.1 (C4), 177.1 (C1); LRMS (ESI-ve): 205 ($[M-H]^-$, 100%).

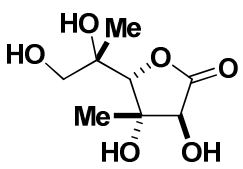
3,5-Di-*C*-methyl-L-mannono-1,5-lactone 189



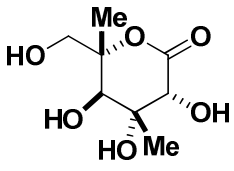
3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-manono lactone **124** (32 mg, 0.13 mmol) was dissolved in water (0.5 mL) and Dowex® (50W X8 H^+ form, 25 mg) was added whilst stirring at room temperature. T.l.c. analysis (ethyl acetate) after 16 hours showed complete consumption of starting material (R_f 0.60) and the formation of a single product (R_f 0.08). The resin was removed by filtration and the solvents concentrated under reduced pressure to afford 3,5-di-*C*-methyl-L-mannono-1,5-lactone **189** as a colourless oil (27 mg, quant.). The six ring lactone was shown by HMBC analysis.

HRMS (ESI+ve): found 229.0681$[M+Na]^+$; $C_8H_{14}Na_1O_6$ requires 229.0683; $[\alpha]_D^{21}$ -21.8 (*c*, 0.75 methanol); ν_{max} (thin film): 3420 (s, br, OH), 1773 (s, C=O); δ_H (CD_3OD, 500 MHz): 1.35 (3H, s, CH_3-5), 1.53 (3H, s, CH_3-3), 3.52 (1H, d, H6, $J_{6,6'}$ 11.0), 3.69 (1H, d, H6', $J_{6',6}$ 11.0), 4.22 (1H, s, H2), 4.32 (1H, s, H4); δ_C (CD_3OD, 125 MHz): 22.2 (CH_3-5), 23.0 (CH_3-3), 67.8 (C6), 75.5 (C5), 78.7 (C3), 76.0 (C2), 84.9 (C4), 177.3 (C1); LRMS (ESI-ve): 205 ($[M-H]^-$, 100%).

3,5-Di-*C*-methyl-L-glucono-1,4-lactone 187 and 3,5-di-*C*-methyl-L-mannono-1,5-lactone 189



gluco

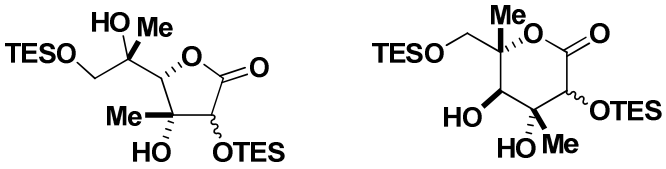


manno

2,4-Di-*C*-methyl-L-arabinose **110** (0.859 g, 4.83 mmol) was dissolved in water (9 mL) and stirred with sodium cyanide (0.307 g, 2.85 mmol) at room temperature for 24 hours, then heated to 90°C for 24 hours. After this time, the reaction mixture was concentrated under reduced pressure to give 1.35 g of crude material. This was dissolved in anhydrous methanol (18 mL) and 4Å beaded

molecular sieves (0.4 g) and acetyl chloride (0.58 mL, 8.12 mmol) were added. The reaction mixture was stirred under argon for 18 hours, after which time t.l.c. analysis (ethyl acetate) revealed three products with R_f 0.13, 0.08 and 0.00 respectively. The reaction mixture was concentrated under reduced pressure, preadsorbed on silica gel and purified by column chromatography (ethyl acetate → 10% methanol in ethyl acetate) to afford 0.567 g of mixed lactones **187** and **189**, with NMR data identical to that listed above.

3,5-Di-*C*-methyl-2,6-di-*O*-triethylsilyl-L-glucono and mannono lactones

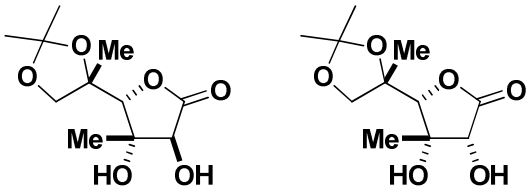


A sample of mixed 3,5-di-*C*-methyl-L-glucono **187** and 3,5-di-*C*-methyl-L-mannono **189** lactones (46 mg, 0.22 mmol) was dissolved in anhydrous *N*,*N*-dimethylformamide (4 mL), and to it was added 4Å beaded molecular sieves (0.2 g), imidazole (61 mg, 0.89 mmol) and triethylsilyl chloride (112 µL, 0.67 mmol). The reaction was stirred under argon for 2 hours, after which time t.l.c. analysis (ethyl acetate/cyclohexane, 1:4) revealed complete consumption of starting materials (R_f 0.00) and the formation of two products (R_f 0.63, 0.57). The reaction mixture was filtered, concentrated under reduced pressure, preadsorbed on silica and purified by column chromatography (ethyl acetate/cyclohexane, 1:10) to give mixed 3,5-di-*C*-methyl-2,6-di-*O*-triethylsilyl-L-glucono and mannono lactones (44 mg, 45%), as a colourless oil, in a 5:4 ratio; '**δ**' donates the major component, a 6 ring lactone and '**γ**' denotes the minor lactone, found in a 5 ring form.

HRMS (ESI+ve): found 493.3124 $[M+MeCN+NH_4]^+$; $C_{22}H_{49}N_2O_6Si_2$ requires 493.3126; ν_{max} (thin film): 3341 (br, OH), 1785 (s, C=O); δ_H ($CDCl_3$, 500 MHz): 0.61-0.83 (24H, m, $Si(C\mathbf{H}_2CH_3)_3^{\delta\ \&\ \gamma}$), 0.96-1.04 (36H, m, $Si(CH_2CH_3)_3^{\delta\&\gamma}$), 1.33 (3H, s, CH_3-5^{γ}), 1.37 (3H, s, CH_3-5^{δ}), 1.39 (3H, s, CH_3-3^{γ}), 1.51 (3H, s, CH_3-3^{δ}), 3.17 (1H, s, OH-3^{δ}), 3.22 (1H, s, OH-5^{γ}), 3.59 (1H, d, $H6^{\gamma}$, *J* 10.1), 3.60

(1H, d, H6$^{\delta}$, $J_{6,6'}$ 9.9), 3.68 (2H, a-d, OH-5$^{\delta}$ & H6'$^{\gamma}$, *J* 10.7), 3.73 (1H, d, H6'$^{\delta}$, $J_{6',6}$ 9.9), 4.13 (1H, s H2$^{\delta}$), 4.18 (1H, s, H4$^{\gamma}$), 4.27 (1H, s, H4$^{\delta}$), 4.30 (1H, s, H2$^{\gamma}$), 4.60 (1H, s, OH-3$^{\gamma}$); δ_C ($CDCl_3$, 125 MHz): 4.6, 4.7, 4.8, 4.9, 5.0 (Si(**C**$H_2CH_3)_3^{\delta\&\gamma}$), 6.5, 6.56, 6.59, 6.7 (Si(CH_2**C**$H_3)_3^{\delta\&\gamma}$), 21.6 (CH_3-5$^{\gamma}$), 22.2 (CH_3-5$^{\delta}$), 22.5 (CH_3-3$^{\gamma}$), 24.1 (CH_3-3$^{\delta}$), 67.8 (C6$^{\gamma}$), 68.3 (C6$^{\delta}$), 74.0 (C5$^{\delta}$), 75.2 (C5$^{\gamma}$), 75.6 (C2$^{\delta}$), 76.6 (C2$^{\gamma}$), 77.3 (C3$^{\delta}$), 78.2 (C3$^{\gamma}$), 82.5(C4$^{\delta}$), 85.5 (C4$^{\gamma}$), 173.0 (C1$^{\gamma}$), 173.8 (C1$^{\delta}$). LRMS (ESI+ve): 452 ([M+NH_4]$^+$, 52%), 886 ([2M+NH_4]$^+$, 83%), 891 ([2M+Na]$^+$, 100%); LRMS (ESI-ve): 433 ([M-H]$^-$, 100%).

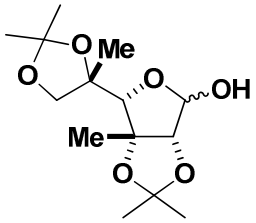
3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-glucono 123 and mannono 124 lactones



A sample of mixed 3,5-di-*C*-methyl-L-glucono **187** and mannono **189** lactones (0.243 g, 1.18 mmol) was dissolved in acetone (9 mL) and stirred at room temperature with anhydrous copper(II) sulfate (0.375 g, 2.36 mmol) and a catalytic concentrated sulfuric acid (0.05 mL). T.l.c. analysis (ethyl acetate) after 16 h revealed the presence of two major products (R_f 0.68, 0.60) and residual starting material (R_f 0.13, 0.08). The reaction mixture was heated to 50°C for 4 h after which time, no starting material remained. The solution was cooled to room temperature, neutralised with solid sodium carbonate, and the salts filtered through a pad of Celite®, eluting with acetone. Evaporation of solvent afforded a brown crude which was preadsorbed on silica and purified by flash column chromatography (ethyl acetate/cyclohexane, 2:3) to yield 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-L-glucono lactone **123** (71 mg, 24%), 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-L-mannono lactone **124** (69 mg, 24%) and a mixed fraction containing both products (82 mg, 28%).

The NMR data was identical to that listed above.

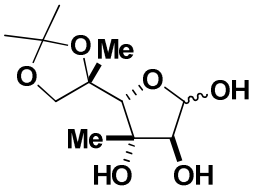
2,3:5,6-Di-*O*-isopropylidene-3,5-di-*C*-methyl-L-mannose 127



2,3:5,6-Di-*O*-isopropylidene-3,5-di-*C*-methyl-L-mannono lactone **125** (7 mg, 24 μmol) was dissolved in dichloromethane (0.5 mL) and cooled to -78°C. Di*iso*butylaluminium hydride solution (1 M in toluene; 26 μL, 26 μmol) was added and the reaction mixture stirred under argon. T.l.c. analysis (ethyl acetate/cyclohexane, 1:4) after one hour revealed complete consumption on starting material (R_f 0.21) and the formation of a single product (R_f 0.18). The reaction was quenched with methanol until the evolution of gas had ceased and stirred with sodium potassium tartrate solution (5 mL) for 20 minutes. The mixture was partitioned between dichloromethane and water (10 mL), and the aqueous phase extracted a further two times (2 x 10 mL). The combined organic phases were dried (magnesium sulfate), filtered and concentrated under reduced pressure to afford 2,3;5,6-di-*O*-isopropylidene-3,5-di-*C*-methyl-L-mannose **127** (7 mg, 99%) as a colourless oil, in a 7:1 ratio of anomers.

HRMS (ESI+ve): found 311.1465 $[M+Na]^+$; $C_{14}H_{24}Na_1O_6$ requires 311.1465; $[\alpha]_D^{18}$ -22.7 (*c*, 0.65 chloroform); ν_{max} (thin film): 3340 (br, OH). δ_H (C_6D_6, 500 MHz): *(NMR data for major anomer only)* 1.09 (3H, s, $C(CH_3)_2$ [2,3]), 1.28 (3H, s, $C(CH_3)_2$ [2,3]), 1.38 (3H, s, CH_3-3), 1.49 (3H, s, CH_3-5), 1.56 (3H, s, $C(CH_3)_2$ [5,6]), 1.59 (3H, s, $C(CH_3)_2$ [5,6]), 2.47 (1H, s, OH), 3.96 (1H, d, H6, $J_{6,6'}$ 8.5), 4.18 (1H, s, H2), 4.21 (1H, s, H4), 4.50 (1H, d, H6', $J_{6',6}$ 8.5), 5.31 (1H, s, H1). δ_C (C_6D_6, 125 MHz): 24.0 (CH_3-5), 25.7 (CH_3-3), 26.2 ($C(\mathbf{C}H_3)_2$ [5,6]), 26.5 ($C(\mathbf{C}H_3)_2$ [5,6]), 26.6 ($C(\mathbf{C}H_3)_2$ [2,3]), 28.7 ($C(\mathbf{C}H_3)_2$ [2,3]), 71.5 (C6), 81.9 (C5), 87.5 (C4), 88.1 (C3), 92.2 (C1), 99.6 (C2), 108.5 ($\mathbf{C}(CH_3)_2$ [2,3]), 112.3 ($\mathbf{C}(CH_3)_2$ [5,6]).

3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-glucose 129

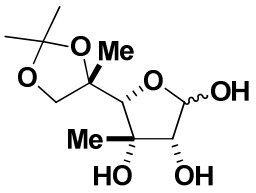


3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-glucono lactone **123** (113 mg, 0.50 mmol) was dissolved in anhydrous tetrahydrofuran (1 mL) and cooled to -60°C. Di*iso*butylaluminium hydride solution (1.5 M in toluene; 0.92 mL, 1.38 mmol) was added and the reaction mixture stirred under nitrogen. T.l.c. analysis (ethyl acetate) after one hour revealed partial consumption of starting material (R_f 0.68) and the formation of a single product (R_f 0.30). A further 0.31 mL of regent was added and the reaction warmed to -50°C. T.l.c. analysis (ethyl acetate) after 30 minutes revealed starting material remained and a final portion (0.31 mL) of reagent was added. T.l.c. analysis (ethyl acetate) after one a further 30 minutes showed complete consumption of starting material. The reaction mixture was quenched with methanol until the evolution of gas had ceased and diluted with ethyl acetate (2 mL). sodium hydrogen carbonate (sat. aq., 1 mL), and potassium dihydrogen orthophosphate (100 mg) were added, and the resulting gel stirred for 30 minutes. The mixture was dried (magnesium sulfate) and filtered through a pad of Celite®, eluting with ethyl acetate (50 mL). The solvents were concentrated under reduced pressure, and the crude purified by column chromatography (ethyl acetate/cyclohexane 1:1 → ethyl acetate) afford 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-L-glucose **129** (67 mg, 59%) as a colourless oil, in a 1:1 ratio of anomers.

HRMS (ESI+ve): found 271.1145 $[M+Na]^+$; $C_{11}H_{20}Na_1O_6$ requires 271.1152; $[\alpha]_D^{25}$ -15.6 (*c*, 0.675 ethyl acetate); ν_{max} (thin film): 3444 (br, OH); δ_H (C_6D_6, 500 MHz): 1.37 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.40 (3H, s, CH_3-$3^{\mathbf{B}}$), 1.44 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.46 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.50 (6H, s, CH_3-$3^{\mathbf{A}}$ & $C(CH_3)_2^{\mathbf{A}}$), 1.55 (3H, s, CH_3-$5^{\mathbf{B}}$), 1.58 (3H, s, CH_3-$5^{\mathbf{A}}$), 2.38 (1H, br-s, OH), 2.69 (1H, s, OH), 3.08 (1H, s, OH), 3.58 (1H, s, $H4^{\mathbf{A}}$), 3.71 (1H, d, $H6^{\mathbf{B}}$, $J_{6,6'}$ 8.7), 3.78 (1H, d, $H6^{\mathbf{A}}$, $J_{6,6'}$ 8.5), 3.91 (1H, d, OH-$1^{\mathbf{A}}$, *J* 3.8), 3.99-4.03 (4H, m, $H2^{\mathbf{B}}$ & $H4^{\mathbf{B}}$ & $H2^{\mathbf{A}}$ & OH), 4.16 (1H, d, $H6'^{\mathbf{B}}$, $J_{6',6}$ 8.8), 4.25 (1H, d,

H6'^{A}, $J_{6',6}$ 8.7), 5.09 (1H, s, H1^{B}), 5.31 (41H, a-d, H1^{A} *J* 4.1); δ_C (C_6D_6, 125 MHz): 21.0 (CH_3-3^{B}), 22.1 (CH_3-5^{B}), 23.3 (CH_3-5^{A}), 24.4, 26.9 (CH_3-3^{A} & $C(CH_3)_2$ ^{A}), 26.8 ($C(CH_3)_2$^{B}), 27.1 ($C(CH_3)_2$^{B}), 27.3 ($C(CH_3)_2$^{A}), 74.0 (C6^{A}), 74.4 (C6^{B}), 80.0 (C3^{A}), 80.6 (C3^{B}), 80.7 (C2^{A}), 82.7 (C5^{A}), 82.8 (C5^{B}), 83.8 (C2^{B}), 85.6 (C4^{B}), 87.2 (C4^{A}), 96.1 (C1^{A}) , 102.5 (C1^{B}), 109.4 ($C(CH_3)_2$^{A}), 109.8 ($C(CH_3)_2$^{B}); LRMS (ESI-ve): 247 ([M-H]$^{-}$, 100%).

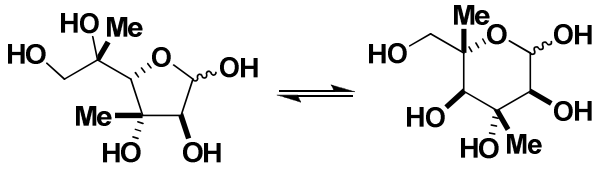
3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-mannose 130



3,5-Di-*C*-methyl -5,6-*O*-isopropylidene-L-mannono lactone **124** (93mg, 0.38 mmol) was dissolved in anhydrous tetrahydrofuran (1 mL) and cooled to -78°C. Di*iso*butylaluminium hydride solution (1.5 M in toluene; 1.01 mL, 1.51 mmol) was added and the reaction mixture stirred under nitrogen. T.l.c. analysis (ethyl acetate) after 90 minutes revealed partial consumption of starting material (R_f 0.60) and the formation of a single product (R_f 0.30). A further 0.25 mL of regent was added and the reaction warmed to -60°C. T.l.c. analysis (ethyl acetate) after a further hour showed complete consumption of starting material. The reaction mixture was quenched with methanol until the evolution of gas had ceased and diluted with ethyl acetate (2 mL). Sodium bicarbonate solution (sat. aq., 1 mL), and potassium dihydrogen orthophosphate (100 mg) were added, and the resulting gel stirred for 30 minutes. The mixture was dried (magnesium sulfate) and filtered through a pad of Celite®, eluting with ethyl acetate (50 mL). The solvents were concentrated under reduced pressure, and the crude purified by column chromatography (ethyl acetate/cyclohexane 1:1 → ethyl acetate) to afford 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-L-mannose **130** (67 mg, 71%) as a colourless oil, in a 2:1 ratio of anomers.

HRMS (ESI-ve): found 247.1177 $[M-H]^-$; $C_{11}H_{19}O_6$ requires 247.1187; $[\alpha]_D^{22}$ -6.7 (*c*, 4.08 ethyl acetate) & $[\alpha]_D^{21}$ -0.9 (*c*, 0.88 ethyl acetate); ν_{max} (thin film): 3445 (br, OH). δ_H (C_6D_6, 500 MHz): 1.29 (3H, s, CH_3-3$^{\mathbf{A}}$), 1.48 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.49 (3H, s, CH_3-3$^{\mathbf{B}}$), 1.52 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.56 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.58 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.60 (3H, s, CH_3-5$^{\mathbf{A}}$), 1.60 (3H, s, CH_3-5$^{\mathbf{B}}$), 3.07 (1H, s, OH$^{\mathbf{A}}$), 3.40 (1H, s, H4$^{\mathbf{A}}$), 3.52 (1H, m, OH$^{\mathbf{A}}$), 3.77 (1H, s, H2$^{\mathbf{A}}$), 3.88 (1H, d, H6$^{\mathbf{A}}$, $J_{6,6'}$ 8.7), 3.90 (1H, d, H6$^{\mathbf{B}}$, $J_{6,6'}$ 8.8), 3.91 (1H, m, H4$^{\mathbf{B}}$), 3.96 (1H, s, OH$^{\mathbf{B}}$), 4.20 (1H, s, H2$^{\mathbf{B}}$), 4.38 (1H, d, H6'$^{\mathbf{B}}$, $J_{6',6}$ 8.8), 4.42 (1H, m, OH$^{\mathbf{A}}$), 4.46 (1H, d, H6'$^{\mathbf{A}}$, $J_{6',6}$ 8.7), 5.25 (1H, s, H1$^{\mathbf{A}}$), 5.49 (1H, a-d, H1$^{\mathbf{B}}$, *J* 2.8); δ_C (C_6D_6, 100 MHz): 22.7, 22.9 (CH_3-5$^{\mathbf{A\&B}}$), 23.8 ($C(\mathbf{C}H_3)_2)^{\mathbf{B}}$ & CH_3-3$^{\mathbf{A}}$), 26.9 ($C(\mathbf{C}H_3)_2^{\mathbf{A}}$) 27.3 ($C(\mathbf{C}H_3)_2$B), 27.5 ($C(\mathbf{C}H_3)_2^{\mathbf{A}}$ & CH_3-3$^{\mathbf{B}}$), 72.9 (C6$^{\mathbf{A}}$), 73.2 (C6$^{\mathbf{B}}$), 76.7 (C4$^{\mathbf{A}}$), 77.1 (C5$^{\mathbf{A}}$), 77.5 (C5$^{\mathbf{B}}$), 82.4 (C4$^{\mathbf{B}}$), 82.8 (C3$^{\mathbf{A}}$), 83.0 (C3$^{\mathbf{B}}$), 86.5 (C2$^{\mathbf{B}}$), 86.6 (C2$^{\mathbf{A}}$), 95.9 (C1$^{\mathbf{A}}$), 101.6 (C1$^{\mathbf{B}}$), 109.1 ($\mathbf{C}(CH_3)_2^{\mathbf{A}}$), 109.3 ($\mathbf{C}(CH_3)_2^{\mathbf{B}}$); LRMS (ESI+ve): 271 ($[M+Na]^+$, 75%); LRMS (ESI-ve): 247 ($[M-H]^-$, 75%), 495 ($[2M-H]^-$, 100%).

3,5-Di-*C*-methyl-L-glucose 97

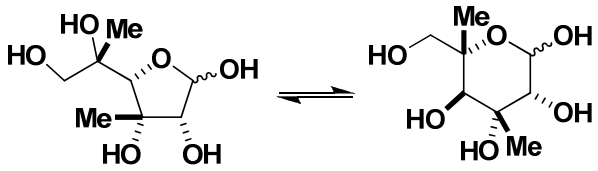


3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-glucose **129** (9 mg, 36 µmol) was dissolved in water (1 mL) and Dowex® (50W X8 H^+ form, 80 mg) was added, the reaction mixture was stirred at room temperature for 2 hours after which time t.l.c. analysis (ethyl acetate) showed complete consumption of starting material (R_f 0.28) and the formation of a single product (R_f 0.00). The resin was removed by filtration and the solvents removed under reduced pressure to afford 3,5-di-*C*-methyl-L-glucose **97** (7 mg, 93%) as a colourless oil.

HRMS (ESI+ve): found 231.0838 $[M+Na]^+$; $C_8H_{16}Na_1O_6$ requires 231.0839; $[\alpha]_D^{25}$ +3.8 (*c*, 0.81 water); ν_{max} (thin film): 3385 (br, OH); See appendix for NMR data; LRMS (ESI+ve): 231 ($M+Na^+$,

100%), 272 (M+MeCN+NH_4^+, 90%), 439 (2M+Na^+, 100%); LRMS (ESI-ve): 207 ([M-H]$^-$, 58%), 243 ([M+Cl]$^-$, 100%).

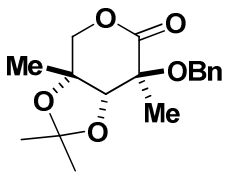
3,5-Di-*C*-methyl-L-mannose 128



3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-mannose **130** (27 mg, 0.11 mmol) was dissolved in water (1 mL) and Dowex® (50W X8 H^+ resin, 80 mg) was added. T.l.c. analysis (ethyl acetate) after 20 hours showed complete consumption of starting material (R_f 0.28) and the formation of a single product (R_f 0.00). The resin was removed by filtration and the solvent removed under reduced pressure. The crude was re-dissolved in methanol and preadsorbed onto silica. Purification by flash column chromatography (ethyl acetate → 20% methanol in ethyl acetate) afforded 3,5-di-*C*-methyl-L-mannose **128** (14 mg, 62%) as a colourless oil.

HRMS (ESI+ve): found 231.0835 [M+Na]$^+$; $C_8H_{16}Na_1O_6$ requires 231.0839; $[\alpha]_D^{25}$ -7.0 (*c*, 0.84 water); ν_{max} (thin film): 3385 (br, OH); See appendix for NMR data; LRMS (ESI+ve): 231 (M+Na^+, 92%), 272 ([M+MeCN+NH_4]$^+$, 87%), 439 ([2M+Na]$^+$, 100%); LRMS (ESI-ve): 207 ([M-H]$^-$, 100%).

2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-D-arabinono-1,5-lactone

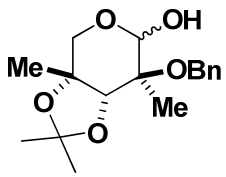


2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **141** (716 mg, 3.31 mmol) was dissolved in *N*,*N*-dimethylformamide (8 mL) with 4Å molecular sieves (50 mg). Benzyl bromide (0.48 mL, 3.98 mmol) and sodium hydride (60% suspension in mineral oil; 159 mg, 3.98 mmol)

were added and the solution stirred at room temperature for 2 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 2:1) after this time revealed complete consumption of starting material (R_f 0.29) and the formation of a single product (R_f 0.63). The reaction mixture was filtered, diluted with water (40 mL) and extracted with dichloromethane (3 x 40 mL). The combined organic phases were dried (sodium sulfate), filtered and the solvents concentrated under reduced pressure to give the crude lactone. This was purified by elution through a silica plug (ethyl acetate/cyclohexane, 1:10) to afford 2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-D-arabinono-1,5-lactone (771 mg, 76%) as a white solid.

HRMS (ESI+ve): found 329.1352 $[M+Na]^+$; $C_{17}H_{22}Na_1O_5$ requires 329.1359; m.p. 64-66°C; $[\alpha]_D^{18}$ -64.4 (*c*, 0.89 in $CHCl_3$); ν_{max} (thin film): 1755 (s, C=O); LRMS (ESI+ve): 365 ($[M+NH_4+MeCN]^+$, 100%); The ^1H and ^{13}C NMR spectra were identical to those of the enantiomer **113** above.

2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-D-arabinose

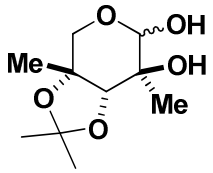


2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-D-arabinono-1,5-lactone (686 mg, 2.24 mmol) was dissolved in dichloromethane (7 mL) and cooled to -78°C. Di*iso*butylaluminium hydride solution (1.5 M in toluene; 1.64 mL, 2.47 mmol) was added and the reaction mixture stirred under argon. T.l.c. analysis (ethyl acetate/cyclohexane, 1:6) after 90 min revealed complete consumption of starting material (R_f 0.32) and the formation of a single product (R_f 0.15). The reaction was quenched with methanol until the evolution of gas had ceased, diluted with dichloromethane (50 mL) and stirred with sodium potassium tartrate solution (50 mL) for 90 minutes. The mixture was partitioned between DCM (15 mL) and water (15 mL) and the aqueous phase extracted with dichloromethane (2 x 50 mL). The combined organic fractions were dried (sodium sulfate), filtered

and concentrated under reduced pressure to afford 2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-D-arabinose (690 mg, quant.), a colourless oil.

HRMS (ESI+ve): found 331.1513 $[M+Na]^+$; $C_{17}H_{24}Na_1O_5$ requires 331.1516; m.p. 68-72°C; $[\alpha]_D^{18}$ -16.7 (*c*, 1.23 in $CHCl_3$); ν_{max} (thin film): 3424 (br, OH); LRMS (ESI+ve): 367 ($[M+NH_4+MeCN]^+$, 100%); The ^1H and ^{13}C NMR spectra were identical to those of the enantiomer **114** above.

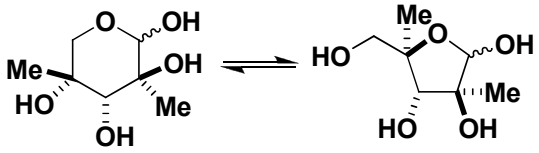
2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-D-arabinose



2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose (1.07 g, 3.46 mmol) was dissolved in ethanol (42 mL). Palladium hydroxide (20% on carbon; 213 mg) was added to the solution whilst stirring. The reaction mixture was degassed and stirred under hydrogen; T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 16 hours, revealed complete consumption of starting material (R_f 0.85) and the formation of a single product (R_f 0.34). The reaction mixture was degassed and filtered through a pad of Celite®, eluting with methanol. The solvents were concentrated under reduced pressure and the crude purified by column chromatography (ethyl acetate/cyclohexane, 1:4 → 1:1) to afford 2,4-di-*C*-methyl-3,4-*O*-isopropylidene-D-arabinose (605 mg, 80%) as a white crystalline solid.

HRMS (ESI-ve): found 217.1075 $[M-H]^-$; $C_{10}H_{17}O_5$ requires 217.1081; m.p. 81-83°C; $[\alpha]_D^{20}$ -24.2 (*c*, 0.635 chloroform); ν_{max} (thin film): 3331 (s, br, OH); LRMS (ESI-ve): 217 ($[M-H]^-$, 100%); The ^1H and ^{13}C NMR spectra were identical to those of the enantiomer **110** above.

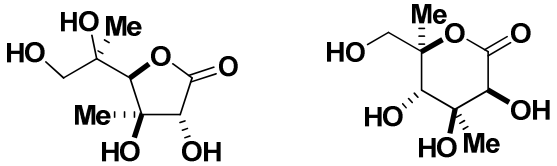
2,4-Di-*C*-methyl-D-arabinose 142



2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose (595 mg, 2.73 mmol) was dissolved in water (10 mL) and Dowex® (50W X8 H^+ form, 10 g) was added. The reaction mixture was heated to 90°C for 90 minutes. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time showed complete consumption of starting material (R_f 0.35) and the formation of a single product (R_f 0.05). The resin was removed by filtration and the solvents removed under reduced pressure to afford 2,4-di-*C*-methyl-D-arabinose **142** (470 mg, 97%) as a cream coloured crystalline solid.

HRMS (ESI-ve): found 177.0768 $[M-H]^-$; $C_7H_{13}O_4$ requires 177.0769; m.p. 126-128°C; $[\alpha]_D^{21}$ -20.7 (*c*, 0.66 methanol); ν_{max} (thin film): 3419 (s, br, OH); LRMS (ESI-ve): 177 ($[M-H]^-$, 100%); The ^1H and ^{13}C NMR spectra were identical to those of the enantiomer **119** above.

3,5-Di-*C*-methyl-D-glucono and mannono lactones

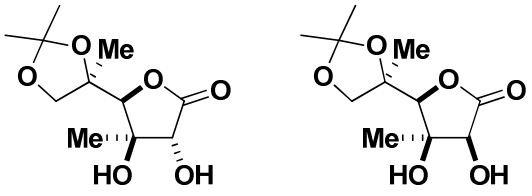


gluco *manno*

2,4-Di-*C*-methyl-D-arabinose **142** (225 mg, 1.26 mmol) was dissolved in water (3 mL) and stirred with sodium cyanide (81 mg, 1.64 mmol) at room temperature for 24 hours, then heated to 90°C for 24 hours. After this time the reaction mixture was concentrated under reduced pressure to give 311 mg of crude material. This was dissolved in anhydrous methanol (4 mL), and 4Å beaded molecular sieves (0.2 g) and acetyl chloride (0.13 mL, 1.90 mmol) were added. The reaction mixture was stirred under argon for 24 hours, after which time t.l.c. analysis (ethyl acetate) revealed three products (R_f 0.13, 0.08, 0.00). The reaction mixture was concentrated under reduced pressure,

preadsorbed and purified by column chromatography (ethyl acetate → 10% methanol in ethyl acetate) to afford 138 mg of mixed lactones (53%). The ^{1}H and ^{13}C NMR spectra were identical to those of the enantiomers **187** and **189** above.

3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-D-glucono and mannono lactones



A sample of mixed 3,5-di-*C*-methyl-D-glucono and mannono lactones (138 mg, 0.67 mmol) was dissolved in acetone (6 mL) and stirred at room temperature with anhydrous copper(II) sulfate (214 mg, 1.34 mmol) and catalytic concentrated sulfuric acid (0.05 mL). T.l.c. analysis (ethyl acetate) after 16 h revealed the presence of two major products (R_f 0.68, 0.60) and residual starting material (R_f 0.13, 0.08). The reaction mixture was heated to 50°C for 4 h after which time, no starting material remained. The solution was cooled to room temperature, neutralised with solid sodium carbonate, and the salts filtered through a pad of Celite®, eluting with acetone. Evaporation of solvent afforded a brown crude which was preadsorbed on silica and purified by flash column chromatography (ethyl acetate/cyclohexane, 2:3) to yield 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-D-glucono lactone (46 mg, 28%), 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-D-mannono lactone (38 mg, 23%) and a mixed fraction containing both products (15 mg, 9%).

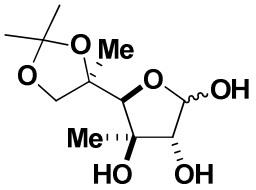
Data for the gluco *lactone*: HRMS (ESI+ve): found 269.1002 $[M+Na]^+$; $C_{11}H_{18}Na_1O_6$ requires 269.0996; m.p. 78-82°C[†]; $[\alpha]_D^{18}$ +31.4 (*c*, 1.26 chloroform); ν_{max} (thin film): 3334 (br, OH), 1771

[†] Due to the small sample size, recrystallisation was not possible, hence the difference between this value and that of its enantiomer.

(s, C=O); LRMS (ESI-ve): 245 ([M-H]$^-$, 55%), 305 ([M+CH_3CO_2]$^-$, 100%); The ^{1}H and ^{13}C NMR spectra were identical to those of the enantiomer **123** above.

Data for the manno *lactone*: HRMS (ESI+ve): found 269.0993 [M+Na]$^+$; $C_{11}H_{18}Na_1O_6$ requires 269.0996; $[\alpha]_D^{20}$ +14.9 (*c*, 1.26 acetone); ν_{max} (thin film): 3334 (br, OH), 1776 (s, C=O); LRMS (ESI-ve): 245 ([M-H]$^-$, 92%); The ^{1}H and ^{13}C NMR spectra were identical to those of the enantiomer **124** above.

3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-D-glucose

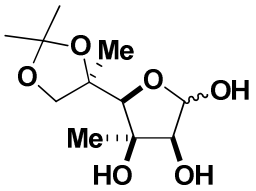


3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-D-glucono lactone (20 mg, 0.081 mmol) was dissolved in anhydrous tetrahydrofuran (1 mL) and cooled to -50°C. Di*iso*butylaluminium hydride solution (1.5 M in toluene; 0.22 mL, 0.325 mmol) was added and the reaction mixture stirred under nitrogen. T.l.c. analysis (ethyl acetate) after one hour revealed complete consumption of starting material (R_f 0.68) and the formation of a single product (R_f 0.28). The reaction mixture was quenched with methanol until the evolution of gas had ceased and diluted with ethyl acetate (2 mL). Sodium hydrogen carbonate solution (sat. aq.) (0.5 mL), and potassium dihydrogen orthophosphate (100 mg) were added, and the resulting gel stirred for 30 minutes. The mixture was dried (magnesium sulfate) and filtered through a pad of Celite®, eluting with ethyl acetate (20 mL). The solvents were concentrated under reduced pressure, and the crude purified by column chromatography (ethyl acetate/cyclohexane, 1:1 → ethyl acetate) afford 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-D-glucose (13 mg, 64%) as a colourless oil, in a 1:1 ratio of anomers.

HRMS (ESI+ve): found 271.1153 [M+Na]$^+$; $C_{11}H_{20}Na_1O_6$ requires 271.1152; $[\alpha]_D^{20}$ +20.9 (*c*, 0.66 ethyl acetate); ν_{max} (thin film): 3406 (br, OH); LRMS (ESI-ve): 247 ([M-H]$^-$, 35%), 293

([M+HCOO]$^-$, 50%). The ^{1}H and ^{13}C NMR spectra were identical to those of the enantiomer **129** above.

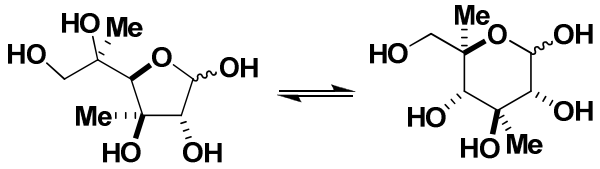
3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-D-mannose



3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-D-mannono lactone (31 mg, 0.13 mmol) was dissolved in anhydrous tetrahydrofuran (1 mL) and cooled to -50°C. Di*iso*butylaluminium hydride solution (1.5 M in toluene; 0.34 mL, 0.50 mmol) was added and the reaction mixture stirred under nitrogen. T.l.c. analysis (ethyl acetate) after 1 hour revealed partial consumption of starting material (R_f 0.60) and the formation of a single product (R_f 0.28). A further 85 µL of regent was added and t.l.c. analysis (ethyl acetate) after a further 30 minutes showed complete consumption of starting material. The reaction mixture was quenched with methanol until the evolution of gas had ceased and diluted with ethyl acetate (2 mL). Sodium hydrogen carbonate solution (sat. aq., 1 mL), and potassium dihydrogen orthophosphate (100 mg) were added, and the resulting gel stirred for 30 minutes. The mixture was dried (magnesium sulfate) and filtered through a pad of Celite®, eluting with ethyl acetate (20 mL). The solvents were concentrated under reduced pressure, and the crude purified by column chromatography (ethyl acetate/cyclohexane 1:1 → ethyl acetate) afford 3,5-di-*C*-methyl-5,6-*O*-isopropylidene-D-glucose (10 mg, 32%) as a colourless oil, in a 1:1 ratio of anomers.

HRMS (ESI+ve): found 271.1155 [M+Na]$^+$; $C_{11}H_{20}Na_1O_6$ requires 271.1152; $[\alpha]_D^{21}$ +2.8 (*c*, 1.07 ethyl acetate); ν_{max} (thin film): 3406 (br, OH); LRMS (ESI-ve): 187 (100%), 247 ([M-H]$^-$, 100%). The ^{1}H and ^{13}C NMR spectra were identical to those of the enantiomer **129** above.

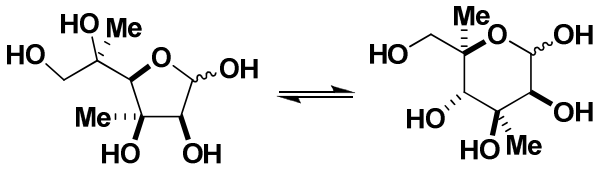
3,5-Di-*C*-methyl-D-glucose 1



3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-D-glucose (11 mg, 44 µmol) was dissolved in water (1 mL) and Dowex® (50W X8 H^+ form, 80 mg) was added. T.l.c. analysis (ethyl acetate) after 90 minutes showed complete consumption of starting material (R_f 0.28) and the formation of a single product (R_f 0.00). The resin was filtered away and the solvents removed under reduced pressure to afford crude 3,5-di-*C*-methyl-D-glucose (8 mg). This was preadsorbed onto silica and purified by column chromatography (ethyl acetate → 20% methanol in ethyl acetate) to afford 3,5-di-*C*-methyl-D-glucose **1** (4 mg, 43%) as a colourless oil.

HRMS (ESI+ve): found 231.0838 $[M+Na]^+$; $C_8H_{16}Na_1O_6$ requires 231.0839; $[\alpha]_D^{25}$ -18.7 (*c*, 0.70 water); ν_{max} (thin film): 3383 (br, OH); LRMS (ESI-ve): 207 ($[M-H]^-$, 100%). The ^1H and ^{13}C NMR spectra were identical to those of the enantiomer **97** above.

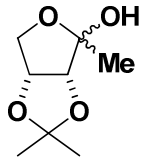
3,5-Di-*C*-methyl-D-mannose 144



3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-D-mannose (10 mg, 40 µmol) was dissolved in water (0.5 mL) and Dowex® (50W X8 H^+ resin, 40 mg) was added. T.l.c. analysis (ethyl acetate) after 20 hours showed complete consumption of starting material (R_f 0.28) and the formation of a single product (R_f 0.00). The resin was removed by filtration and the solvents concentrated under reduced pressure. The crude was re-dissolved in methanol and preadsorbed onto silica. Purification by flash column chromatography (ethyl acetate → 20% methanol in ethyl acetate) afforded 3,5-di-*C*-methyl-D-mannose **144** (6 mg, 72%) as a pale brown oil.

HRMS (ESI+ve): found 231.0835 $[M+Na]^+$; $C_8H_{16}Na_1O_6$ requires 231.0839; ν_{max} (thin film): 3387 (br, OH); LRMS (ESI-ve): 207 ($[M-H]^-$, 100%). The ^1H and ^{13}C NMR spectra were identical to those of the enantiomer **128** above. Due to the small sample size, the optical rotation was not measurable.

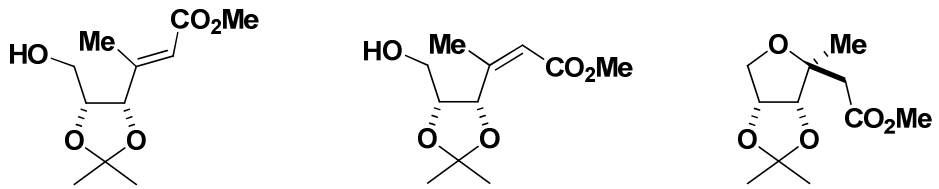
1-Deoxy-3,4-*O*-isopropylidene-D-*erythro*-pentulofuranose 92



2,3-*O*-Isopropylidene-D-erythronolactone (1.07 g, 6.77 mmol) was dissolved in dry tetrahydrofuran (10 mL), the solution was cooled to -78°C and methyl magnesium bromide (3 M solution in ether, 2.37 mL, 7.11 mmol) was added dropwise. The reaction was stirred at -78°C for 45 minutes after which time ammonium chloride (sat aq., 10 mL) was added and the reaction allowed to warm to room temperature. The mixture was partitioned between ethyl acetate (30 mL) and water (30 mL). The aqueous layer was extracted with ethyl acetate (2 x 30 mL) and then the combined organics were dried (sodium sulfate) and concentrated under reduced pressure. The crude was purified by column chromatography (ethyl acetate/cyclohexane, 1:2) to give the ketose **92** as a white crystalline solid in an 8:1 mixture of anomers (909 mg, 77%).

m.p. 82 - 85°C, [Lit.[166] 87-89°C]; ν_{max}(thin film): 3423 (m, br, OH); δ_H ($CDCl_3$, 400 MHz): *(NMR data for major anomer only):* 1.31 (3H, s, $C(CH_3)_2$), 1.46 (3H, s, $C(CH_3)_2$), 1.51 (3H, s, CH_3), 3.89 (1H, d, H5, $J_{5,5'}$ 10.2), 3.97 - 4.00 (1H, dd, H5', *J* 3.8, $J_{5,5'}$ 10.3), 4.38 (1H, d, H3, $J_{3,4}$ 6.0), 4.82 - 4.85 (1H, m, H4); δ_C ($CDCl_3$, 100 MHz): 22.3 (C1), 25.0 ($C(\mathbf{C}H_3)_2$), 26.3 ($C(\mathbf{C}H_3)_2$), 70.9 (C5), 80.8 (C4), 85.0 (C3), 105.9 (C2), 112.3 ($\mathbf{C}(CH_3)_2$); LRMS (ESI-ve): 173 ($[M-H]^-$, 100%).

(4*S*, 5*R*, *E*)-Methyl 6-hydroxy-4,5-*O*-isopropylidene-3-methyl hex-2-enoate 150, (4*S*, 5*R*, *Z*)-methyl 6-hydroxy-4,5-*O*-isopropylidene-3-methyl hex-2-enoate and methyl 3,6-anhydro-2-deoxy 4,5-*O*-isopropylidene-3-*C*-methyl-D-allonate (or D-*ribo*-hexanoate) 151



$[Bu_3PCH_2CO_2Me]^+Br^-$ (2.40 g, 6.77 mmol) was dissolved in dichloromethane (30 mL) and shaken with sodium hydroxide (1 M aq., 30 mL) for 5 min. The organic layer was washed with brine (2 x 20 mL), dried (magnesium sulfate) and the solvents concentrated under reduced pressure. A solution of 1-deoxy-3,4-isopropylidine-D-erythro-pentolo furanose **92** (471 mg, 2.71 mmol) in toluene (5.5 mL) was added to the residue and the reaction mixture stirred at 40°C for 48 hours. T.l.c. analysis (ethyl acetate/cyclohexane 1:1) showed the formation of two UV active products (R_f 0.42 and 0.38). Purification by flash column chromatography (ethyl acetate/cyclohexane, 1:4 → 1:1) afforded (4*S*, 5*R*, *Z*)-methyl 6-hydroxy-4,5-*O*-isopropylidene-3-methyl hex-2-enoate (R_f 0.42, 89 mg, 14%), (4*S*, 5*R*, *E*)-methyl 6-hydroxy-4,5-*O*-isopropylidene-3-methyl hex-2-enoate **150** (R_f 0.38, 497 mg, 80%) as colourless oils. If the reaction was allowed to remain at 40°C for greater than 48 hours a third product, a pale yellow oil, was obtained which was found to be methyl 3,6-anhydro-2-deoxy 4,5-*O*-isopropylidene-3-*C*-methyl-D-allonate **151**.

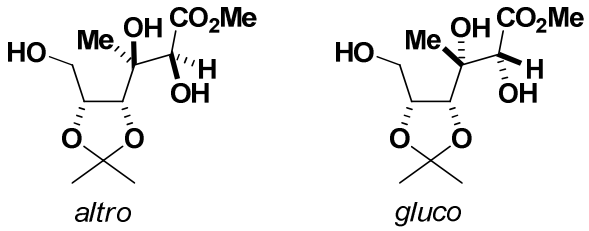
Data for ***(4S, 5R, E)-methyl 6-hydroxy-4,5-O-isopropylidene-3-methyl hex-2-enoate***: HRMS (ESI+ve) found: 253.1046 $[M+Na]^+$; $C_{11}H_{18}Na_1O_5$ requires: 253.1046; $[\alpha]_D^{17}$ +41.7 (*c*, 1.28 chloroform); ν_{max} (thin film): 3423 (s, br, OH), 1719 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.31 (3H, s, $C(CH_3)_2$), 1.45 (3H, s, $C(CH_3)_2$), 2.05 (3H, a-d, CH_3-3, *J* 1.3), 2.52 (1H, br, s, OH), 3.31 (1H, dd, H6, $J_{6,5}$ 4.8, $J_{6,6'}$ 11.4), 3.35 (1H, dd, H6', $J_{5,6'}$ 7.7, $J_{6',6}$ 11.4), 3.62 (3H, s, OMe), 4.31 (1H, ddd, H5, $J_{5,6}$ 4.9, $J_{5,4}$ 6.9, $J_{6',5}$ 7.5), 4.58 - 4.61 (1H, a-dd, H4, *J* 1.1, *J* 6.7), 5.97 (1H, a-t, H2, *J* 1.4); δ_C ($CDCl_3$, 100 MHz): 21.0 (CH_3-3), 27.8 ($C(\mathbf{C}H_3)_2$), 25.9 ($C(\mathbf{C}H_3)_2$), 51.1 (OMe), 61.7 (C6), 78.2

(C5), 82.9 (C4), 109.3 (**C**$(CH_3)_2$), 115.8 (C2), 157.7 (C3), 166.7 (C1); LRMS (ESI-ve): 229 ($[M-H]^-$, 70%).

Data for ***(4S, 5R, Z)-methyl 6-hydroxy-4,5-O-isopropylidene-3-methyl hex-2-enoate***: HRMS (ESI+ve) found: 253.1046 $[M+Na]^+$; $C_{11}H_{18}Na_1O_5$ requires: 253.1046; $[\alpha]_D^{21}$ +45.7 (*c*, 0.6 chloroform); ν_{max} (thin film): 3444 (s, br, OH), 1644 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.40 (3H, s, $C(CH_3)_2$), 1.54 (3H, s, $C(CH_3)_2$), 1.97 - 1.98 (3H, m, CH_3-3), 3.45 (1H, dd, H6, $J_{6,5}$ 5.9, $J_{6,6'}$ 11.7), 3.59 (1H, dd, H6', $J_{6',5}$ 3.7, $J_{6',6}$ 11.7), 3.69 (3H, s, OMe), 4.68 (1H, ddd, H5, $J_{5,6'}$ 3.7, $J_{5,6}$ 5.8, $J_{5,4}$ 8.0), 5.67 (1H, d, H4, $J_{4,5}$ 8.0), 5.84 - 5.85 (1H, m, H2); δ_C ($CDCl_3$, 100 MHz): 22.1 (C(**C**$H_3)_2$), 22.2 (C(**C**$H_3)_2$), 26.5 (CH_3-3), 51.3 (OMe), 61.5 (C6), 76.2 (C4), 78.7 (C5), 108.6 (**C**$(CH_3)_2$), 117.0 (C2), 159.0 (C3), 166.4 (C1); LRMS (ESI+ve): 280 ($[M+MeOH+NH_4]^+$, 100%).

Data for ***151****:* HRMS (ESI+ve) found: 253.1049 ($M+Na^+$); $C_{11}H_{18}Na_1O_5$ requires: 253.1046; $[\alpha]_D^{15}$ -17.8 (*c*, 1.13 chloroform); ν_{max} (thin film): 3454 (m, br, OH), 1740 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.33 (3H, s, $C(CH_3)_2$), 1.41 (3H, s, CH_3-3), 1.52 (3H, s, $C(CH_3)_2$), 2.42 (2H, a-s, H2, H2'), 3.68 (3H, s, OMe), 3.80 (1H, dd, H6, $J_{6,5}$ 4.2, $J_{6,6'}$ 11.0), 3.93 (1H, d, H6', $J_{6',6}$ 10.9), 4.58 (1H, d, H4, $J_{4,5}$ 6.2), 4.86 (1H, ddd, H5, *J* 0.7, $J_{5,6}$ 4.2, $J_{5,4}$ 6.0); δ_C ($CDCl_3$, 100 MHz): 20.2 (CH_3-3), 26.2 (C(**C**$H_3)_2$), 26.8 (C(**C**$H_3)_2$), 41.0 (C2), 51.8 (OMe), 71.0 (C6), 81.7 (C5), 83.5 (C3), 84.6 (C4), 112.5 (**C**$(CH_3)_2$), 170.6 (C1); LRMS (ESI+ve): 253 ($[M+Na]^+$, 100%).

Methyl 4,5-*O*-isopropylidene-3-*C*-methyl-D-altronoate 152 and methyl 4,5-*O*-isopropylidene-3-*C*-methyl-D-gluconate 153



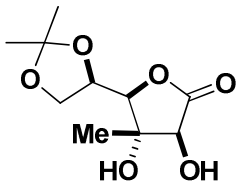
Alkene **150** (59 mg, 0.26 mmol) was dissolved in *tert*-butyl alcohol (0.95 mL) and water (0.95 mL), stirring under an atmosphere of argon. Citric acid (40 mg, 0.19 mmol) was added and allowed to dissolve before the addition of potassium osmate dihydrate (1 mg, 2.6 µmol) and 4-methylmorpholine *N*-oxide (33 mg, 0.28 mmol). The suspension was stirred at room temperature for 16 hours. After this time, t.l.c. analysis (ethyl acetate) showed complete consumption of starting material (R_f 0.64) and the formation of two products (R_f 0.43 and 0.34). The reaction mixture was concentrated under reduced pressure and the residue purified by flash column chromatography (ethyl acetate) to afford methyl 4,5-*O*-isopropylidene-3-*C*-methyl-D-gluconoate **153** (14 mg, 21%) and methyl 4,5-*O*-isopropylidene-3-*C*-methyl-D-altronoate **152** (43 mg, 63%) both as colourless oils.

*Data for **152** (major product, R_f 0.43)*: Found: C, 49.00; H, 8.11; $C_{11}H_{20}O_7$ requires: C, 49.49; H, 7.63; HRMS (ESI+ve) found: 287.1111 $[M+Na]^+$; $C_{11}H_{20}Na_1O_7$ requires: 287.1101; $[\alpha]_D^{17}$ +17.8 (*c*, 1.65 acetone); ν_{max} (thin film): 3418 (s, br, OH), 1738 (s, C=O); δ_H (acetone-d_6, 400 MHz): 1.31 (3H, s, $C(CH_3)_2$), 1.32 (3H, s, $C(CH_3)_2$), 1.41 (3H, s, CH_3-3), 3.57 - 3.62 (1H, m, H6), 3.70 (3H, s, OMe), 3.90 - 4.00 (1H, m, H6'), 4.15 (1H, d, H2, $J_{2,2OH}$ 8.0), 4.24 (1H, s, OH-6), 4.34-4.35 (1H, m, H4, H5), 4.41 (1H, d, OH-2, $J_{OH\text{-}2,2}$ 8.2), 4.44 (1H, s, OH-3); δ_C (acetone-d_6, 100 MHz): 20.2 (CH_3-3), 27.2 ($C(\mathbf{C}H_3)_2$), 27.0 ($C(\mathbf{C}H_3)_2$), 51.5 (OMe), 61.1 (C6), 75.0 (C3), 76.2 (C5), 78.7 (C4), 79.0 (C2), 107.4 ($\mathbf{C}(CH_3)_2$), 172.7 (C1); LRMS (ESI-ve): 263 ($[M-H]^-$, 100%).

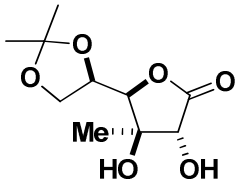
*Data for **153** (minor product R_f 0.34)*: Found: C, 49.00; H, 8.11; $C_{11}H_{20}O_7$ requires: C, 49.49; H, 7.63; HRMS (ESI+ve) found: 287.1111 $[M+Na]^+$; $C_{11}H_{20}Na_1O_7$ requires: 287.1101; $[\alpha]_D^{18}$ +19.6 (*c*

0.55 acetone); ν_{max} (thin film): 3418 (s, br, OH), 1738 (s, C=O); δ_H (acetone-d_6, 400 MHz): 1.30 (3H, s, $C(CH_3)_2$), 1.41 (3H, s, $C(CH_3)_2$), 1.33 (3H, s, CH_3-3), 2.82 (1H, s, OH), 2.85 (1H, s, OH), 3.57 - 3.62 (1H, m, H6), 3.67 - 3.73 (1H, m, H6'), 3.75 (3H, s, OMe), 4.10-4.12 (1H, m, H5), 4.26 (1H, dd, H4, *J* 1.2, 5.5), 4.48 (1H, a-d, H2, *J* 5.1), 4.43 - 4.51 (1H, m, OH). δ_C (acetone-d_6, 100 MHz): 23.3 (CH_3-3), 27.7 ($C(\mathbf{C}H_3)_2$), 26.0 ($C(\mathbf{C}H_3)_2$), 52.4 (OMe), 62.6 (C6), 74.2 (C3), 76.23 (C5), 78.9 (C4), 79.0 (C2), 108.0 ($\mathbf{C}(CH_3)_2$), 166.7 (C1); LRMS (ESI-ve): 263 ($[M-H]^-$, 100%).

5,6-*O*-Isopropylidene-3-*C*-methyl-D-glucono-1,4-lactone 155 and 5,6-*O*-isopropylidene-3-*C*-methyl-D-altrono-1,4-lactone 154



altro



gluco

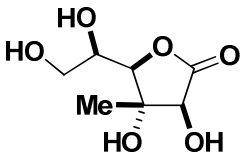
A mixture of methyl 4,5-*O*-isopropylidene-3-*C*-methyl-D-altronate **152** and methyl 4,5-*O*-isopropylidene-3-*C*-methyl-D-gluconate **153**, in a 4:3 ratio (135 mg, 0.51 mmol) were dissolved in water (4 mL) and stirred with Dowex® (50W X8 H^+ form) resin (100 mg) for 48 hours. After this time, the resin was removed by filtration, and the solution concentrated under reduced pressure to give a colourless oil (98 mg, 100%). The crude mixture of 3-*C*-methyl-D-glucono/altrono-1,5-lactone and 3-*C*-methyl-D-glucono/altrono-1,4-lactone was used without further purification. This crude was treated with acetone (2 mL), anhydrous copper(II) sulfate (163 mg, 1.02 mmol) and a catalytic quantity of concentrated sulfuric acid. T.l.c. analysis (ethyl acetate) after 48 hours revealed complete consumption of starting material (R_f 0.05, 0.15) and the presence of two major products (R_f 0.71, 0.59). The reaction mixture was neutralised with excess sodium carbonate and the salts filtered away through a pad of Celite®. The filtrate was concentrated under reduced pressure to leave an oily a residue (124 mg). Purification by flash column chromatography (ethyl

acetate/cyclohexane, 2:1) afforded 5,6-*O*-isopropylidene-3-*C*-methyl-D-glucono-1,4-lactone **155** (24 mg, 25%, R_f 0.71) and 5,6-*O*-isopropylidene-3-*C*-methyl-D-altrono-1,4-lactone **154** (53 mg, 54%, R_f 0.59).

*Data for **155** (minor product, R_f 0.71)*: HRMS (ESI-ve) found: 249.0970 $[M+OH]^-$; $C_{10}H_{17}O_7$ requires: 249.0969; $[\alpha]_D^{21}$ +74.7 (*c*, 0.25 acetone); ν_{max} (thin film): 3452 (s, br, OH), 1772 (s, C=O); δ_H (acetone-d_6, 400 MHz): 1.32 (3H, s, $C(CH_3)_2$), 1.39 (3H, s, $C(CH_3)_2$), 1.47 (3H, s, CH_3-3), 3.88 (1H, d, H2, $J_{2,OH-2}$ 5.0), 3.94 (1H, dd, H6, *J* 5.8, $J_{6,6'}$ 8.6), 4.10 (1H, dd, H6', *J* 6.5, $J_{6',6}$ 8.6), 4.33 (1H, d, H4, *J* 6.3), 4.47 (1H, a-q, H5, *J* 6.2), 4.59 (1H, s, OH-3), 5.62 (1H, d, OH-2, $J_{OH-2,2}$ 5.1); δ_C (acetone-d_6, 100 MHz): 23.3 ($C(\mathbf{C}H_3)_2$), 25.6 (CH_3-3), 27.0 ($C(\mathbf{C}H_3)_2$), 66.9 (C6), 73.6 (C5), 76.8 (C3), 77.1 (C2), 85.8 (C4), 109.6 ($\mathbf{C}(CH_3)_2$), 175.4 (C1); LRMS (ESI-ve): 203 (100%), 231 ($[M-H]^-$, 73%), 249 ($[M+OH]^-$, 50%), 291 ($[M+OCOCH_3]^-$, 45%), 463 ($[2M-H]^-$, 20%).

*Data for **154** (major product, R_f 0.59):* HRMS (ESI-ve) found: 231.0860 $[M-H]^-$; $C_{10}H_{15}O_6$ requires: 231.0863; $[\alpha]_D^{19}$ +50.4 (*c*, 0.46 acetone); ν_{max} (thin film): 3420 (s, br, OH), 1788 (s, C=O); δ_H (acetone-d_6, 400 MHz): 1.28 (3H, s, CH_3-3); 1.33 (3H, s, $C(CH_3)_2$), 1.42 (3H, s, $C(CH_3)_2$), 3.90 (1H, dd, H6, $J_{6,5}$ 5.2, $J_{6,6'}$ 8.5), 4.12 (1H, dd, H6', $J_{6',5}$ 6.2, $J_{6',6}$ 8.6), 4.19 (1H, d, H4, $J_{4,5}$ 7.4), 4.30 (1H, ddd, H5, $J_{5,6}$ 5.3, $J_{5,6'}$ 6.2, $J_{5,4}$ 7.3), 4.41 (1H, d, H2, $J_{2,OH-2}$ 5.7), 4.44 (1H, s, OH-3), 5.24 (1H, d, OH-2, $J_{OH-2,2}$ 5.8); δ_C (acetone-d_6, 100 MHz): 16.2 (CH_3-3), 25.5($C(\mathbf{C}H_3)_2$), 27.0 ($C(\mathbf{C}H_3)_2$), 67.1 (C6), 74.4 (C5), 76.7 (C2), 78.1 (C3), 83.6 (C4), 110.1 ($\mathbf{C}(CH_3)_2$), 174.3 (C1); LRMS (ESI-ve): 231 ($[M-H]^-$, 100%).

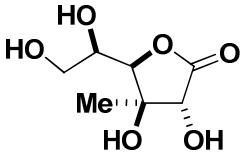
3-*C*-Methyl-D-altrono-1,4-lactone 156



Protected altronolactone **154** (28 mg, 0.12 mmol) was dissolved in water (0.5 mL) and Dowex® (50W X8 H^+ form) was added. The reaction was stirred at room temperature for 16 hours, after which time t.l.c. (ethyl acetate) showed the formation of one major product (R_f 0.15) and a small amount of unreacted starting material (R_f 0.59). After 72 hours the reaction was filtered and concentrated under reduced pressure and purified by column chromatography (20% methanol in ethyl acetate) to give 3-*C*-methyl-D-altrono-1,4-lactone **156** as a colourless oil (21 mg, 88%).

HRMS (ESI+ve) found: 215.0526 $[M+Na]^+$; $C_7H_{12}Na_1O_6$ requires: 215.0526; $[\alpha]_D^{22}$ +63.3 (*c*, 0.15 methanol); ν_{max} (thin film): 3385 (s, br, OH), 1782 (m, C=O); δ_H (D_2O, 500 MHz): δ_H (D_2O, 500 MHz): 1.31 (3H, s, CH_3-3), 3.69 (1H, dd, H6, $J_{6,5}$ 5.6, $J_{6,6'}$12.2), 3.79 (1H, dd, H6', $J_{6',5}$ 2.8, 12.3), 3.92 (1H, ddd, H5, $J_{5,6'}$ 2.8, $J_{5,6}$ 5.6, $J_{5,4}$ 9.2), 4.25 (1H, d, H4, $J_{4,5}$ 9.1), 4.64 (1H, s, H2); δ_C (D_2O, 125 MHz): 14.5 (CH_3-3), 63.4 (C6), 69.7 (C5), 76.3 (C2), 78.7 (C3), 80.5 (C4), 176.5 (C1); LRMS (ESI-ve): 191 ($[M-H]^-$, 100%).

3-*C*-Methyl-D-glucono-1,4-lactone 157

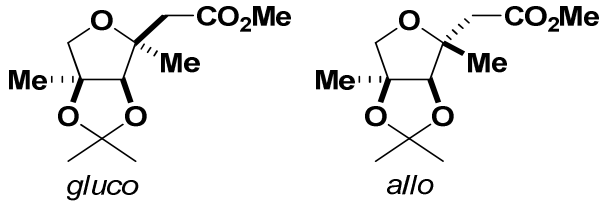


Protected gluconolactone **155** (16 mg, 0.07 mmol) was dissolved in water (1 mL) and Dowex® (50W X8 H^+ form) was added. The reaction was stirred at room temperature for 48 hours, after which time t.l.c. (ethyl acetate) showed the formation of one product (R_f 0.05) and unreacted starting material (R_f 0.71). The reaction was heated to 70°C and after a further 3 hours the reaction mixture was filtered and concentrated under reduced pressure and purified by column

chromatography (10% isopropanol in ethyl acetate) to give 3-*C*-methyl-D-glucono-1,4-lactone **157** as a colourless oil (7 mg, 56%).

HRMS (ESI+ve) found: 215.0533 $[M+Na]^+$; $C_7H_{12}Na_1O_6$ requires: 215.0526; $[\alpha]_D^{20}$ +47.1 (*c*, 0.48 water); ν_{max} (thin film): 3148 (s, br, OH), 1772 (s, C=O); δ_H (D_2O, 500 MHz): 1.35 (3H, s, CH_3), 3.67 (1H, dd, H6, $J_{6,5}$ 3.4, $J_{6,6'}$ 12.1), 3.75 (1H, dd, H6′, $J_{6',5}$ 5.8, $J_{6',6}$ 12.1), 4.00 (1H, ddd, H5, $J_{5,6}$ 3.4, $J_{5,6'}$ 5.9, $J_{5,4}$ 6.9), 4.29 (1H, d, H4, $J_{4,5}$ 7.1), 4.40 (1H, s, H2); δ_C (D_2O, 125 MHz): 19.8 (CH_3), 62.6 (C6), 69.9 (C5), 74.7 (C2), 76.7 (C3), 84.5 (C4), 176.8 (C1); LRMS (ESI-ve): 191 ($[M-H]^-$, 100%).

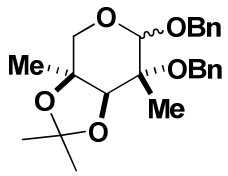
Methyl 3,6-anhydro-2-deoxy-4,5-*O*-isopropylidene-3,5-di-*C*-methyl-L-gluconate and allonate 160



Preparation of Wittig reagent: $[Bu_3PCH_2CO_2Me]^+Br^-$ (504 mg, 1.43 mmol) was dissolved in dichloromethane (10 mL) and shaken vigorously for 10 minutes with sodium hydroxide solution (1M aq., 10 mL). The organic layer was washed with brine (2 x 10 mL), dried ($MgSO_4$) and the solvents concentrated under reduced pressure. A solution of 1-deoxy-3,-4-*O*-isopropylidene-4-*C*-methyl-L-ribulose **102** (108 mg, 0.57 mmol) in toluene (1.5 mL) was added to the ylid and the reaction mixture heated to 40°C for 48 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed the formation of a single product (R_f 0.69, faintly UV active) and almost complete consumption of starting material (R_f 0.60). The solvents were concentrated under reduced pressure and purification by column chromatography (ethyl acetate/cyclohexane, 1:8 → 1:6) afforded and inseparable mixture of the title compounds **160** (104 mg, 70%) as a colourless oil in a 1:1.2 ratio and unreacted starting material **102** (19 mg).

HRMS (FI+ve): found 244.1306 $[M]^{\cdot}$; $C_{12}H_{20}O_5$ requires 244.1311; $[\alpha]_D^{21}$ +33.6 (*c*, 0.93 chloroform); ν_{max} (thin film): 1740 (s, C=O); δ_H (C_6D_6, 500 MHz): 1.30 (3H, s, CH_3-5$^{\mathbf{B}}$), 1.32 (3H, s, CH_3-3$^{\mathbf{B}}$), 1.35 (3H, s, CH_3-5$^{\mathbf{A}}$), 1.38 (3H, s, $C(CH_3)_2{}^{\mathbf{B}}$), 1.42 (3H, s, $C(CH_3)_2{}^{\mathbf{A}}$), 1.58 (3H, s, $C(CH_3)_2{}^{\mathbf{B}}$), 1.61 (3H, s, $C(CH_3)_2{}^{\mathbf{A}}$), 1.77 (3H, s, CH_3-3$^{\mathbf{A}}$), 2.28 (1H, d, H2$^{\mathbf{A}}$, J_{gem} 13.6), 2.42 (1H, d, H2'$^{\mathbf{A}}$, J_{gem} 13.6), 2.89 (1H, d, H2$^{\mathbf{B}}$, J_{gem} 16.4), 3.29 (1H, d, H2'$^{\mathbf{B}}$, J_{gem} 16.7), 3.41 (3H, s, OMe$^{\mathbf{A}}$), 3.42 (3H, s, OMe$^{\mathbf{B}}$), 3.45 (1H, d, H6$^{\mathbf{B}}$, J_{gem} 10.1), 3.55 (1H, d, H6$^{\mathbf{A}}$, J_{gem} 10.4), 4.02 (1H, d, H6'$^{\mathbf{B}}$, J_{gem} 10.1), 4.07 (1H, d, H6'$^{\mathbf{A}}$, J_{gem} 10.4), 4.20 (1H, s, H4$^{\mathbf{B}}$), 4.31 (1H, s, H4$^{\mathbf{A}}$); δ_C (C_6D_6, 125 MHz): 20.1 (CH_3-3$^{\mathbf{B}}$), 20.6 (CH_3-3$^{\mathbf{A}}$), 24.2 (CH_3-5$^{\mathbf{B}}$), 24.3 (CH_3-5$^{\mathbf{A}}$), 27.6 (2 x $C(CH_3)_2{}^{\mathbf{B}}$), 27.7 ($C(CH_3)_2{}^{\mathbf{A}}$), 27.8 ($C(CH_3)_2{}^{\mathbf{A}}$), 40.8 (C2$^{\mathbf{A}}$), 41.0 (C2$^{\mathbf{B}}$), 50.9 (OMe$^{\mathbf{B}}$), 51.2 (OMe$^{\mathbf{A}}$), 76.6 (C6$^{\mathbf{B}}$), 77.2 (C6$^{\mathbf{A}}$), 83.7 (C3$^{\mathbf{B}}$), 84.4 (C3$^{\mathbf{A}}$), 90.1, 90.4, (C5$^{\mathbf{A\&B}}$) 90.7 (C4$^{\mathbf{B}}$), 91.1 (C4$^{\mathbf{A}}$), 112.1 ($C(CH_3)_2{}^{\mathbf{B}}$), 112.5 ($C(CH_3)_2{}^{\mathbf{A}}$), 170.2 (C1$^{\mathbf{A}}$), 171.1 (C1$^{\mathbf{B}}$).

Benzyl 2-*O*-Benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinopyranoside 167

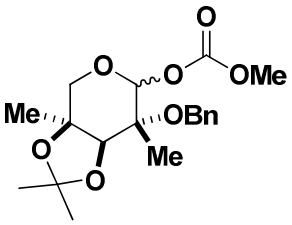


Lactol **114** (25 mg, 81 µmol) was dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL). Barium oxide (50 mg, 0.33 mmol), barium hydroxide octahydrate (13 mg, 41 µmol) and benzyl bromide (29 µL, 0.24 mmol) were added and the reaction flask wrapped in foil and placed in an ultrasonication bath at 25°C. T.l.c. analysis (ethyl acetate/cyclohexane, 1:6) after 1 hour revealed complete consumption of starting material (R_f 0.22) and the formation of a single compound (R_f 0.59). The reaction mixture was diluted with water (10 mL) and extracted with dichloromethane (3 x 10 mL). The combined organic fractions were dried (sodium sulfate), filtered, and concentrated under reduced pressure; subsequent purification by flash column chromatography (ethyl

acetate/cyclohexane, 1:10) gave benzyl glycoside **167** (26 mg, 80%) as a colourless oil and as a single anomer.

HRMS (ESI+ve): found 421.1978 $[M+Na]^+$; $C_{24}H_{30}Na_1O_5$ requires 421.1985; $[\alpha]_D^{18}$ +39.6 (*c*, 0.80 chloroform); δ_H (C_6D_6, 500 MHz): 1.40 (3H, s, $C(CH_3)_2$), 1.50 (3H, s, CH_3-4), 1.60 (3H, s, CH_3-2), 1.61 (3H, s, $(CH_3)_2$), 3.79 (1H, d, H5, $J_{5,5'}$ 11.5), 3.96 (1H, s, H3), 4.16 (1H, d, H5', $J_{5',5}$ 11.4), 4.52 (1H, d, anomeric CH_2Ph, J_{gem} 11.8), 4.72 (1H, d, CH_2Ph, J_{gem} 11.4), 4.91 (1H, s, H1), 5.04 (1H, d, anomeric CH_2Ph, J_{gem} 12.0), 5.24 (1H, d, CH_2Ph, J_{gem} 11.5), 7.19-7.32 (6H, m, Ar-H), 7.39-7.41 (2H, m, Ar-H), 7.46-7.48 (2H, m, Ar-H); δ_C (C_6D_6, 100 MHz): 18.4 (CH_3-2), 24.4 (CH_3-4), 26.9 ($C(\mathbf{C}H_3)_2$), 27.9 ($C(\mathbf{C}H_3)_2$), 67.4 (C5), 70.4 ($\mathbf{C}H_2Ph$), 70.5 ($\mathbf{C}H_2Ph$), 74.8 (C2), 79.2 (C4), 84.4 (C3), 102.0 (C1), 108.5 ($\mathbf{C}(CH_3)_2$), 127.3, 127.6, 127.8, 127.9, 128.0, 128.2, 128.4, 128.5 (Ar-C), 138.8 (Ar-C_{quat}), 140.5 (Ar-C_{quat}); LRMS (ESI+ve): 416 ($[M+NH_4]^+$, 100%).

Methoxy formyl 2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinopyranoside 162

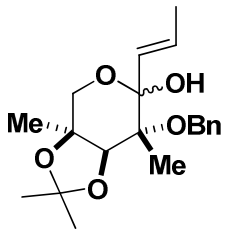


Lactol **114** (50 mg, 0.16 mmol) was dissolved in dichloromethane (0.1 mL). Triethylamine (2 µL, 0.1 µmol) and methyl cyanoformate (14 µL, 17 mmol) were added and the reaction mixture stirred at room temperature. T.l.c analysis (ethyl acetate/cyclohexane, 1:4) after 10 minutes revealed partial consumption of starting material (R_f 0.19) and the formation of a single compound (R_f 0.49). A further 2 µL of triethylamine was added and t.l.c. analysis (ethyl acetate/cyclohexane, 1:4) after 10 minutes revealed complete consumption of starting material (R_f 0.19) and a single compound (R_f 0.49). The solvents were concentrated under reduced pressure and the crude purified by column chromatography (ethyl acetate/cyclohexane, 1:9) to afford glycoside **162** as a colourless oil (56 mg, 97%)

HRMS (ESI+ve): found 389.1598 $[M+Na]^+$; $C_{19}H_{26}Na_1O_7$ requires 389.1576; $[\alpha]_D^{25}$ +42.3 (*c*, 0.73 methanol); ν_{max} (thin film): 1759 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.31 (3H, s, CH_3-4), 1.38 (3H, s, $C(CH_3)_2$), 1.47 (3H, s, $C(CH_3)_2$), 1.54 (3H, s, CH_3-2), 3.62 (1H, d, H5, $J_{5,5'}$ 11.6), 3.85 (3H, s, OMe), 3.92 (1H, s, H3), 4.05 (1H, d, H5', $J_{5',5}$ 11.6), 4.62 (1H, d, CH_2Ph, J_{gem} 11.3), 4.90 (1H, d, CH_2Ph, J_{gem} 11.3), 5.78 (1H, s, H1), 7.27-7.35 (5H, m, Ar-H); δ_C ($CDCl_3$, 100 MHz): 18.4 (CH_3-4), 24.1 (CH_3-2), 26.6 ($C(\mathbf{C}H_3)_2$), 27.3 ($C(\mathbf{C}H_3)_2$), 54.9 (OMe), 66.8 (CH_2Ph), 70.0 (C5), 74.5 (C4), 79.3 (C2), 82.9 (C3), 98.5 (C1), 109.1 ($\mathbf{C}(CH_3)_2$), 127.2, 127.3, 128.3, 128.4 (Ar-C), 139.1 (Ar-$C_{quat.}$), 155.4 (C=O); LRMS (ESI+ve): 384 ($[M+NH_4]^+$, 100%), 389 ($[M+Na]^+$, 65%), 750 ($[2M+NH_4]^+$, 100%), 755 ($[2M+Na]^+$, 90%).

1-*C*-[(*E*)-Prop-1'-enyl]-2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinopyranose

171



Benzylated lactone **113** (2.01 g, 6.55 mmol) was dissolved in diethyl ether (20 mL) and cooled to -40°C under argon. Allyl magnesium bromide solution (1 M in Et_2O, 7.2 mL, 7.21 mmol) was added and the temperature maintained at -40°C. IR analysis after 1 hour revealed the complete absence of a carbonyl peak (~1750 cm^{-1} region). The reaction mixture was quenched with saturated ammonium chloride solution (50 mL), diluted with dichloromethane (100 mL) and washed with water (5 x 100 mL). The combined organic fractions were dried (sodium sulfate), filtered and the solvent concentrated under reduced pressure. Purification of the crude by flash column chromatography (ethyl acetate/cyclohexane 1:10) afforded lactol **170** (1.66 g) as colourless oil.

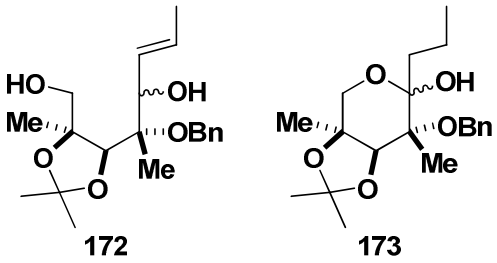
*Selected data for **170**:* ν_{max} (thin film): 3483 (s, br, O-H); LRMS (ESI-ve): 349 (100% $M-H^+$).

Lactol **170** was immediately dissolved in tetrahydrofuran (17 mL) and stirred under argon with 1,8-diazabicyclo[5.4.0]undec-7-ene (1.07 mL, 7.16 mmol). T.l.c. analysis (ethyl acetate/cyclohexane, 1:4) after 2 hours revealed partial consumption of starting material (R_f 0.38) and the formation of a single major product (R_f 0.31). The solvents were concentrated under reduced pressure to afford lactol **171** (1.09 g) and a mixed fraction containing **171** and starting material (587 mg) in approximately a 1:1 ratio, affording **171** in 60% over two steps. **171** exists as a mixture of the open chain (OC) form, A and B anomers, in a 1:1:0.5 ratio.

HRMS (ESI+ve): found 371.1824 $[M+Na]^+$; $C_{20}H_{28}Na_1O_5$ requires 371.1829; $[\alpha]_D^{25}$ -42.8 (*c*, 1.18 methanol); ν_{max} (thin film): 1695 (small, s, C=O), 1374 (s, C=C); δ_H (CD_3OD, 500 MHz): 1.27 (3H, s, CH_3-4$^{\mathbf{B}}$), 1.31 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.37 (3H, s, CH_3-4$^{\mathbf{OC}}$), 1.40 (9H, s, CH_3-2$^{\mathbf{B}}$ & $C(CH_3)_2^{\mathbf{OC\ \&\ B}}$), 1.43 (3H, s, CH_3-4$^{\mathbf{A}}$), 1.44 (6H, s, CH_3-2$^{\mathbf{A}}$ & $(C(CH_3)_2^{\mathbf{A}}$), 1.49 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.51 (3H, s, $C(CH_3)_2^{\mathbf{OC}}$), 1.59 (3H, s, CH_3-2$^{\mathbf{OC}}$), 1.74 (3H, dd, CH_3-3$^{\mathbf{'B}}$, *J* 1.4, $J_{CH3\text{-}3',2'}$ 6.7), 1.77 (3H, dd, CH_3-3$^{\mathbf{'A}}$, *J* 1.4, $J_{CH3\text{-}3',2'}$ 6.6), 1.92 (3H, dd, CH_3-3$^{\mathbf{'OC}}$, $J_{CH3\text{-}3,1'}$ 1.1, $J_{CH3\text{-}3,2'}$ 6.7), 3.31 (1H, d, H5$^{\mathbf{A}}$, $J_{5,5'}$ 11.8), 3.43 (1H, d, H5$^{\mathbf{B}}$, $J_{5,5'}$12.0), 3.54 (1H, d, H5$^{\mathbf{OC}}$, $J_{5,5'}$ 11.9), 3.68 (1H, d, H5$^{\mathbf{'OC}}$, $J_{5',5}$ 12.0), 3.90 (1H, d, H5$^{\mathbf{'B}}$, $J_{5',5}$ 12.0), 3.95 (1H, s, H3$^{\mathbf{OC}}$), 4.08 (1H, d, H5$^{\mathbf{'A}}$, $J_{5',5}$ 11.5), 4.11 (1H, s, H3$^{\mathbf{A}}$), 4.19 (1H, s, H3$^{\mathbf{B}}$), 4.48 (1H, d, $CH_2Ph^{\mathbf{OC}}$, J_{gem} 10.8), 4.54 (1H, d, $CH_2Ph^{\mathbf{A}}$, J_{gem} 11.5), 4.61 (1H, d, $CH_2Ph^{\mathbf{A}}$, J_{gem} 11.3), 4.62 (1H, d, $CH_2Ph^{\mathbf{OC}}$, J_{gem} 10.8), 4.63 (1H, d, $CH_2Ph^{\mathbf{B}}$, J_{gem} 11.3), 4.85 (1H, d, $CH_2Ph^{\mathbf{B}}$, J_{gem} 11.2), 5.72-5.76 (2H, m, H1$^{\mathbf{'A\ \&\ B}}$), 6.01-6.05 (2H, dd, H2$^{\mathbf{'A}}$, $J_{2',CH3\text{-}3'}$ 6.6, *J* 15.5), 5.93-5.99 (2H, dd, H2$^{\mathbf{'B}}$, $J_{2',CH3\text{-}3'}$ 6.6, *J* 15.5), 6.85-6.88 (1H, dd, H1$^{\mathbf{'OC}}$, $J_{1',CH3\text{-}3}$ 1.6, $J_{1',2'}$ 15.5), 6.96-7.03 (1H, dq, H2$^{\mathbf{'OC}}$, $J_{2',CH3\text{-}3}$ 6.9, $J_{2',1'}$ 15.3), 7.25-7.43 (15H, m, Ar-H$^{\mathbf{A\&B\&OC}}$); δ_C (CD_3OD, 125 MHz): 17.2 (CH_3-2$^{\mathbf{A}}$), 17.7 (CH_3-2$^{\mathbf{B}}$), 17.8 (CH_3-3$^{\mathbf{'A\&B}}$), 18.6 (CH_3-3$^{\mathbf{'OC}}$), 18.7 (CH_3-2$^{\mathbf{OC}}$), 20.2 ($C(\mathbf{C}H_3)_2^{\mathbf{B}}$), 23.7 ($C(\mathbf{C}H_3)_2^{\mathbf{A}}$), 24.3($CH_3-4^{\mathbf{A}}$), 24.6 ($CH_3-4^{\mathbf{B}}$), 26.7 ($C(\mathbf{C}H_3)_2^{\mathbf{A}}$), 27.1 ($C(\mathbf{C}H_3)_2^{\mathbf{OC}}$), 27.3 ($C(\mathbf{C}H_3)_2^{\mathbf{B}}$), 28.2 ($CH_3-4^{\mathbf{OC}}$), 28.6 ($C(\mathbf{C}H_3)_2^{\mathbf{OC}}$), 65.7 (C5$^{\mathbf{A}}$), 66.1 ($CH_2Ph^{\mathbf{A}}$), 66.4 (C5$^{\mathbf{OC}}$), 67.4 ($CH_2Ph^{\mathbf{B}}$), 68.5 (C5$^{\mathbf{B}}$ & $CH_2Ph^{\mathbf{OC}}$), 77.5 (C4$^{\mathbf{A}}$ & C2$^{\mathbf{A}}$), 78.5 (C2$^{\mathbf{B}}$), 80.9 (C4$^{\mathbf{B}}$), 82.1 (C3$^{\mathbf{A}}$), 83.1 (C3$^{\mathbf{B}}$), 84.66 (C2$^{\mathbf{OC}}$ & C4$^{\mathbf{OC}}$), 88.2 (C3$^{\mathbf{OC}}$), 98.9 (C1$^{\mathbf{B}}$), 99.6 (C1$^{\mathbf{A}}$), 108.1 ($\mathbf{C}(CH_3)_2^{\mathbf{A}}$), 109.3

(**C**$(CH_3)_2$^{OC}), 109.9 (**C**$(CH_3)_2$^{B}), 126.9, 128.2, 128.3, 128.4, 128.6, 128.6, 128.9, 128.9, 129.0, 129.3, 129.3, 129.4, 129.5, 132.4 (C=C^{A} & C=C^{OC} & Ar-C$^{A\&B\&OC}$), 132.4 (C=C^{B}), 134.2 (C=C^{B}), 134.2 (C=C^{A} or C=C^{OC} or Ar-C$^{A\ or\ B\ or\ OC}$), 139.3 (Ar-C_{quat}^{OC}), 140.2 (Ar-C_{quat}^{B}), 140.5 (Ar-C_{quat}^{A}), 144.3 (C=C^{OC}), 202.7 (C1^{OC}). LRMS (ESI-ve): 347 ([M-H]$^-$, 100%).

1-*C*-[(*E*)-Prop-1'-enyl]-2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinitol 172 and 1-*C*-Propanyl-2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose 173



172 **173**

Method 1: Lactol **171** (104 mg, 0.30 mmol) was dissolved in methanol (0.8 mL) and cooled to -10°C. Cerium trichloride (111 mg, 0.30 mmol) was added and the reaction mixture stirred for 5 minutes under argon, then sodium borohydride (11 mg, 0.30 mmol) was added and the reaction stirred at -10°C. T.l.c analysis (ethyl acetate/cyclohexane, 1:1) after 2 hours revealed partial consumption of starting material (R_f 0.73) and the formation of a single major product (R_f 0.60). The reaction was quenched by the addition of water (1 mL) and the products extracted with ethyl acetate (3 x 5 mL). The combined organic fractions were dried (sodium sulfate), filtered, and concentrated under reduced pressure. Purification by column chromatography (ethyl acetate/cyclohexane, 1:5 → 1:3) gave starting material (40 mg) and arabinitol **172** (50 mg, 48%, 78% based on recovered starting material) as a colourless oil in a 1:0.85 ratio of diastereomers A:B.

Method 2: Lactol **171** (88 mg, 0.25 mmol) was dissolved in dry tetrahydrofuran (0.9 mL) and cooled to 0°C under argon. $LiAlH_4$ solution (2 M in THF; 139 µL, 0.28 mmol) was added and the reaction mixture stirred at 0°C for 2 hours, after which time t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) revealed the complete consumption of starting material (R_f 0.73) and the

formation of two products (R_f 0.60 & 0.85). The reaction was quenched with ammonium chloride (sat., aq., 1 mL) and the products extracted with ethyl acetate (3 x 5 mL). The combined organic fractions were dried (sodium sulfate), filtered, and concentrated under reduced pressure. Purification by column chromatography (ethyl acetate/cyclohexane, 1:5 → 1:3) gave arabinitol **172** (R_f 0.60, 49 mg, 55%) in a 1:1 a mixture of diastereomers and **173** (R_f 0.85, 42 mg, 48%) in a 2:1 mixture of diastereomers, both as colourless oils.

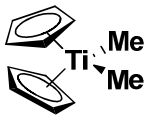
Method 3: Lactol **171** (95 mg, 0.27 mmol) was dissolved in dry tetrahydrofuran (1 mL) and cooled to 0°C under argon. Red-Al® solution (70% in toluene; 90 µL, 0.32 mmol) was added and the reaction mixture stirred at 0°C for 1 hour, after which time t.l.c. analysis revealed the complete consumption of starting material (R_f 0.73) and the formation of two products (R_f 0.60 & 0.85). The reaction was quenched with sodium potassium tartrate solution (sat., aq., 5 mL) and the products extracted with ethyl acetate (3 x 5 mL). The combined organic fractions were dried (sodium sulfate), filtered, and concentrated under reduced pressure. Purification by column chromatography (ethyl acetate/cyclohexane 1:5 → 1:3) gave arabinitol **172** (R_f 0.60, 49 mg, 51%) in a 1:1 mixture of diastereomers and **173** (R_f 0.85, 40 mg, 42%) in a 2:1 mixture of diastereomers.

*Data for **172**:* HRMS (ESI+ve): found 373.1824 $[M+Na]^+$; $C_{20}H_{28}Na_1O_5$ requires 373.1829; ν_{max} (thin film): 3453 (s, br, OH), 1379 (s, C=C). δ_H ($CDCl_3$, 500 MHz): 1.29 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.31 (3H, s, CH_3-$2^{\mathbf{B}}$), 1.32 (3H, s, CH_3-$4^{\mathbf{B}}$), 1.36 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.42 (3H, s, CH_3-$2^{\mathbf{A}}$), 1.43 (3H, s, CH_3-$4^{\mathbf{A}}$), 1.51 (6H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.77 (6H, s, CH_3-$3'^{\mathbf{A\&B}}$), 3.24 (1H, a-dd, $OH^{\mathbf{B}}$, *J* 5.9, *J* 9.1), 3.32-3.36 (1H, m, $H5^{\mathbf{A}}$), 3.39-3.44 (1H, m $H5'^{\mathbf{A}}$), 3.45-3.51 (2H, m, OH), 3.71-3.76 (2H, m, $H5^{\mathbf{B}}$ & $H5'^{\mathbf{B}}$), 3.77 (1H, s, $H3^{\mathbf{A}}$), 3.84 (1H, s, $H3^{\mathbf{B}}$), 4.36-4.39 (1H, m, $H1^{\mathbf{B}}$), 4.55 (1H, d, $CH_2Ph^{\mathbf{B}}$, J_{gem} 9.9), 4.79 (1H, dd, $H1^{\mathbf{A}}$, *J* 1.7, *J* 7.9), 4.85 (1H, d, $CH_2Ph^{\mathbf{B}}$, J_{gem} 9.9), 4.94 (1H, d, $CH_2Ph^{\mathbf{A}}$, J_{gem} 11.4), 5.05 (1H, d, $CH_2Ph^{\mathbf{A}}$, J_{gem} 11.4), 5.49-5.59 (2H, m, $H1'^{\mathbf{A\&B}}$), 5.83-5.93 (2H, m, $H2'^{\mathbf{A\&B}}$), 7.29-7.40 (10H, m, Ar-$H^{\mathbf{A\&B}}$); δ_C ($CDCl_3$, 125 MHz): 16.8 (CH_3-$2^{\mathbf{B}}$), 18.0, 18.6 (CH_3-$3'^{\mathbf{A\&B}}$), 23.2, 23.3 (CH_3-$2^{\mathbf{A}}$ & CH_3-$4^{\mathbf{A}}$ & CH_3-$4^{\mathbf{B}}$), 26.4 ($C(CH_3)_2^{\mathbf{A}}$), 27.4 ($C(CH_3)_2^{\mathbf{A}}$), 28.3 (2 x $C(CH_3)_2^{\mathbf{B}}$), 64.7

($CH_2Ph^{\mathbf{B}}$), 65.4 ($C5^{\mathbf{B}}$), 66.1 ($C5^{\mathbf{A}}$), 67.5 ($CH_2Ph^{\mathbf{A}}$), 73.9 ($C3^{\mathbf{B}}$), 76.8 ($C3^{\mathbf{A}}$), 77.3 ($C2^{\mathbf{B}}$), 79.1 ($C2^{\mathbf{A}}$), 82.7 ($C4^{\mathbf{B}}$), 83.1 ($C4^{\mathbf{A}}$), 85.7 ($C1^{\mathbf{B}}$), 86.5 ($C1^{\mathbf{A}}$), 106.0, 106.7 ($\mathbf{C}(CH_3)_2^{\mathbf{A\&B}}$), 127.7, 128.0, 128.1, 128.3, 128.6, 128.7, 128.8, 128.9, 129.6, 130.7 (Ar-$C^{\mathbf{A\&B}}$ and C=$C^{\mathbf{A\&B}}$), 137.3, 138.1 (Ar-$C_{quat}{}^{\mathbf{A\&B}}$); LRMS (ESI-ve): 349 ($[M-H]^-$, 100%), 395 ($[M+HCOO]^-$, 85%), 409 ($[M+ CH_3COO]^-$, 85%).

*Data for **173**:* HRMS (ESI+ve): found 409.2690 $[M+MeCN+NH_4]^+$; $C_{22}H_{37}N_2Na_1O_5$ requires 409.2697; $[\alpha]_D^{20}$ -13.1 (*c*, 1.12 chloroform); ν_{max} (thin film): 3454 (s, br, OH); δ_H (C_6D_6, 500 MHz): 1.13-1.19 (6H, m, CH_3-$3'^{\mathbf{A\&B}}$), 1.31 (3H, s, $C(CH_3)_2{}^{\mathbf{A}}$), 1.34 (3H, s, $C(CH_3)_2{}^{\mathbf{B}}$), 1.42 (3H, s, $C(CH_3)_2{}^{\mathbf{A}}$), 1.43 (3H, s, CH_3-$2^{\mathbf{B}}$), 1.46 (3H, s, CH_3-$4^{\mathbf{B}}$), 1.50 (3H, s, CH_3-$4^{\mathbf{A}}$), 1.56 (3H, s, CH_3-$2^{\mathbf{A}}$), 1.60 (3H, s, $C(CH_3)_2{}^{\mathbf{B}}$), 1.86-1.88 (1H, m, $H1'^{\mathbf{B}}$), 1.89-1.95 (1H, m, $H1'^{\mathbf{A}}$), 2.06-2.14 (4H, m, $H2'^{\mathbf{A\&B}}$ & $H2''^{\mathbf{A\&B}}$), 2.26-2.31 (1H, m, $H1''^{\mathbf{A}}$), 2.37-2.43 (1H, m, $H1''^{\mathbf{B}}$), 3.69 (1H, d, $H5^{\mathbf{A}}$, $J_{5,5'}$ 12.6), 3.74 (1H, d, $H5^{\mathbf{B}}$, $J_{5,5'}$ 12.0), 3.79 (1H, d, $H5'^{\mathbf{B}}$, $J_{5',5}$ 12.1), 4.03 (1H, s, $H3^{\mathbf{B}}$), 4.04 (1H, s, $H3^{\mathbf{A}}$), 4.17 (1H, $H5'^{\mathbf{A}}$, $J_{5',5}$ 12.1), 4.26 (1H, d, $CH_2Ph^{\mathbf{B}}$, J_{gem} 11.0), 4.33 (1H, d, $CH_2Ph^{\mathbf{A}}$, J_{gem} 11.4), 4.42 (1H, d, $CH_2Ph^{\mathbf{B}}$, J_{gem} 11.2), 4.45 (1H, d, $CH_2Ph^{\mathbf{A}}$, J_{gem} 10.1), 4.54 (1H, d, $OH^{\mathbf{B}}$, *J* 1.7), 4.83 (1H, d, $OH^{\mathbf{A}}$, *J* 1.7), 7.22-7.40 (10H, m, Ar-$H^{\mathbf{A\&B}}$); δ_C (C_6D_6, 125 MHz): 15.2 ($C2'^{\mathbf{A}}$), 16.4 ($C2'^{\mathbf{B}}$), 17.6 (CH_3-$2^{\mathbf{B}}$), 18.0 (CH_3-$2^{\mathbf{A}}$), 23.9 (CH_3-$4^{\mathbf{A}}$), 24.3 (CH_3-$4^{\mathbf{B}}$), 26.8 ($C(\mathbf{C}H_3)_2{}^{\mathbf{A}}$), 27.1 ($C(\mathbf{C}H_3)_2{}^{\mathbf{B}}$), 27.7 ($C(\mathbf{C}H_3)_2{}^{\mathbf{B}}$), 28.1 ($C(\mathbf{C}H_3)_2{}^{\mathbf{A}}$), 36.4 ($C1'^{\mathbf{A}}$), 39.4 ($C1'^{\mathbf{B}}$), 65.1 ($\mathbf{C}H_2Ph^{\mathbf{A}}$), 65.4 ($C5^{\mathbf{A}}$), 65.5 ($\mathbf{C}H_2Ph^{\mathbf{B}}$), 67.8 ($C5^{\mathbf{B}}$), 75.3 ($C2^{\mathbf{A}}$), 77.2 ($C4^{\mathbf{A}}$), 77.7 ($C2^{\mathbf{B}}$), 78.7 ($C4^{\mathbf{B}}$), 81.4 ($C3^{\mathbf{A}}$), 81.9 ($C3^{\mathbf{B}}$), 98.5 ($C1^{\mathbf{B}}$), 100.4 ($C1^{\mathbf{A}}$), 108.3 ($\mathbf{C}(CH_3)_2{}^{\mathbf{B}}$), 108.74 ($\mathbf{C}(CH_3)_2{}^{\mathbf{A}}$), 127.2, 127.4, 127.6, 127.8, 127.9, 128.0, 128.2, 128.8 (Ar-$C^{\mathbf{A\&B}}$), 138.4 (Ar-$C_{quat}{}^{\mathbf{B}}$), 139.0 (Ar-$C_{quat}{}^{\mathbf{A}}$); LRMS (ESI+ve): 373 ($[M+Na]^+$, 55%), 723 ($[2M+Na]^+$, 50%); LRMS (ESI-ve): 349 ($[M-H]^-$, 100%).

Dimethyl titanocene



All solvents used in this procedure were distilled over calcium hydride before use.

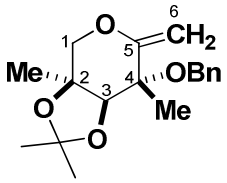
To a flame dried 500 mL round bottomed flask was added Cp_2TiCl_2 (10.17 g, 40.8 mmol) and diethyl ether (200 mL) under argon. The suspension was cooled to -78°C and methyl lithium (1.6 M in Et_2O; 54.9 mL, 87.8 mmol) was added slowly. The reaction mixture was allowed to warm to 0°C over 1 hour, quenched with water (90 mL) and the layers immediately separated (by use of a separating funnel). The organic phase was washed with brine (30 mL), dried (sodium sulfate), filtered into a foil covered flask and concentrated under reduced pressure. The orange solid was then suspended in pentane (200 mL), and cooled from room temperature to -78°C over 1 hour to form long orange crystals. The excess pentane was removed *via* cannula and the crystals dried for 10 minutes under vacuum* (7.42 g, 87%) before being dissolved in toluene (200 mL) and stored at -20°C.

*CAUTION: the solid can be explosive

δ_H ($CDCl_3$, 400 MHz): -0.14 (6H, s, CH_3), 6.07 (10H, s, Cp); {Lit.[167] δ_H ($CDCl_3$, 250 MHz) -0.05 (6H, s, CH_3), 6.05 (10H, s, Cp)}.

1,5-Anhydro-4-*O*-benzyl-6-deoxy-2,4-di-*C*-methyl-2,3-*O*-isopropylidene-L-*lyxo*-hex-5-enitol

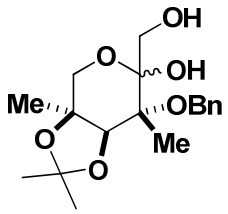
180



To a round bottomed flash wrapped in foil was added 2-*O*-benzyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene L-arabinono-1,5-lactone **113** (308 mg, 1.01 mmol) and Cp_2TiMe_2 (0.13 M in toluene; 27 mL, 3.52 mmol). The mixture was heated to reflux under argon for 90 minutes, after which time t.l.c. analysis (ethyl acetate/cyclohexane, 1:6) revealed complete consumption of starting material (R_f 0.38), and the formation of a single product (R_f 0.72). The solvent was removed under vacuum, and the crude purified by flash column chromatography (ethyl acetate/cyclohexane, 1:20) to give vinyl ether **180** (258 mg, 84%) as a pale yellow oil.

HRMS (FI+ve): found 304.1678 $[M]^{\cdot}$; $C_{18}H_{24}O_4$ requires 304.1675; $[\alpha]_D^{18}$ +36.0 (*c*, 0.22 ethyl acetate); ν_{max} (thin film): 1639 (s, C=C); δ_H (C_6D_6, 500 MHz): 1.23 (3H, s, CH_3-4), 1.41 (3H, s, $C(CH_3)_2$), 1.52 (3H, s, CH_3-2), 1.65 (3H, s, $C(CH_3)_2$), 3.97 (1H, d, CH_2Ph, J_{gem} 11.4), 4.00 (1H, s, H3), 4.12 (1H, d, CH_2Ph, J_{gem} 11.5), 4.39 (1H, s, H6), 4.41 (1H, d, H1, $J_{1,1'}$ 11.4), 4.53 (1H, d, H1', $J_{1',1}$ 11.2), 5.02 (1H, s, H6'), 7.21-7.33 (5H, m, Ar-H); δ_C (C_6D_6, 125 MHz): 20.0 (CH_3-2), 25.3 (CH_3-4), 27.5 ($C(\mathbf{C}H_3)_2$), 28.0 ($C(\mathbf{C}H_3)_2$), 64.8 (C1), 71.1 ($\mathbf{C}H_2Ph$), 76.7 (C2), 80.2 (C4), 84.0 (C3), 91.7 (C6), 109.7 ($\mathbf{C}(CH_3)_2$), 127.7, 127.9, 128.1, 128.6 (Ar-C), 139.0 (Ar-C_{quat}), 155.7 (C5); LRMS (ESI+ve): 305 ($[M+H]^+$, 60%).

3-*O*-Benzyl-3,5-di-*C*-methyl-4,5-*O*-isopropylidene-L-fructose 181

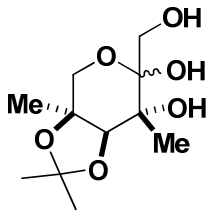


Ruthenium trichloride (0.1 M aq.; 0.47 mL, 47 µmol) was added to sodium periodate (215 mg, 1.01 mmol) in water (0.9 mL) and cooled to 0°C. The alkene **180** (204 mg, 0.67 mmol) was dissolved in acetonitrile (5.4 mL) and injected in one portion. The reaction mixture was stirred at 0°C for 5 minutes before quenching with saturated aqueous sodium thiosulfate (5 mL). T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) revealed complete consumption of starting material (R_f 0.82) and the formation of a single product (R_f 0.43). The reaction mixture was diluted with water (5 mL) and washed with ethyl acetate (3 x 10 mL). The combined organic fractions were dried (sodium sulfate), filtered and concentrated under reduced pressure before purification of the crude by flash column chromatography (ethyl acetate/cyclohexane, 1:3) to give 3-*O*-benzyl-3,5-di-*C*-methyl-4,5-*O*-isopropylidene-L-fructose **181** (149 mg, 66%) as a white solid, in a 3:1 ratio of anomers.

HRMS (ESI+ve): found 361.1622 $[M+Na]^{+;}$ $C_{18}H_{26}Na_1O_6$ requires 361.1622; $[\alpha]_D^{25}$ -9.81 (*c*, 0.84 chloroform); m.p. 149-150°C; ν_{max} (thin film): 3423 (br, OH); δ_H (C_6D_6, 500 MHz): 1.30 (3H, s, CH_3-3$^{\mathbf{B}}$), 1.41 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.41 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.49 (3H, s, $C(CH_3)_2^{\mathbf{B}}$), 1.56 (3H, s, $C(CH_3)_2^{\mathbf{A}}$), 1.59 (3H, s, CH_3-3$^{\mathbf{A}}$), 1.61 (3H, s, CH_3-5$^{\mathbf{A}}$), 1.62 (3H, s, CH_3-5$^{\mathbf{B}}$), 2.18 (1H, dd, OH-1$^{\mathbf{A}}$, $J_{OH,1'}$ 3.9, J 9.4), 2.67 (1H, q, OH-1$^{\mathbf{B}}$, J 4.0), 3.51-3.55 (2H, m, H6$^{\mathbf{B}}$ & H6'$^{\mathbf{B}}$), 3.69-3.77 (3H, m, H1$^{\mathbf{B}}$, H1'$^{\mathbf{B}}$ & H1$^{\mathbf{A}}$), 3.80 (1H, d, H6$^{\mathbf{A}}$, $J_{6,6'}$ 12.3), 3.89 (1H, dd, H1'$^{\mathbf{A}}$, $J_{1',OH}$ 3.7, J 11.7), 3.95 (1H, d, H6'$^{\mathbf{A}}$, $J_{6',6}$ 12.1), 4.06 (1H, s, H4$^{\mathbf{A}}$), 4.14 (1H, s, H4$^{\mathbf{B}}$), 4.43 (1H, s, OH-2$^{\mathbf{A}}$), 4.53 (1H, d, $CH_2Ph^{\mathbf{A}}$, J_{gem} 11.0), 4.57 (1H, d, $CH_2Ph^{\mathbf{B}}$, J_{gem} 10.7), 4.65 (1H, d, $CH_2Ph^{\mathbf{A}}$, J_{gem} 11.0), 4.77 (1H, d, $CH_2Ph^{\mathbf{B}}$, J_{gem} 10.7), 4.88 (1H, s, OH-2$^{\mathbf{B}}$), 7.28-7.40 (10H, m, Ar-H$^{\mathbf{A\&B}}$); δ_C (C_6D_6, 125 MHz): 17.8 (CH_3-3$^{\mathbf{B}}$), 18.0 (CH_3-5$^{\mathbf{A}}$), 23.5 ($C(CH_3)_2^{\mathbf{A}}$), 24.0 (CH_3-5$^{\mathbf{B}}$), 26.3 ($C(CH_3)_2^{\mathbf{B}}$), 26.7 ($C(CH_3)_2^{\mathbf{A}}$), 26.8 ($C(CH_3)_2^{\mathbf{B}}$), 26.9 (CH_3-3$^{\mathbf{A}}$), 64.6 (C1$^{\mathbf{A}}$), 65.1, 65.2 ($CH_2Ph^{\mathbf{A}}$, C6$^{\mathbf{A}}$), 65.9, 66.3 ($CH_2Ph^{\mathbf{B}}$, C6$^{\mathbf{B}}$), 67.1

($C1^{B}$), 75.8 ($C5^{A}$), 76.6($C5^{B}$), 77.2 ($C3^{A}$), 79.4 ($C3^{B}$), 80.5 ($C4^{A}$), 81.1 ($C4^{B}$), 96.9 ($C2^{B}$), 97.8 ($C2^{A}$), 108.5 (**C**$(CH_3)_2^{A}$), 109.0 (**C**$(CH_3)_2^{B}$), 127.1, 127.4, 127.6, 128.1, 128.5, 128.7 (Ar-$C^{A\&B}$), 137.2 (Ar-C_{quat}^{B}), 137.8 (Ar-C_{quat}^{A}); LRMS (ESI-ve): 397 ($[M+CH_3CO_2]^-$, 100%), 337 ($[M-H]^-$, 60%), 373 ($[M+Cl]^-$, 50%).

3,5-Di-*C*-methyl-4,5-*O*-isopropylidene-L-fructose 179

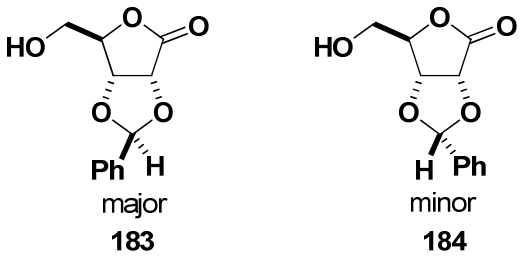


3-*O*-Benzyl-3,5-di-*C*-methyl-4,5-*O*-isopropylidene-L-fructose **181** (129 mg, 0.38 mmol) was dissolved in ethanol (5 mL) and methanol (1 mL). Palladium hydroxide (20% on carbon; 26 mg) was added to the solution whilst stirring. The reaction mixture was degassed and stirred under hydrogen; T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after two hours revealed complete consumption of starting material (R_f 0.37) and the formation of a single product (R_f 0.14). The reaction mixture was degassed, and filtered through a pad of Celite®, eluting with methanol. The solvents were concentrated under reduced pressure and the crude purified by flash column chromatography (ethyl acetate/cyclohexane, 1:1) to afford 3,5-di-*C*-methyl-4,5-*O*-isopropylidene-L-fructose **179** (76 mg, 80%) as a white crystalline solid, found in a 1:1 ratio of anomers.

HRMS (ESI+ve): found 271.1152 $[M+Na]^+$; $C_{11}H_{20}Na_1O_6$ requires 271.1152; $[\alpha]_D^{25}$ +31.9 (*c*, 0.27 chloroform); m.p. 74°C; ν_{max} (thin film): 3424 (br, OH), 1644 (s, C=O); δ_H (C_6D_6, 500 MHz): 1.24 (3H, s, $C(CH_3)_2^{B}$), 1.32 (3H, s, $C(CH_3)_2^{A}$), 1.34 (3H, s, CH_3-3^{A}), 1.37 (3H, s, CH_3-5^{A}), 1.38 (3H, s, $C(CH_3)_2^{B}$), 1.53 (3H, s, $C(CH_3)_2^{A}$), 1.58 (3H, s, CH_3-3^{B}), 1.61 (3H, a-d, CH_3-5^{B}, *J* 0.9), 2.11 (1H, dd, OH-1^{B}, *J* 5.7, *J* 8.5), 2.44 (1H, dd, OH-1^{A}, $J_{OH,1'}$ 2.7, $J_{OH,1}$ 10.2), 3.15 (1H, s, OH-3^{A}), 3.60 (1H, dd, $H6^{B}$, *J* 0.9, $J_{6,6'}$ 12.0), 3.62 (1H, a-d, $H1^{A}$, *J* 10.4), 3.66 (1H, d, $H6^{A}$, $J_{6,6'}$ 12.0), 3.74-3.77 (3H, m, OH-2^{A}, OH-3^{B}, $H1^{B}$), 3.77 (1H, s, $H4^{B}$), 3.92 (1H, s, $H4^{A}$), 3.95 (1H, d, $H6'^{A}$, $J_{6',6}$ 12.0), 3.98

(1H, m, H1'^{B}), 3.99 (1H, a-dd, H1'^{A}, $J_{1',OH}$ 2.7, J 10.9), 4.21 (1H, dd, H6'^{B}, J 0.9, $J_{6',6}$ 12.0), 4.84 (1H, s, OH-2^{B}); δ_C (C_6D_6, 125 MHz): 23.8 (CH_3-3^{B}), 23.9 (CH_3-3^{A}), 24.6 (CH_3-5^{B}), 24.7 (CH_3-5^{A}), 26.3 ($C(CH_3)_2^{A}$), 26.6 ($C(CH_3)_2^{B}$), 27.1 ($C(CH_3)_2^{A}$), 28.1 ($C(CH_3)_2^{B}$), 65.9, 66.0 (C6^{B}, C1^{B}) , 67.6 (C1^{A}), 67.8 (C6^{A}), 71.1 (C3^{A}), 71.4 (C3^{B}), 76.6 (C5^{B}), 80.0 (C5^{A}), 83.7 (C4^{A}), 85.1 (C4^{B}), 96.2 (C2^{A}), 96.6 (C2^{B}), 108.8 (**C**$(CH_3)_2^{A}$), 108.3 (**C**$(CH_3)_2^{B}$; LRMS (ESI-ve): 247 ([M-H]$^-$, 71%); LRMS (ESI+ve): 312 ([M+MeCN+Na]$^+$, 100%).

2,3-*O*-Benzylidene-2-*C*-methyl-D-ribono-1,4-lactones 183 and 184



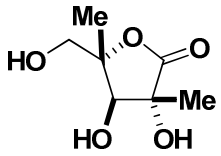
major **183** minor **184**

2-*C*-Methyl-D-ribono-1,4-lactone (6.11 g, 0.42 mol) was suspended in benzaldehyde (65 mL) and to it was added hydrochloric acid (37% aq., 5 mL). The suspension was stirred at room temperature under argon for 20 hours, during which time the starting material dissolved and the solution turned pale yellow in colour. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed complete consumption of starting material (R_f 0.00) and two major products (R_f 0.74 & 0.44). The solvents were concentrated under reduced pressure and the crude preadsorbed on silica and purified by column chromatography (ethyl acetate/cyclohexane, 1:6→1:1). This gave 2.32 g (24%) of the compound with R_f 0.74, as a pale yellow foam. By ^{1}H NMR, it was shown to be a mixture of 4 different 1,4 lactones (HMBC analysis and C=O 1785cm^{-1}), but further structural elaboration was not possible. The second component, with R_f 0.44 was found to be a mixture of the lactones shown above (6.99 g, 71%). This was shown by ^{1}H NMR to be a 2:1 ratio of major to minor components, A:B.

δ_H ($CDCl_3$, 500 MHz): 1.74 (6H, s, CH_3-2$^{A\&B}$), 2.64 (2H, br-s, OH$^{A\&B}$), 3.83-3.89 (2H, m, H5$^{A\&B}$), 4.02 (1H, dd, H5'^{A}, $J_{5',4}$ 2.8, *J* 12.3), 4.05 (1H, dd, H5'^{B}, *J* 2.5, *J* 12.6), 4.64 (1H, t, H4^{A}, $J_{4,5'}$ 2.5), 4.65 (1H, d, H3^{B}, *J* 1.1), 4.66 (1H, a-d, H3^{A}, *J* 0.5), 4.69-4.70 (1H, m, H4^{B}), 7.39-7.50 (10H, m, Ar-H$^{A\&B}$); δ_C ($CDCl_3$, 100 MHz): 18.0 (CH_3-2^{A}), 18.3 (CH_3-2^{B}), 62.2 (C5^{A}), 62.4 (C5^{B}), 81.8, 84.5 (C3^{B}, C4^{B}), 82.4, 83.8 (C3^{A}, C4^{A}), 82.8 (C2^{B}), 83.3 (C2^{A}), 104 (Ph**C**H^{B}), 105.4 (Ph**C**H^{A}), 126.71, 126.74, 128.5, 130.0 (Ar-C$^{A\&B}$), 135.0 (Ar-C_{quat}^{B}), 135.1 (Ar-C_{quat}^{A}), 175.1 (C1^{A}), 175.7 (C1^{B}).

A sample of the major component was crystallised from a mixture of the two using diethyl ether and 40°-60° petrol and slow evaporation. HRMS (ESI+ve): found 273.0727 $[M+Na]^+$; $C_{13}H_{14}Na_1O_5$ requires 273.0733; m.p.: 96 - 99°C; ν_{max} (thin film): 3490 (s, br, OH), 1781 (s, C=O); $[\alpha]_D^{18}$ -37.8 (*c*, 0.86 chloroform); LRMS (ESI-ve): 267 ($[M-OH]^-$, 100%), 499 ($[2M-H]^-$, 45%).

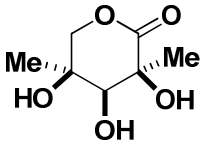
2,4-Di-*C*-methyl-L-arabinono-1,4-lactone 190



2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** (82 mg, 0.38 mmol) was dissolved in water (0.2 mL) and trifluoroacetic acid (0.6 mL) was added whilst stirring at room temperature. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 16 h showed starting material (R_f 0.54) and the formation of a single product (R_f 0.04). A further 0.6 mL of trifluoroacetic acid was added and the solution stirred for a further 24 h. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time showed the complete consumption of starting material and a single product. The solvents were removed under reduced pressure and the resulting crude was dissolved in water (5 mL) and washed with dichloromethane (3 x 5 mL). The aqueous phase was concentrated to give 2,4-di-*C*-methyl-L-arabinono-1,4-lactone **190** as a white amorphous solid (62 mg, 93%). The γ lactone was identified by HMBC analysis.

HRMS (ESI-ve): found 175.0601 $[M-H]^-$; $C_7H_{11}O_5$ requires 175.0605; m.p. 104-106°C; $[\alpha]_D^{21}$ -9.0 (*c*, 0.97 methanol); ν_{max} (thin film): 3385 (s, br, OH), 1765 (s, C=O); δ_H (CD_3OD, 400 MHz): 1.31 (3H, s, CH_3-4), 1.40 (3H, s, CH_3-2), 3.57 (1H, d, H5, $J_{5,5'}$ 12.1) 3.65 (1H, d, H5', $J_{5',5}$ 12.1), 4.27 (1H, s, H3); δ_C (CD_3OD, 125 MHz): 17.2 (CH_3-4), 20.6 (CH_3-2), 67.0 (C5), 76.5 (C3), 76.9 (C2), 87.5 (C4), 179.5 (C1); LRMS (ESI-ve): 175 ($[M-H]^-$, 80%), 235 ($[M+CH_3COO]^-$, 100%).

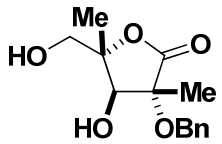
2,4-Di-*C*-methyl-L-ribono-1,5-lactone 191



2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone **104** (74 mg, 0.34 mmol) was dissolved in water (2 mL) and Dowex® (50W X8 H^+ form, 500 mg) was added whilst stirring at 50°C. T.l.c. analysis (ethyl acetate) after 3 hours showed starting material (R_f 0.48) and the formation of a single product (R_f 0.26). The temperature was increased to 90°C, and t.l.c. analysis after a further 4 hours revealed complete conversion of the starting material into a single product. The resin was removed by filtration and the solvent concentrated under reduced pressure to afford 64 mg of an amorphous yellow solid. This was purified by flash column chromatography (ethyl acetate) to yield 2,4-di-*C*-methyl-L-ribono-1,5-lactone, **191** as a white solid (51 mg, 84%). The δ lactone was identified by HMBC analysis.

HRMS (ESI+ve): found 199.0580 $[M+Na]^+$; $C_7H_{12}Na_1O_5$ requires 199.0577; m.p. 97-99°C; $[\alpha]_D^{21}$ -37.0 (*c*, 1.125 methanol); ν_{max} (thin film): 3454 (s, br, OH), 1763 (s, C=O); δ_H (CD_3OD, 400 MHz): 1.35 (3H, s, CH_3-4), 1.40 (3H, s, CH_3-2), 3.49 (1H, d, H5, $J_{5,5'}$ 12.4), 3.61 (1H, d, H5', $J_{5',5}$ 12.4), 4.05 (1H, s, H3); δ_C (CD_3OD, 125 MHz): 17.5 (CH_3-4), 22.4 (CH_3-2), 66.2 (C5), 73.6 (C2 & C3), 88.6 (C4), 178.1 (C1); LRMS (ESI-ve): 175 ($[M-H]^-$, 94%), 221 ($[M+CH_3CH_2O]^-$, 100%), 351 ($[2M-H]^-$, 75%).

2-*O*-Benzyl-2,4-di-*C*-methyl-L-arabinono-1,4-lactone 192



N-Iodosuccinamide (693 mg, 3.08 mmol) and tetra butyl ammonium iodide (227 mg, 0.62 mmol) were dissolved in dichloromethane (7 mL), and a suspension of 2-*O*-benzyl-2,4-di-*C*-methyl-L-arabinose **113** (165 mg, 0.62 mmol) in dichloromethane was added to it. The dark brown reaction mixture was stirred at room temperature for 2 hours, after which time t.l.c. analysis (ethyl acetate/cyclohexane, 3:2) revealed the consumption of starting material (R_f 0.11) and the formation of a single major product (R_f 0.26). The reaction mixture was quenched with saturated sodium thiosulfate solution (10 mL), and the aqueous phase extracted with dichloromethane (3 x 10 mL), and the combined organic layers were dried (magniesium sulfate), filtered and the solvents concentrated under reduced pressure. Purification of the crude by flash column chromatography (ethyl acetate/cyclohexane, 2:1) afforded 2-*O*-benzyl-2,4-di-*C*-methyl-L-arabinono-1,4-lactone, **192** as a white solid (93 mg, 57%). The γ lactone was identified by HMBC analysis.

Found: C, 63.06; H, 6.80; $C_{14}H_{18}O_5$ requires: C, 63.15; H, 6.81; HRMS (ESI+ve): found 284.1492 $[M+NH_4]^+$; $C_{14}H_{22}N_1O_5$ requires 284.1493; $[\alpha]_D^{24}$ -10.3 (*c*, 1.09 1,4-dioxane); m.p. 100°C; ν_{max} (thin film): 3418 (br, OH), 1760 (s, C=O); δ_H (DMSO-d_6, 400 MHz): 1.22 (3H, s, CH_3-4), 1.36 (3H, s, CH_3-2), 3.41 (1H, dd, H5, $J_{5,OH}$ 6.3, $J_{5,5'}$ 12.0), 3.51 (1H, dd, H5', $J_{OH,5}$ 5.3, $J_{5',5}$ 12.0), 4.46 (1H, d, OCH_2Ph, J_{gem} 11.2), 4.57 (1H, d, H3, $J_{3,OH}$ 5.6), 4.61 (1H, d, OCH_2Ph, J_{gem} 11.2), 5.30 (1H, a-t, OH-5, *J* 5.8), 5.82 (1H, d, OH-3, $J_{OH,3}$ 5.6), 7.26-7.37 (5H, m, Ar-H); δ_C (DMSO-d_6, 100 MHz): 17.5 (CH_3-4), 17.9 (CH_3-2), 65.2 (C5), 65.8 (O**C**H_2Ph), 70.7 (C3), 81.5 (C2), 86.3 (C4), 127.5, 127.7, 128.3 (Ar-C), 138.2 (Ar-C_{quat}), 174.9 (C1); LRMS (ESI-ve): 301 ($[M-Cl]^-$, 100%).

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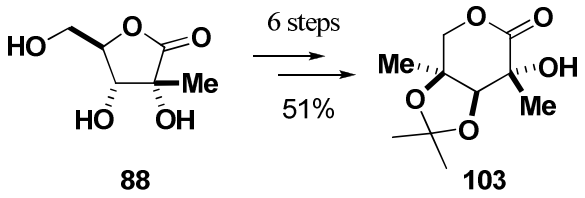
Chapter 3

Synthetic Utility of Di-C-Methyl Branched Sugar Lactones

The second half of this project investigated the chemistry of isopropylidene protected 2,4-di-*C*-methyl-L-arabinono-1,5-lactone **103**, a key intermediate in the synthesis of 3,5-di-*C*-methyl-L-glucose **97**, produced rapidly and in high yield from 2-*C*-methyl-D-ribono-1,4-lactone **88**, itself a readily available methyl branched sugar lactone (**Figure 3.1**).[1]



88 **103**

Figure 3.1: The synthesis of 2,4-di-*C*-methyl-L-arabinono-1,5-lactone **103**.

Branched carbohydrate building blocks serve as extremely useful starting materials for the synthesis of densely functionalised homochiral targets with quaternary centres.[2] The advantage of carbohydrates as starting materials derives from the pre-existence of a sequence of chiral centres, which would otherwise need to be installed through a series of stereoselective reactions.[3] Furthermore, the existence here of a quaternary centre α- to the lactone functionality makes epimerisation at C2, either by acid or base catalysis unlikely. A wide range of targets from 2,4-di-*C*-methyl-L-arabinono-1,5-lactone were therefore proposed (**Figure 3.2**). These include a deoxy sugar **121**, piperidines **194**, **195** and **196**, hydroxy proline **197** and pyrrolidine **198**.

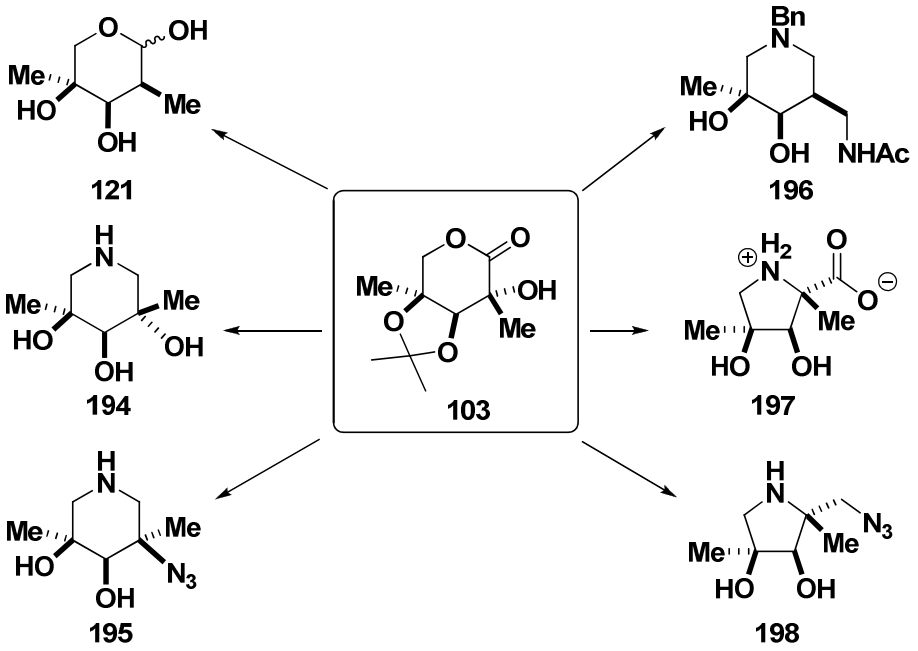
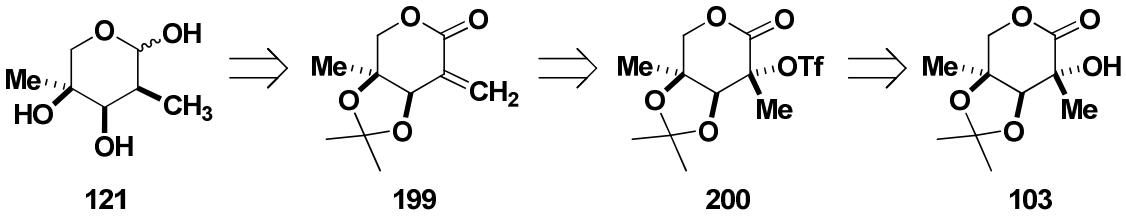


Figure 3.2: Proposed targets from protected 2,4-di-*C*-methyl-L-arabinonolactone **103**.

The biological significance of deoxy sugars is well known, with 2-deoxy-D-ribose forming the sugar backbone of perhaps the most important biological molecule, DNA. Furthermore, deoxy sugars are often found in bacterial cell wall polysaccharides, with or without additional methyl branch points.[4,5,6] Proline analogue **197** is of interest as an unnatural protein building block; its unusual structure could influence protein folding, helping the understanding of this natural process. Proline analogues are also of interest as potential chiral catalysts and as therapeutic agents for a range of diseases.[7,8,9] Piperidines **194**, **195** and **196** and pyrrolidines such as **198** represent two members of a class of sugar derivatives known as imino (or aza) sugars. Several imino sugars are known to act as enzyme inhibitors, as they can compete with the parent monosaccharide to occupy the active site.[10] Many unnatural L- imino sugars have been found to inhibit enzymes through a non competitive mechanism (see **Chapter 1**, section **1.5.1**).[11,12,13] As such, their synthesis has received much attention in recent years.[14]

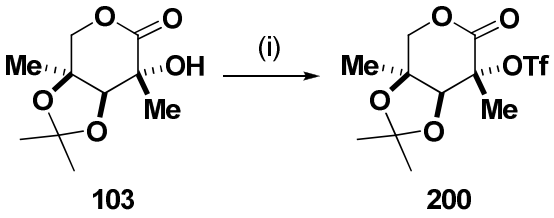
3.1 Synthesis of 2-Deoxy-2,4-Di-C-Methyl-L-Arabinose, 121

Deoxygenation of protected 2,4-di-*C*-methyl arabinono-1,5-lactone **103** was envisaged *via* alkene **199**, itself formed from triflate **200** (**Scheme 3.1**). This short sequence relies on selective hydrogenation of alkene **199** to give the *arabino* stereochemistry.



Scheme 3.1: Retrosynthetic analysis of 2-deoxy-2,4-di-*C*-methyl-L-arabinose, **121**.

Following a literature procedure,[15] lactone **103** was treated with trifluoromethanesulfonic anhydride in anhydrous dichloromethane at -20°C (**Scheme 3.2**). T.l.c. analysis showed the reaction to be sluggish, most likely because of the steric hindrance imposed by the tertiary centre. Almost complete esterification was eventually achieved using forcing conditions- three separate additions of triflic anhydride were required every two hours. Addition of reagent was performed at 0°C, and the reactions subsequently warmed to room temperature each time.



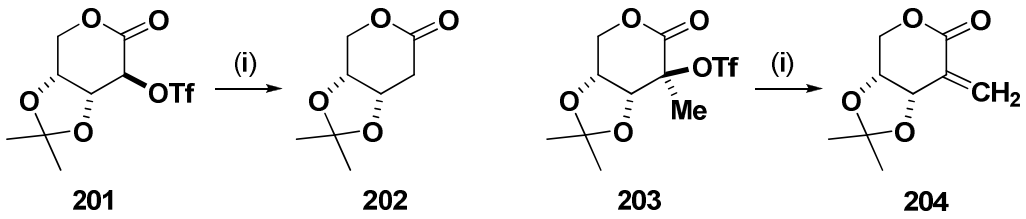
Reagents and conditions: (i) Tf_2O, py, DCM 0°C→RT, reagents added every 2 h; total reaction time 6 h.

Scheme 3.2: Difficult triflation of the alcohol group in lactone **103**.

Triflate **200** was purified by flash column chromatography – despite the high degree of instability of this compound to decomposition, complete esterification could never be achieved, and it was necessary to remove the traces of unreacted alcohol at this stage, as separation further down the sequence proved difficult. The triflate was immediately dissolved in anhydrous DMF and stirred at room temperature. After 16 hours, remarkably, t.l.c. analysis revealed only one major product, found to be the elimination product **199**, a stable crystalline solid, formed in up to 63% yield;

however this result was found to fluctuate wildly depending on scale. This may be due to decomposition of either the triflate or the alkene by the triflic acid generated by the reaction. To test this and attempt to improve the yield, several catalysts were screened. Addition of a variety of bases was tried, in an attempt to raise the pH of the reaction mixture, however only complex mixtures were observed, without the isolation of meaningful quantities of alkene **199**.

Work by Stewart had shown that the arabino triflate **201**, when treated with lithium iodide underwent a rapid deoxygenation reaction to deoxy lactone **202** (**Scheme 3.3**).[16] Attempts to expand this methodology to branched arabino system **203** resulted in elimination to **204**, not deoxygenation.[17]



201 **202** **203** **204**

Reagents and conditions: (i) LiI.3H_2O, butanone, reflux.

Scheme 3.3: Atttempted one-step deoxygenation.

Replication of these conditions on the di-*C*-methyl system also resulted in elimination, rather than the desired deoxygenation. A considerable number of side products were also visible by NMR and complete purification of the product was considerably more difficult. Any product obtained in this way could never be hydrogenated successfully, possibly due to iodide residues poisoning the catalyst.

Hydrogenation of **199** obtained from the uncatalysed reaction in the presence of palladium in ethanol gave a single compound **205** in 72% yield; a significant nOe between H2 and H5 was strongly suggestive of the production of the more stable *arabino* product **205** even though this would require hydrogenation from the most hindered face (**Figure 3.3**).

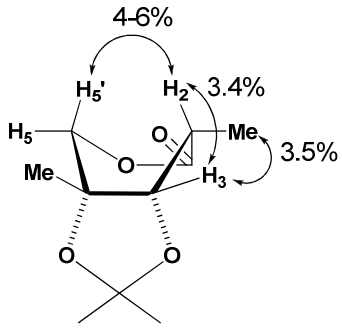


Figure 3.3: nOe enhancements obtained from lactone **205** (D-enantiomer drawn for clarity).

Reduction of **205** by DIBAL gave the lactol **206** in 59% yield. This crystallized as the β-pyranose anomer, and the crystal structure clearly shows that no epimerisation at C2 had occurred (**Figure 3.4**). Removal of the isopropylidene protecting group by acidic resin gave 2-deoxy-2,4-di-*C*-methyl-L-arabinose **121** in 82% yield.

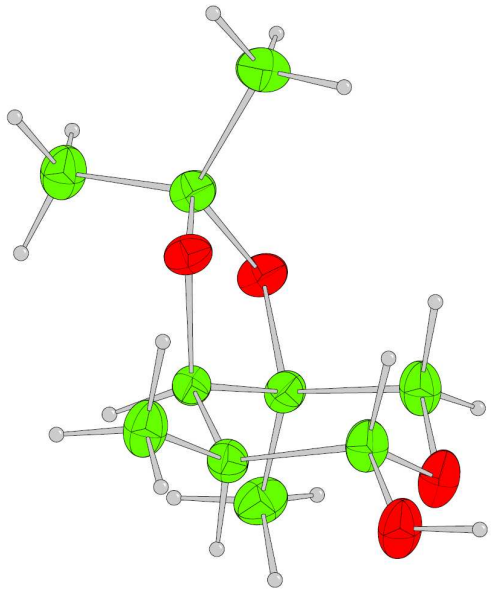


Figure 3.4: Lactol **206** with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

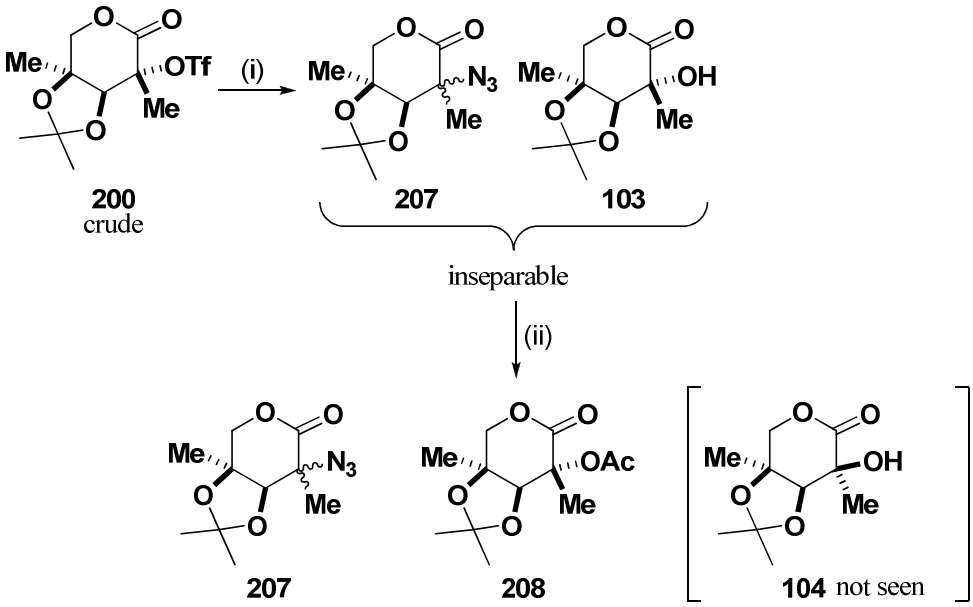
Detailed conformational analysis of the novel deoxy sugar was undertaken. This was compared with arabinose, 2-*C*-methyl arabinose and 2,4-di-*C*-methyl arabinose (see **Chapter 2**). Arabinose is known to exist mainly in the pyranose form (approximately 95% of material), 2-*C*-methyl arabinose was found to reduce this by half to only 49% pyranose. However, 2,4-di-*C*-methyl arabinose and 2-deoxy-2,4-di-*C*-methyl arabinose were found to both favour the pyranose conformation (75 and

76% respectively); deoxygenation at C2 was shown to produce little effect on the pyranose/furanose ratio of the free sugar. For full NMR assignment, please see the **Appendix** to the rear of this work.

3.2 Introduction of Nitrogen into Arabinono-1,5-lactone 103

It was envisaged that nitrogen could be introduced into arabinono-1,5-lactone **103** *via* a triflation and azide displacement sequence. This combination of alcohol activation, followed by sodium azide displacement has been used to great effect in the synthesis of many imino sugars,[14] and is particularly suitable here as a preparation for triflate **200** had already been established.

Esterification of the alcohol with triflic anhydride was preformed as previously, and, as before complete esterification was not possible. Nevertheless, the crude was taken directly with sodium azide in DMF. It was thought that column chromatography of the unstable triflate would be unnecessary here as it was anticipated that any unreacted alcohol would be easily separable from the azide in the next step. Unfortunately, this was not found to be the case as, by t.l.c. analysis, the desired azide product **207** co-spotted with the starting alcohol **103**. Treatment of the azide/alcohol mixture with acetic anhydride to try and effect separation *via* acetate protection of the free hydroxyl was less successful than anticipated, as, surprisingly, by t.l.c. analysis the acetate and azide were very close in R_f; column chromatography did however afford limited separation (**Scheme 3.4**).



Reagents and Conditions: (i) NaN_3, DMF, RT, 16 h; (ii) Ac_2O, Py, 36 h, heating.

Scheme 3.4: Attempt to perform the triflate/azide displacements steps with no purification of the triflate.

The crystal structure of acetate **208** was obtained, to ensure this was formed from starting material, and did not have the inverted configuration at C2 **104** (**Figure 3.5**). Insufficient pure azide product was obtained to confirm stereochemistry at C2 at this point, however, NMR analyses confirmed it to be a single compound, rather than a mixture of stereoisomers.

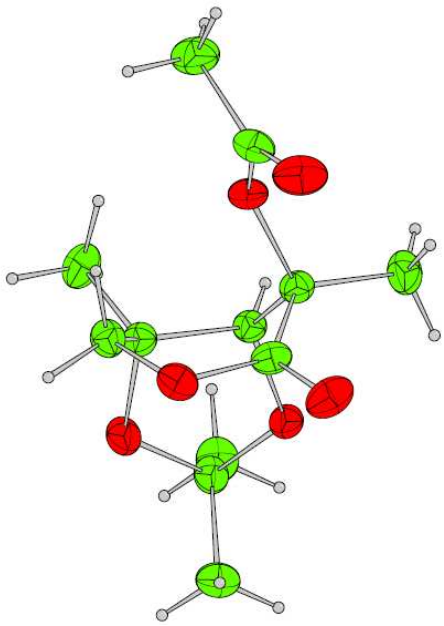


Figure 3.5: Acetate **208** with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

Purification of the triflate, followed by treatment with sodium azide in *N*,*N*-dimethylformamide gave a single azide **207**, in up to 80% yield from the triflate. X-ray crystallography and nOe studies (**Figure 3.6**) confirmed that this azide had *ribo* stereochemistry at C2.

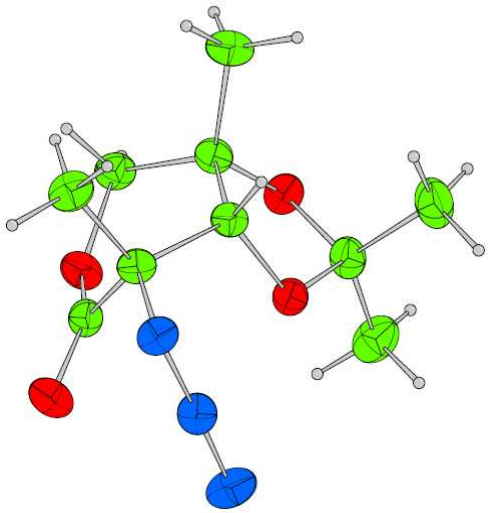
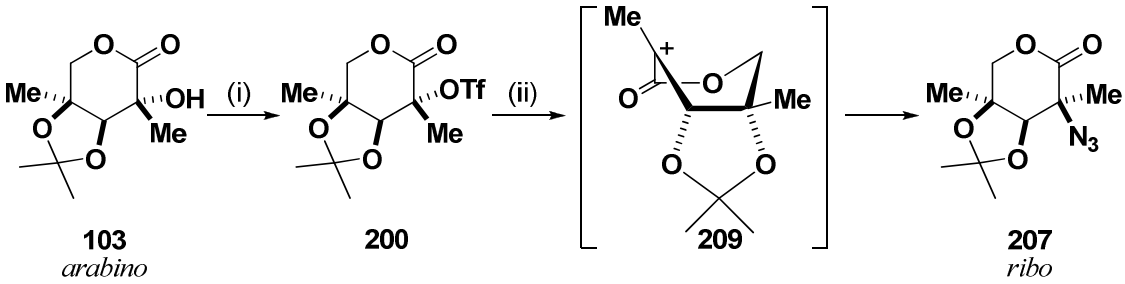


Figure 3.6: Azide **207**, confirmed to be *ribo*. Displacement ellipsoids are drawn at the 50%probability level. H atoms are shown as spheres of arbitrary radius.

Detailed mechanistic studies to determine whether this is an S_N1 or S_N2 process were beyond the scope of this investigation, however nucleophilic displacement at a tertiary centre with a β-O atom is likely to be an S_N1 reaction, *via* carbocation **209**. Azide **207** would then be formed by nucleophilic attack from the least hindered face to give the less stable *ribo* product (**Scheme 3.5**). Complete inversion of stereochemistry in an S_N1 reaction is uncommon, as racemisation is predicted, whereas inversion is predicted in an S_N2 mechanism. S_N2 reactions at tertiary centres are not common, and tend to be slow (for example bridgehead positions), however this reaction took several hours in a polar aprotic solvent (DMF) so an S_N2 process cannot be ruled out.

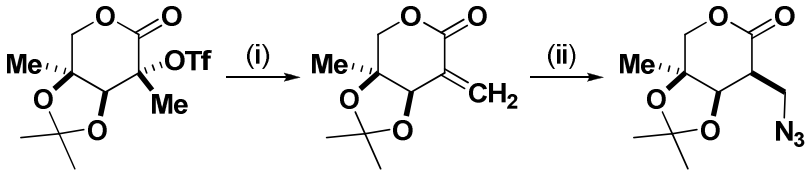


103 *arabino* **200** **209** **207** *ribo*

Reagents and conditions: (i) Tf_2O, Py, DCM, -20°C, 6 h; (ii) NaN_3, DMF, RT, 16 h.

Scheme 3.5: Formation of azide **207**.

It was envisaged that a second azide might be accessible from triflate **200** by combining the degradation and azide addition steps. Purified triflate **200** was stirred in DMF for 16 hours as before; t.l.c. analysis after this time indicated the reaction was complete, and to the reaction mixture was added sodium azide. After 3 hours, no UV active products were visible by t.l.c. analysis and purification of the reaction mixture afforded a single azide product **210**, in moderate yield (**Scheme 3.6**).



200 **199** **210** 63%

Regents and conditions: (i) DMF, RT, 16 h; (ii) NaN_3, RT, 3 h.

Scheme 3.6: Michael addition to alkene **199** to generate a second azide **210**.

Recrystallisation of azide **210** to confirm stereochemistry at C2 repeatedly afforded thin needle-like crystals, unsuitable for X-ray analysis, however nOe enhancements were able to confirm this to be the *ribo* product **210** (**Figure 3.7**).

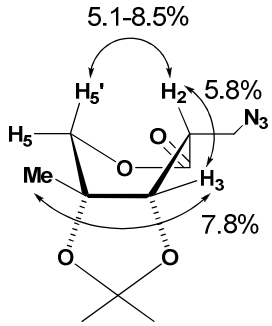
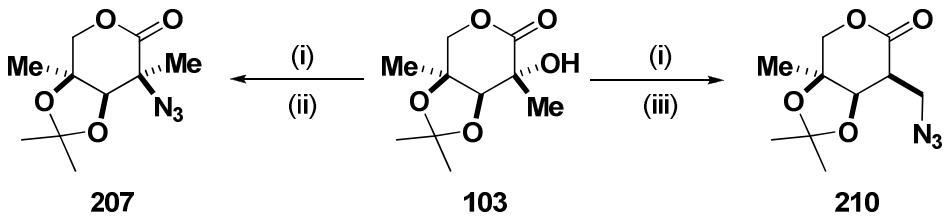


Figure 3.7: nOe enhancements from lactone **210** (D-enantiomer drawn for clarity).

Curiously, it was not possible to perform this Michael addition reaction on purified alkene **199**. All attempts to force the reaction were unsuccessful, including catalysis with strong acids. It may be that despite purifying the triflate, some inorganic impurities were present which helped catalyse the Michael addition reaction, and these were removed during purification of alkene **199**.

In this way, it was possible to access two different azides from arabinono-1,5-lactone **103** (**Scheme 3.7**). It should be noted here that in the analogous singly branched case, it was not possible to obtain this degree of selectivity, and attempts to generate solely the tertiary azide produced the Michael addition product as a contaminant.[17]



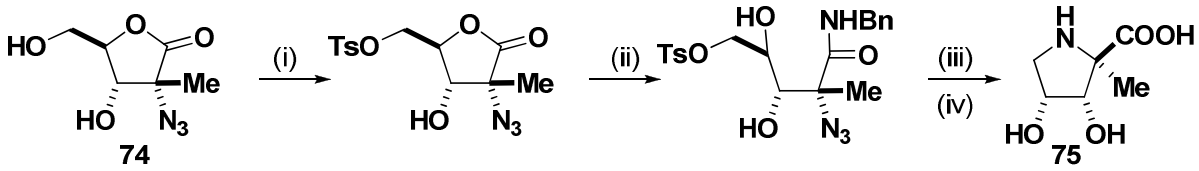
Reagents and Conditions: (i) Tf_2O, Py, DCM, 0°C→RT, 6 h; (ii) NaN_3, DMF, RT, 16 h; (iii) DMF, RT, 16 h then NaN_3, 3 h.

Scheme 3.7: Synthesis of two azides with complete selectivity from arabinono-1,5-lactone **103**.

With two azides in hand, routes to a variety of imino and amino sugars were proposed.

3.3 Branched Proline synthesis from Azide 207

It was envisaged that azide **207** could be used in the synthesis of branched proline derivative **197**, in an analogous fashion to the sequence used by da Cruz *et al* (**Scheme 3.8**).[18]

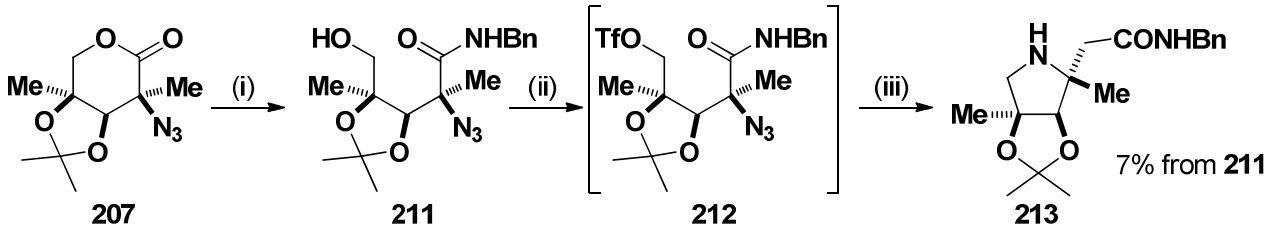


Reagents and Conditions: (i) TsCl, Py, 68% (based on recovered starting material); (ii) NH_2Bn, THF, 82%; (iii) H_2, Pd/C, 1,4-dioxane; (iv) 37% $HCl_{(aq.)}$, 83% over 2 steps.

Scheme 3.8: Synthesis of novel branched hydroxy proline analogues.

Given the moderate yield achieved in the tosylation step, and that the analogous di-branched molecule would have a neopentyl alcohol group at this position due to the extra methyl at the 4-position, it was decided to proceed on the isopropylidene protected azide **207** and introduce a leaving group species to this position after the benzylamine ring opening step.

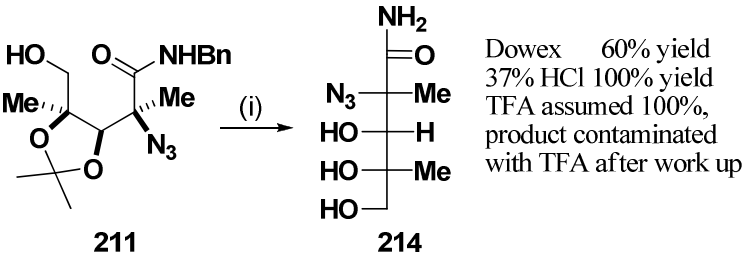
Ring opening of azide **207** with benzylamine in THF at room temperature gave ribonamide **211** in high yield (**Scheme 3.9**). Treatment of ribonamide **211** with touenesulfonyl chloride, to introduce a leaving group was unsuccessful, even with a large excess of reagent and the introduction of the nucleophilic catalyst, *N*,*N*-dimethylamino pyridine (DMAP). This is not wholly unexpected, as when this was carried out on the singly branched system, without the methyl at the 4-position, the reaction was slow with a moderate yield; only 68% yield based on recovered starting material.[17] Treatment of **211** with trifluoromethanesulfonic anhydride and pyridine in dichloromethane at -20°C did show complete conversion of starting material to a sole product by t.l.c. analysis. Attempts to isolate and purify triflate **212** were unsuccessful due to its rapid decomposition; hydrogenation of the crude triflate did result in the in the formation of protected proline **213**, however the yield was very low (**Scheme 3.9**).



Reagents and conditions: (i) NH_2Bn, THF, 40°C, 2 h; (ii) Tf_2O, Py, DCM, -30°C, 1 h; (iii) Pd/C, EtOH, H_2, 16 h.

Scheme 3.9: Route to branched proline derivative **213**.

It was decided to deprotect ribonamide **211**, as the presence of the acetonide group might have been prohibiting ring closure, resulting in a primary amine which would be difficult to isolate and may be responsible for the low yield. Several attempts at the removal of the isopropylidene group were made using either acidic resin in water, mineral acid or trifluoroacetic acid. These all resulted in the removal of the benzyl group as well as the isopropylidene group, to give **214** (**Scheme 3.10**).

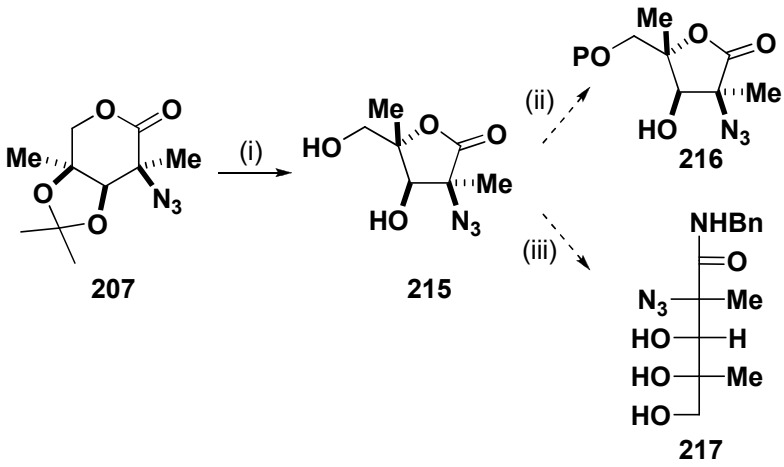


Reagents and Conditions: (i) Dowex® (50W X8 H^+ form), H_2O, 1,4-dioxane, 90°C, 6 h then RT, 16 h *or* 37% $HCl_{(aq)}$, 60°C, 40 h *or* $TFA_{(aq)}$, 50°C, 16 h.

Scheme 3.10: The forcing conditions needed to remove the isopropylidene group resulted in concomitant removal of the benzyl group.

To obtain the desired deprotected ribonamide, isopropylidene removal was attempted prior to ring opening with benzylamine. Removal of the isopropylidene group from azido lactone **207** was achieved in good yield (92%) using acidic resin at high temperature (**Scheme 3.11**). This deprotected lactone **215** was shown by HMBC to adopt a 5 ring structure. Attempts to tosylate or mesitylate the alcohol group at this stage to give **216** were, as anticipated, unsuccessful. Ring opening to **217** was successful, as shown by the crude NMR spectra, IR analysis and mass spectroscopy, however purification of the products was not trivial. Neither column chromatography,

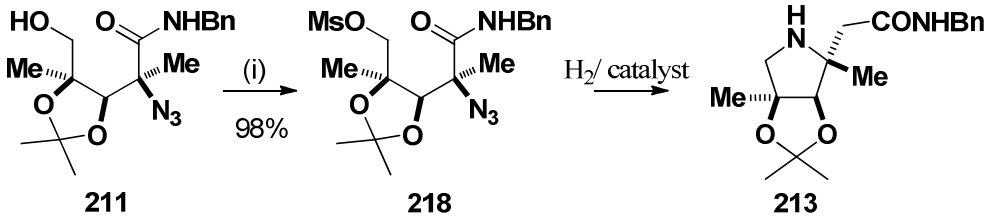
reverse phase chromatography with C-18 silica gel nor ionic resin column chromatography could satisfactorily remove the excess benzylamine.



Reagents and Conditions: (i) Dowex® (50W X8 H^+ form), H_2O, 70°C, 21 h; (ii) TsCl or MstCl, Py, DMAP, DCM; (iii) H_2NBn, THF, RT, 16 h.

Scheme 3.11: Although deprotection of the azide could be readily achieved, synthetic manipulations on the product were difficult to either carry out **216** or purify **217**.

Returning to protected ribonamide **211**, activation of the alcohol group was attempted with methanesulfonyl chloride in pyridine. This reaction, although slower than the triflation, gave a significantly cleaner reaction, and the mesylate product **218** was sufficiently stable to be purified by column chromatography and its structure fully characterised (**Scheme 3.12**).



Reagents and Conditions: (i) MsCl, Py, 0°C→RT, 2.5 h *or* MsCl, Py, DCM, 0°C→RT, 18 h (large scale).

Scheme 3.12: Synthesis of protected proline analogue **213**.

Hydrogenation of mesylate **218** was carried out in both ethyl acetate and ethanol, with selected palladium catalysts (**Table 3.1**).

Catalyst	***Solvent***	***Time***	***Outcome***
Palladium on Carbon	EtOAc	90h	No Reaction
Palladium on Carbon	EtOH	90h	82%
Palladium hydroxide	EtOAc	90h	No Reaction
Palladium hydroxide	EtOH	90h	36%
Palladium black	EtOH	4h	81%

Table 3.1: All hydrogenations carried out at a concentration of 25 mg/mL and with 20% by mass of catalyst

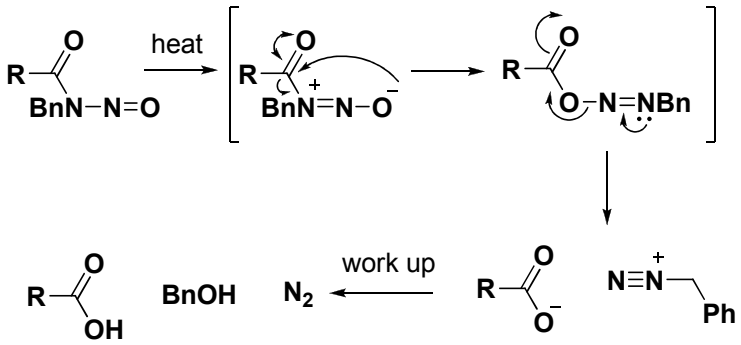
Whilst it is unsurprising that the reaction proceeds fastest with palladium black as the catalyst, it is not obvious why the hydrogenation should not proceed at all in ethyl acetate, but does proceed in absolute ethanol. Solvent effects are well documented in catalytic hydrogenation reactions; if the coordinate link between solvent and catalyst is stronger than the binding of donor or acceptor, then reduction can be significantly inhibited.[19] The relative static permittivity or dielectric constant (DC) of a solvent is a relative measure of its polarity. In this instance, ethanol is much more polar with a DC of 24.3 than ethyl acetate with a DC of 6.0 (both at room temperature). This increased polarity may mean that ethyl acetate binds more tightly to the palladium than ethanol, effectively inhibiting the reaction. This sequence, from alcohol **103** to the protected proline **213** was successfully scaled up to over a gram with no complications.

3.3.1: Deprotection of proline analogue 213

Isopropylidene deprotection of **213** and amide hydrolysis would yield the desired branched hydroxy proline **197**. Two separate approaches were investigated, hydrolysis and nitrosation.

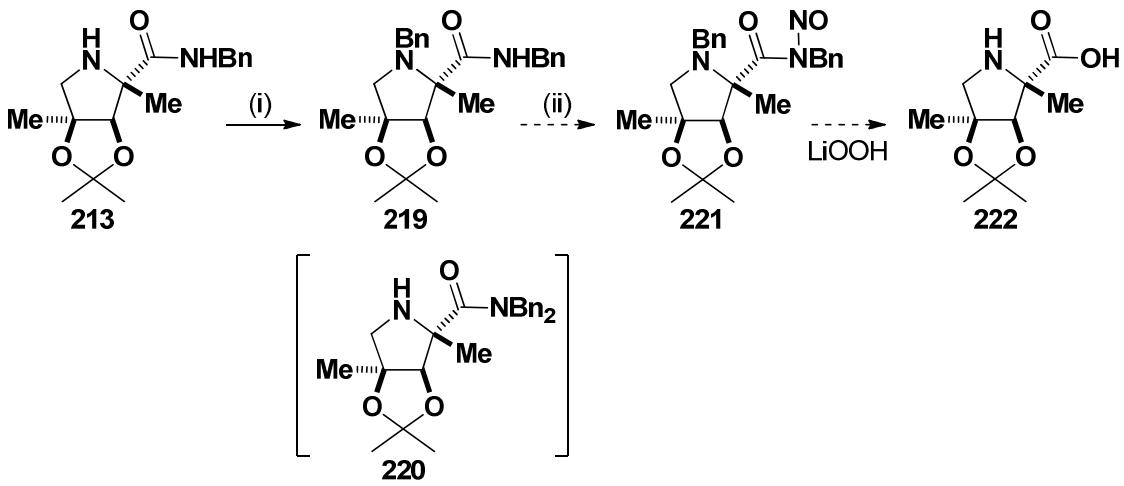
3.3.1a Nitrosation

Nitrosation of the amines, followed by thermal rearrangement has been used in the literature to convert amides into esters or acids (**Scheme 3.13**).[20,21,22,23]



Scheme 3.13: Thermal rearrangement of an *N*-nitroso amide into an acid. The work up conditions determine whether an ester or an acid is observed.

It was proposed that this process might be applied to the amide group in protected proline analogue **213** (**Scheme 3.14)**. Before this could be attempted, the ring nitrogen would need to be protected; a benzyl group was chosen as it should be facile to remove with a hydrogenation reaction. Protected proline **213** was stirred with benzyl bromide and sodium hydride under anhydrous conditions. Use of 1.2 equivalents of benzyl bromide did prevent dibenzylation occurring, but the yield of **219** was moderate, at 43%, with some recovered starting material. Attempting to drive the reaction to completion resulted in the formation of some of the dibenzylated product **220** (**Scheme 3.14**).



Reagents and conditions: (i) BnBr, NaH, DMF, 4Å ms, RT, 16 h; (ii) $NaNO_2$, Ac_2O, AcOH, 0°C→RT.

Scheme 3.14: The protection of the amine in **213** resulted in the formation of desired product **219** and dibenzylated side product **220**. Nitrosation of **219** was difficult to follow.

Attempts to protect the amine with diphenyl diazomethane under neutral conditions were unsuccessful, and no protected material was recovered from this reaction. Alternative protecting

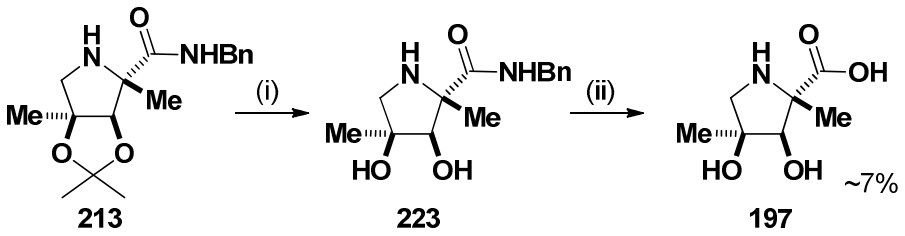
groups, such as Cbz and Boc were briefly investigated, but these too were hampered by concomitant amide protection.

Thus, protected proline analogue **219** was treated with sodium nitrite in acid to form the *N*-nitroso amide compound **221** (**Scheme 3.14**).[23] A change in R_f was observed by t.l.c. analysis of the reaction mixture, however confirmation that the desired compound had been formed was difficult to obtain. No significant shifts in the NMR spectrum were visible, nor were there any indicative signs *via* mass or IR analysis that the desired compound had been formed, however, the compound discoloured rapidly at room temperature, and so it might be that decomposition occurred too quickly for structural confirmation to be obtained. One of the decomposition products might in fact have been the desired rearrangement product, as in certain systems it is known that this process can occur rapidly, even without heating.[23] Crude *N*-nitrosoamide **221** was stirred with hydrogen peroxide and lithium hydroxide in wet THF.[24] The addition of hydrogen peroxide generates the HOO^- anion, which should be a smaller nucleophile than HO^- in aqueous media as a consequence of its smaller solvation energy, and therefore less prone to steric effects.[25] This should hopefully hydrolyse any ester generated from the breakdown of the nitroso compound. Despite repeated attempts, only complex mixtures were recovered, from which none of the desired acid **222** could be seen.

3.3.1b Hydrolysis

As both isopropylidene removal and amide hydrolysis could theoretically be accomplished under acidic conditions, **213** was treated with concentrated (37%) aqueous hydrochloric acid solution at 100°C. Whilst the isopropylidene group was removed, the amide proved to be particularly resistant to acid catalysed hydrolysis, presumably because of its proximity to a tertiary centre. By continuing to heat the reaction mixture in acid for a further 7 days some of the desired compound **197** was

obtained, but yields were very low, approximately 7% from the fully protected material (**Scheme 3.15**).



213 **223** **197**

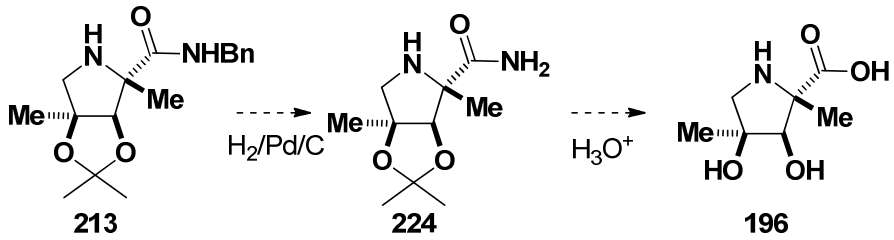
Reagents and Conditions: (i) 37% $HCl_{(aq.)}$, reflux, 48 h; (ii) 37% $HCl_{(aq.)}$, reflux, 7 days

Scheme 3.15: Hydrolysis of the isopropylidene and amide groups with 37% $HCl_{(aq.)}$.

Whilst hydrochloric acid did remove the isopropylidene group, the reaction was slow (48 hours); in an attempt to improve this, aqueous 48% HBr solution was tested. This was indeed found to be much faster, at only 60 minutes, however isolation of **223** was difficult. Neutralisation with sodium hydroxide or sodium carbonate resulted in a large quantity of inorganic salts, which were difficult to separate from the polar product. Elution through a basic resin column (Amberlite CG-400) had little effect on pH. Whilst **223**.HCl salt was stable at room temperature, **223**.HBr rapidly discoloured and decomposed, and for this reason, HCl deprotection was preferred.

Base catalysed amide hydrolysis was attempted on both **213** and **223** using aqueous calcium hydroxide solution at 100°C. However, in both cases only starting material was recovered after several days. In the singly branched case, lithium hydroxide was used to hydrolyse the amide, but that was found to be ineffective here. Fully protected proline **213** was treated with sodium methoxide in methanol to try and effect an esterification reaction; despite prolonged heating, no reaction was observed.

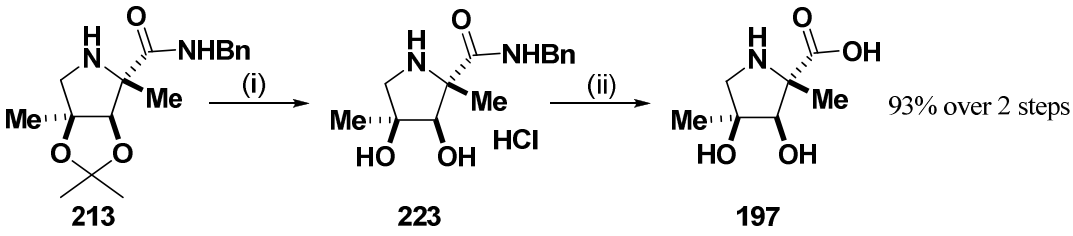
It was postulated that perhaps the presence of the benzyl group was adding to the steric hindrance of the carbonyl centre, hindering hydrolysis and therefore its removal was attempted *via* hydrogenation (**Scheme 3.16**).



Scheme 3.16: Proposed hydrogenation of proline analogue **213** to facilitate amide hydrolysis.

Fully protected proline **213** was stirred under a hydrogen atmosphere with palladium black in ethanol. No reaction was observed, and the addition of formic acid, used in some transfer hydrogenations as a source of hydrogen, did not help the reaction to proceed. A range of solvents and catalysts were tried, to no avail. Transfer hydrogenation with cyclohexene at reflux was similarly unsuccessful. These disappointing results are perhaps unsurprising, as benzyl amides can be difficult to cleave by hydrogenolysis, and severe conditions can be required such as dissolving metal reductions or high pressures.[26]

Returning to the direct hydrolysis of the amide group, KOH in methanol was tried on isopropylidene deprotected product **223**.HCl. This system had been used successfully on hindered amides, difficult to hydrolyse by other means,[27] and was found be efficacious here too (**Scheme 3.17**).



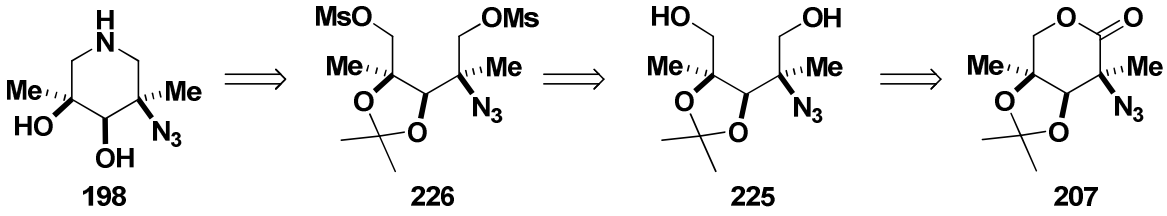
Reagents and Conditions: (i) 37% $HCl_{(aq.)}$, reflux, 140 h; (ii) KOH, MeOH, RT, 96 h.

Scheme 3.17: The optimal conditions were eventually discovered to be acid catalysed isopropylidene removal followed by strongly alkaline conditions to hydrolyse the amide group.

It was not possible to hydrolyse **213** under the same conditions of excess KOH in methanol, so clearly the presence of the isopropylidene hindered amide hydrolysis.

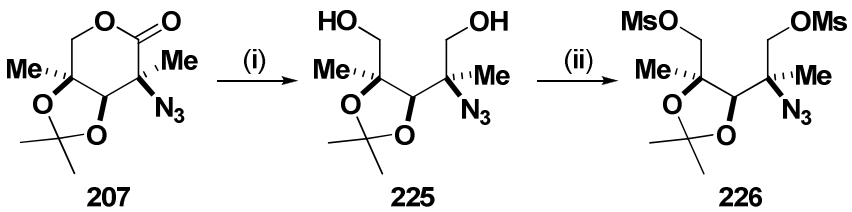
3.4 Branched Piperidine from Tertiary Azide

It was envisaged that azide **207** could be converted into piperidine **198** *via* a short sequence involving ring opening, alcohol activation and ring closure with an amine (**Scheme 3.18**). Similar sequences have been used to great effect in a number of imino sugar syntheses.[28,29,30]



Scheme 3.18: Retrosynthesis of piperidine **198**.

Reduction of lactone **207** was achieved in high yield (83%) using lithium borohydride, with no concomitant azide reduction observed. Given the difficulties observed so far in these systems with triflate instability, a methanesulfonyl group was chosen for activation of the alcohols. Diol **225** was taken with an excess of mesyl chloride in pyridine at room temperature. After two hours the reaction was deemed complete by t.l.c. analysis however, after purification of the crude, it became apparent that the mono and di-mesylated products co-eluted in the chosen solvent system. For this reason, an additional 2 equivalents of mesyl chloride were added to further reactions after one hour, and the reaction continued for a minimum of 4 hours. Larger scale reactions could be performed in dichloromethane as solvent and reacted for 16 hours without compromising the yield. The dimesylated azide **226** could be obtained in up to quantitative yields (**Scheme 3.19**).



Reagents and Conditions: (i) $LiBH_4$, THF, RT, 165 min; (ii) MsCl, Py, 0°C→RT, 5 h *or* MsCl, Py, DCM, 0°C→RT, 16 h.

Scheme 3.19: Facile synthesis of dimesylate **226**.

Benzylamine was chosen to effect the ring closure reaction. The reasons for this were twofold; benzylamine had been used successfully within the group for this type of conversion on several occasions and previous investigations into biological activity had revealed the presence of a benzyl group could boost enzyme inhibition (c.f. **Scheme 1.33**). Thus dimesylate **226** was stirred with benzylamine in tetrahydrofuran at room temperature. No reaction was observed after a prolonged reaction time, and the system was heated to reflux. Evidence of a small quantity of some product was observed by t.l.c. analysis but the reaction was sluggish. This product is believed to be a mixture of mono displaced systems **227** and **228** (**Figure 3.8**).

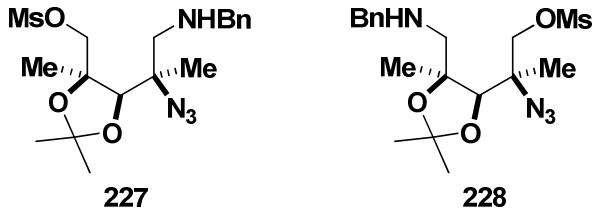
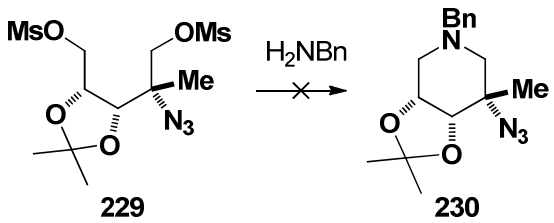


Figure 3.8: Proposed products from the attempted double displacement of dimesylate **226**.

Full structural elucidation at this time was not possible as the product obtained was clearly a mixture of compounds and was also contaminated with residual benzylamine; evidence for these structures comes from LRMS data and ^{13}C DEPT spectra, in which some 'CH_2' peaks appear unchanged from the starting dimesylate. Heating these systems to higher temperatures in neat benzylamine resulted in complex mixtures. It is possible that one of the products was a polymer, but this proposal has little evidence to support it, except for a peak in the LRMS spectrum. This result, although disappointing, is not wholly unsurprising as two neopentyl S_N2 reactions are required, and these are likely to be slow. Also, similar work on the related system **229** had similarly failed to generate the desired piperidine **230** (**Scheme 3.20**).[17]



Scheme 3.20: Double displacement with benzylamine could not be achieved on the analogous singly branched system.

It was thought that the presence of the isopropylidene group may have been preventing ring closure and attempts to remove it were made. Dimesylate **226** was taken with acidic resin and heated to 50°C; after 16 hours a single product was visible by t.l.c. analysis, however NMR and mass spectral analyses revealed a mixture of products including the desired diol **231** (ESI-ve *m/z* 360, $[M-H]^-$). There was evidence of a further product(s) with ESI-ve *m/z* 264, this could correspond to any of the epoxides **232**, **233** or **234** or oxetane **235** shown (**Figure 3.9**).

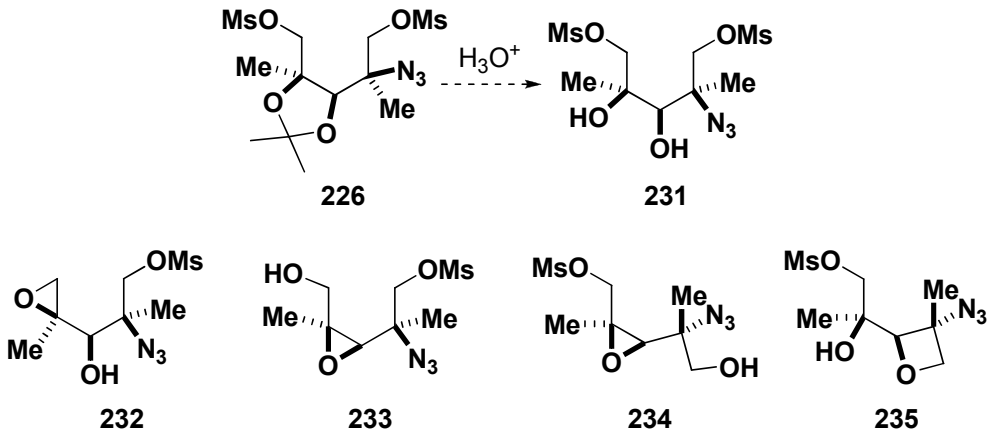
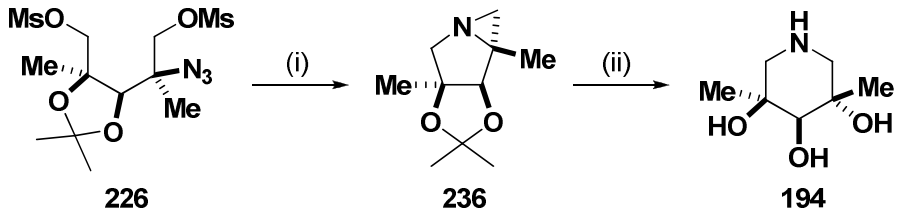


Figure 3.9: Possible products from the isopropylidene deprotection of dimesylate **226**.

The extremely complex nature of this reaction lead to the development of an alternative route *via* aziridine **236** (**Scheme 3.21**). This scheme involves an ambitious double neopentyl S_N2 reaction, already shown to be difficult to achieve, and selective opening of the aziridine in to a six, rather than a five, membered ring, potentially with concomitant isopropylidene deprotection. The *arabino* stereochemistry **194** is predicted as this is the least hindered product, with the methyl groups on opposite faces.

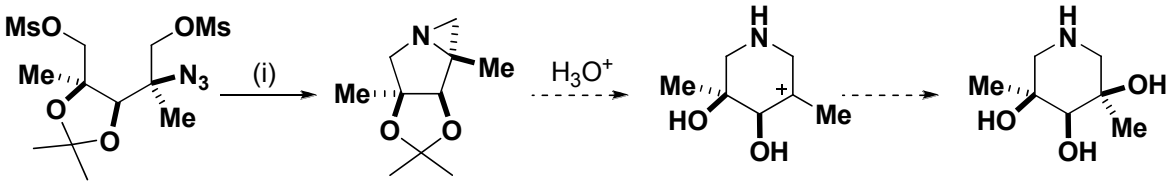


Proposed Reagents and Conditions: (i) Pd/C, H_2; (ii) H_3O^+

Scheme 3.21: Synthesis of piperidine **194** from dimesylate **226** *via* aziridine **236.**

Although the double neopentyl displacement is ambitious, the intramolecular nature of the reactions should promote ring closure. Initial investigations taking dimesylate **226** with hydrogen and palladium in ethanol were disappointing and extensive decomposition was observed. pH Analysis of the reaction mixture showed it to be strongly acidic, suggesting that the mesylate group had been displaced, generating mesic acid which in turn degraded the sugar. Addition of triethylamine circumvented this problem and aziridine **236** was obtained in moderate yield (40%) in just 1 hour. By shortening the reaction time to 30 minutes and replacing the column chromatography purification step with a shorter silica plug, almost quantitative yields were obtained (**Scheme 3.22**).

Aziridine **236** was stirred in 1 M HCl, in the hope that an S_N1 type ring opening would occur. Although the carbocation **237** could in theory lead to a mixture of products, it was anticipated that the inherent steric bulk of the molecule would provide a large degree of stereoselectivity, as had been the case in the formation of azido lactones **207**, **210** and deoxy lactone **205**. Thus *arabino* piperidine **194** was anticipated as this would be the least hindered product (**Scheme 3.22**). Concomitant isopropylidene deprotection would afford the desired product **194** in just two steps from dimesylate **226**.



226 **236** **237** **194**

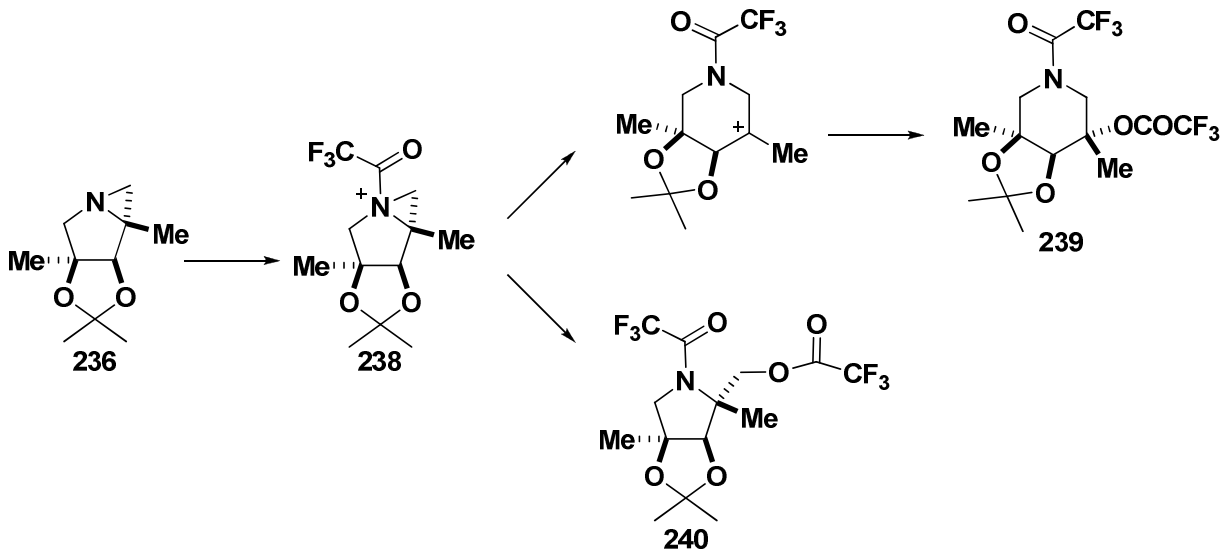
Reagents and Conditions: Pd/C, H_2, TEA, EtOH, 30 min, RT, 96%.

Scheme 3.22: Anticipated *ribo* product **194** in just two steps from **226**.

Regrettably, no useful products could be recovered from his reaction; repeat attempts with milder acids were similarly unsuccessful. In an attempt to promote just the ring opening reaction, and avoid isopropylidene deprotection, aziridine **236** was stirred with wet acetonitrile. Under these

conditions some reaction was observed, as seen by a change of colour and R_f, however, again, the reaction mixture contained an inseparable complex mixture of products.

An alternative approach was investigated, using trifluoroacetic anhydride to activate the nitrogen by generating cation **238**, and thereby promote aziridine ring opening. This would provide a product with a protected nitrogen atom **239**, hopefully simplifying purification procedures; however the trifluoroacetate could compete with an S_N2 style ring opening **240** (**Scheme 3.23**). Trifluoroacetate was chosen as the resulting acetate should hydrolyse rapidly, and trifluoroacetate is a relatively poor nucleophile, compared with, for example, methyl chloroformate where the corresponding nucleophile would be chloride.



Scheme 3.23: TFAA opening of aziridine **236**.

Given the sensitivity of aziridine **236** to acidic conditions, it was taken with trifluoroacetic anhydride in tetrahydrofuran with triethylamine. A reaction was visible on t.l.c. after only 10 minutes. Despite appearing to be a single compound on t.l.c., it was in fact shown by NMR analysis to be a mixture of at least four compounds (**Figure 3.10**).

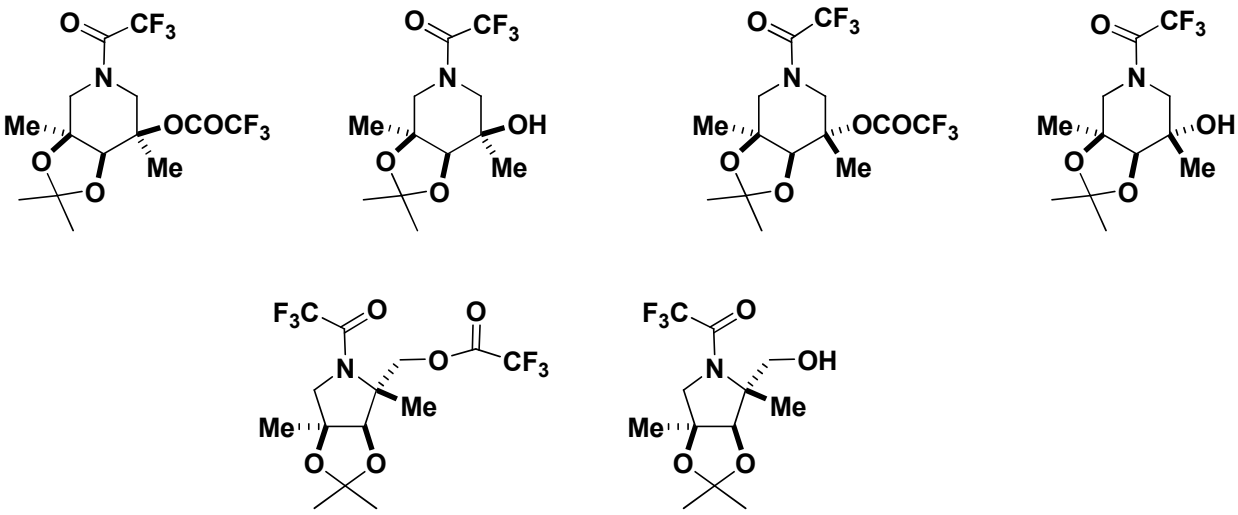
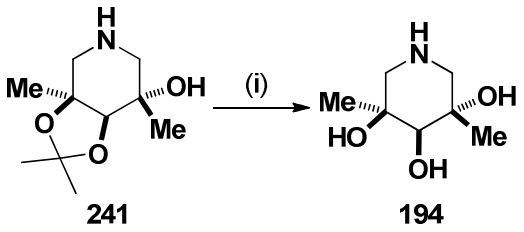


Figure 3.10: Possible products that could arise from TFAA opening of aziridine **236**.

It was not possible to tell at this point which compounds had been formed and separation of the mixture was not trivial, as they co-eluted in a number of solvent systems. Instead, the crude mixture was taken with potassium carbonate in methanol and heated to reflux. It was hoped that this would hydrolyse the trifluoroacetate groups, and therefore reduce the number of potential products to just three. This was indeed seen to the the case, as no carbonyl stretch was visible in the IR spectrum after the reaction. It was shown by NMR to be a mixture of three compounds with one dominant compound, accounting for approximately 80% of the material, still assumed to be the *arabino* piperidine **241**. Once again, purification of the compounds was not possible and so the crude was taken with 1 M HCl to remove the isopropylidene group (**Scheme 3.24**).



Reagents and conditions: (i) 1 M HCl, 60°C, 1 h.

Scheme 3.24: Synthesis of piperidine **194**?

Although this would generate a highly polar compound which would be difficult to purify by standard laboratory techniques, a collaborator of the Fleet group with much experience in the purification of imino sugars had kindly agreed to purify this compound using a selection of ion

exchange resins. Thus a sample of what was believed to be **194** was sent to Jackie Hollingshead and Professor Rob Nash at Summit Corporation Plc. Their purification methods were somewhat successful and have allowed much cleaner ^{1}H and ^{13}C NMR spectra to be obtained (**Figure 3.11**). Analysis was performed by Dr M. Wormald at the Biochemistry Department of the University of Oxford.

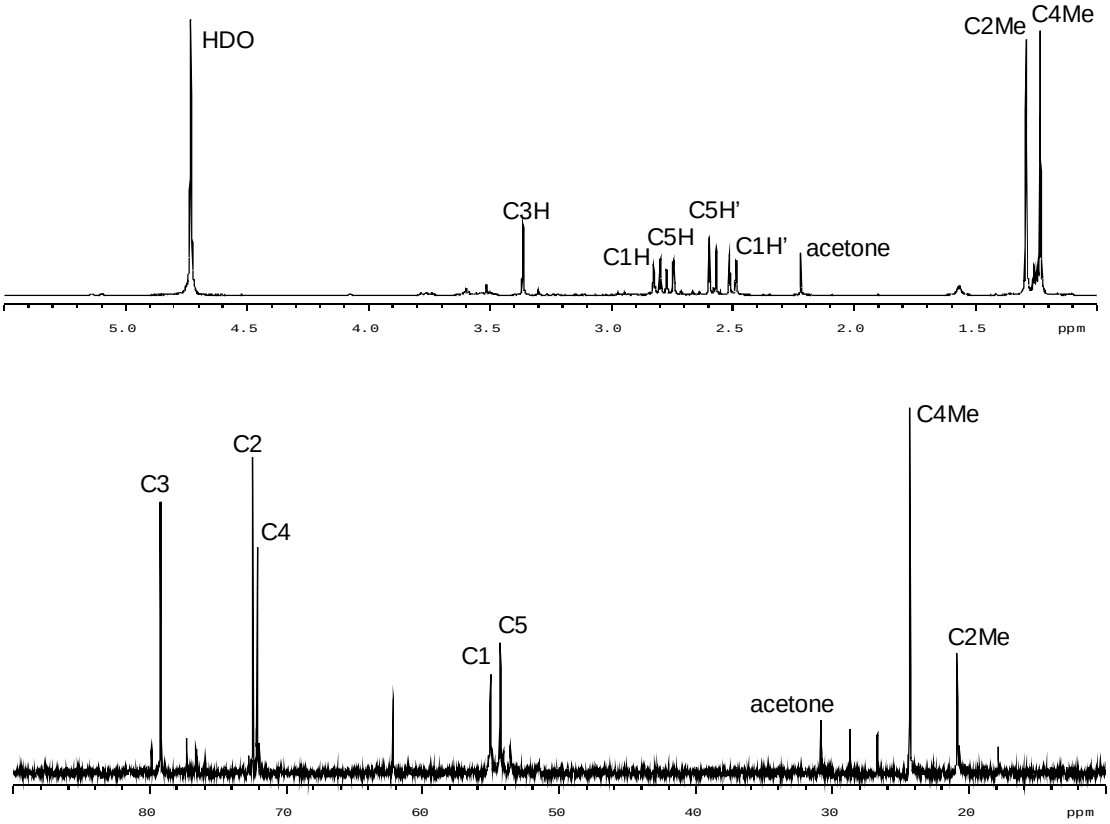


Figure 3.11: ^{1}H and ^{13}C NMR spectra of possible piperidine **194**.

The position of the two CH_2 groups very close to one another in the NMR spectra implies similar chemical environments and therefore a six, rather than a five ring structure is more likely. In a five membered ring system, the CH_2 group outside the ring would be expected to have a significantly different shift to the one inside the ring. HMBC analyses on the purer material were able to confirm the six ring structure by a C1 to H5 and complementary C5 to H1 coupling. The stereochemistry at C2 was elucidated by ROE spectroscopy; strong ROEs between H1', H3 and H5' place these three protons on the same side of the ring and in an axial position (assuming that the ring is in a chair conformation). The strong ROEs from CH_3-4 to H3 and H5' place this methyl group on the same

side of the ring as H3 and therefore equatorial (again assuming a chair conformation). This is also consistent with the moderate ROE from CH_3-4 to H5. The strong ROE from CH_3-2 to H1, the very weak ROE from CH_3-2 to H3 and the absence of a CH_3-2 to H1' ROE place this methyl group on the opposite side of the ring to H3 and thus axial. These results are consistent with the original prediction of the *arabino* compound **194** (**Figure 3.12**).

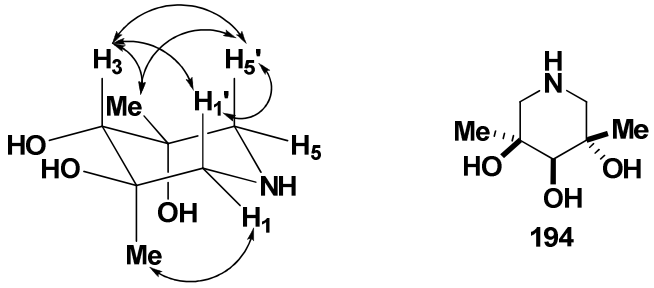
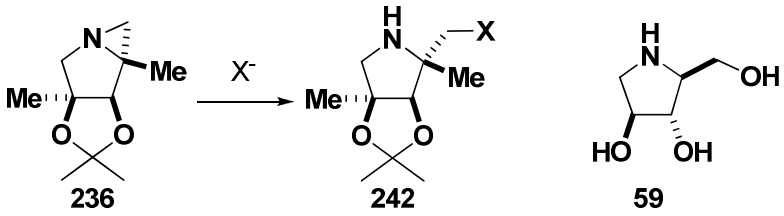


Figure 3.12: ROE enhancements confirm the *arabino* product **194**.

3.4.1 Nucleophilic Aziridine Ring Opening

Attempts were made to open the aziridine ring with a nucleophile, as this would gauge the ease of this reaction occurring and therefore how likely it might be that the previous sequence could have generated the 5 membered ring product. Nucleophilic opening of the aziridine ring would generate a branched DAB analogue of the type **242** (**Scheme 3.25**). DAB **59** is a known potent glycosidase and glycogen phosphorylase inhibitor and synthesis of analogues has received much attention.[31,32,33,34]



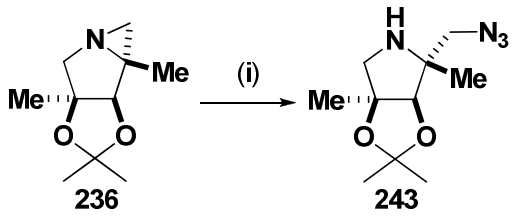
236 242 59

Scheme 3.25: Opening aziridine **236** under basic conditions to generate pyrrolidine type imino sugars **244**.

Most aziridine rings are very stable to nucleophilic conditions; however, the more strained nature of this one led us to believe that it might be achievable if the right conditions could be found. Initial investigations were discouraging as **236** proved to be remarkably resistant to S_N2 style ring

opening, with no reaction observed with sodium or potassium methoxide or hydroxide, or sodium acetate, even after prolonged heating.

Work by Yoshimitsu *et al* had shown the opening of a strained aziridine with sodium azide under remarkably mild conditions, with the reaction occurring at room temperature in DMF.[35] Direct repetition of these conditions was not possible, however by heating the reaction to 100°C, the desired opened compound **243** was obtained in 51% yield, albeit with a reaction time of 6 days (**Scheme 3.26**).



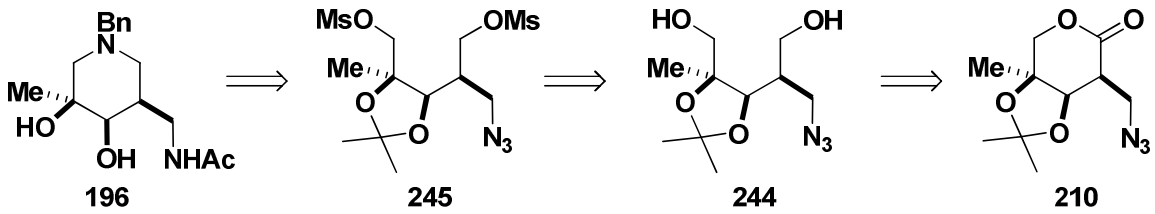
236 243

Reagents and conditions: NaN_3, DMF, trace water, 100°C, 6 days.

Scheme 3.26: Nucleophilic ring opening of aziridine **236**.

3.5 Azidomethyl Branched Piperidine

A second piperidine synthesis was proposed from the Michael addition azide **210**, generating an isofagomine analogue.[36] It was again envisaged that azide **210** could be converted into piperidine **196** *via* a short sequence involving ring opening to diol **244**, alcohol activation to dimesylate **245** and ring closure with an amine. Hydrogenation and acetylation of the azide group and isopropylidene deprotection should then give piperidine **196** (**Scheme 3.27**). This sequence was unsuccessful in the previous system, but it was hoped here that the presence of only one, instead of two tertiary centres could promote ring closure.



Scheme 3.27: Route towards novel piperidine **196**.

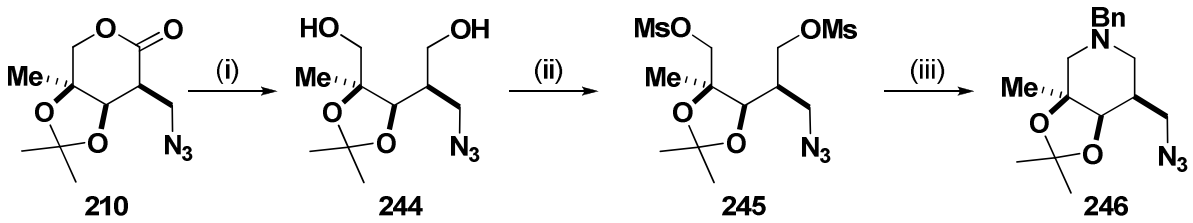
Azido lactone **210** was taken with lithium borohydride, as this reducing agent had been used in the previous system to great effect. It quickly became apparent that this system was unsuitable, as a very large number of side products were observed on t.l.c. analysis. It was believed that these were formed as a result of removal of the acidic hydrogen α- to the lactone functionality, followed by decomposition. It has also been observed that in certain systems, lithium borohydride can reduce the azide group,[37] however this seems highly substrate dependent. Reduction of the lactone to the diol with sodium borohydride was attempted, to reduce the possibility of azide reduction, however again, a complex mixture of products were observed. Adjusting the pH of the sodium borohydride reduction to 5 with acetic acid was trialled as this approach has been used successfully as a more gentle system to reduce lactones, stopping at the lactol stage.[38] T.l.c. analysis of the reaction mixture was more promising, and fewer side products were seen, however the mass recovered was very low. NMR analysis confirmed the major product to be the lactol, but the low mass and contamination, despite looking clean by t.l.c. meant that an alternative was sought.

Reduction of the azide group separately prior reduction of the lactone was put forward as an alternative strategy. Not only would this remove the potential problems of concomitant azide and lactone reduction, but it would allow for a high temperature reduction procedure, as good results have been obtained using refluxing *tert*-butanol and sodium borohydride.[39] Azido lactone **210** was treated with palladium black in ethanol under a hydrogen atmosphere. For reasons that are not fully understood, the reaction, whilst appearing to go ‘spot to spot’ on t.l.c. gave a poor yield of barely

30%, before purification. Satisfactory purification could not be achieved, and as such attention returned to the direct reduction of the lactone.

Use of a non basic reducing agent should in theory prevent deprotonation at C2 and therefore limit side reactions. DIBAL is a commonly used electrophillic reducing agent, capable of reducing lactones to lactols, used in a variety of carbohydrate syntheses.[40] Once reduced to the lactol, the proton α- to the carbonyl should now be less acidic, and a stronger reducing agent should be able to take the lactol down to the diol. The two stage approach to the reduction of the lactone did afford limited success, using lithium borohydride as the second reducing agent, but yields were moderate, at around 41% (**Scheme 3.28**).

Dimesylation was straightforward and activated azide **245** could be obtained in 81% yield (**Scheme 3.28**). Treatment of this dimesylate with benzylamine as before was highly successful, giving a single product **246** in quantitive yield.

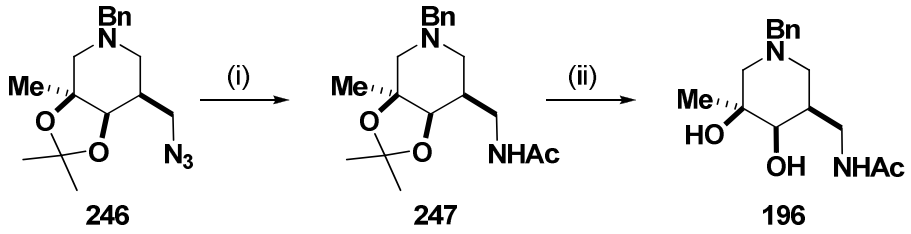


210 **244** **245** **246**

Reagents and Conditions: (i) DIBAL, DCM, -30°C→ -20°C, 2 h then $LiBH_4$, THF, RT, 2 h ; (ii) MsCl, Py, 0°C→RT, 2h; (iii) H_2NBn, 100°C, 20h.

Scheme 3.28: More difficult ring opening then the tertiary azide, but more facile ring closing reaction to give protected piperidine **246**.

Reduction of the azide was now facile, and complete conversion could be achieved in 90 minutes using palladium on carbon in ethanol in a hydrogen atmosphere. Attempts to purify the crude were complicated by the high polarity of the product, and resulted in a greatly compromised yield. Instead the crude was directly treated with acetic anhydride in pyridine, affording **247** in 53% yield over two steps (**Scheme 3.29**). Subsequent isopropylidene removal was surprisingly facile in this instance, and the acetonide was removed in 82% yield to afford **196**.



Reagents and Conditions: (i) Pd/C, H_2, EtOH, RT, 90 min then Ac_2O, Py, RT, 2 h; (ii) TFA, H_2O, RT, 70 min.

Scheme 3.29: Synthesis of benzyl protected piperidine **196**.

3.6 Conclusions and Future Work

In the final section of this project a series of synthetic routes were explored towards a range of branched imino sugars. The synthetic utility of branched sugar lactone **103** was demonstrated through the development of a viable route to novel deoxy sugar **121**, branched proline derivative **197** and piperidines **194** and **196** have been established (**Figure 3.13**).

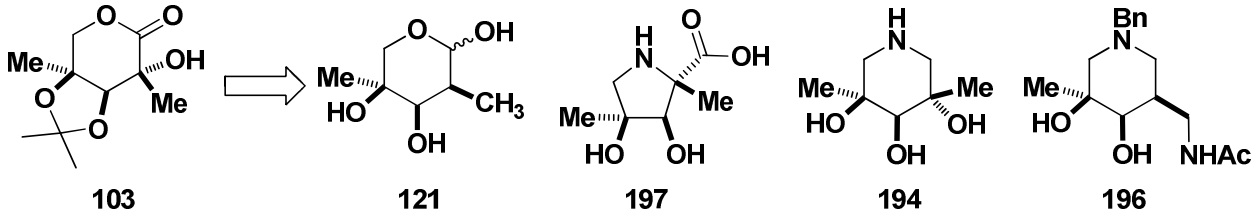


Figure 3.13: Products generated from 2,4-di-*C*-methyl-L-arabinono-1,5-lactone **103**.

Biological testing of these imino sugars has not yet been performed. Future work could focus on the using azide **243** to generate a range of pyrrolidines or triazole derivatives of the type **248** (**Figure 3.14**).

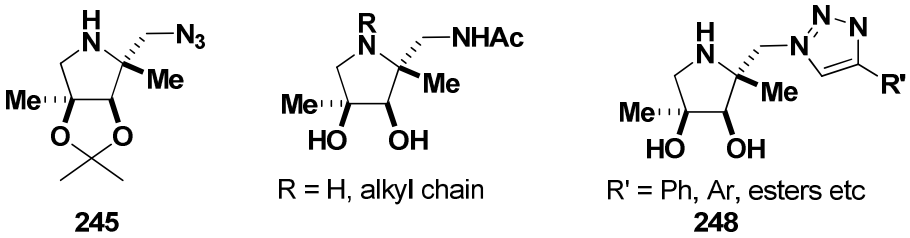
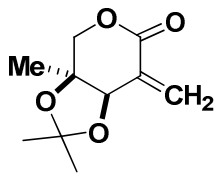


Figure 3.14: Potential products from azide **243**.

3.7 Experimental

For an explanation of the general experimental procedures used see **Chapter 2**, page 120.

2-Deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-2-*C*-methylene-L-erythro-pentono-1,5-lactone 199

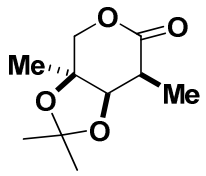


2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** (136 mg, 0.63 mmol) was dissolved in dichloromethane (1.5 mL) and cooled to 0°C. Once cool, pyridine (0.15 mL, 1.89 mmol) and trifluoromethanesulfonic anhydride (0.17 mL, 1.01 mmol) were added dropwise. The reaction mixture was kept under argon, and allowed to warm to room temperature. Two further additions of pyridine (2 x 0.15 mL, 2 x 1.89 mmol) and trifluoromethanesulfonic anhydride (2 x 0.17 mL, 2 x 1.01 mmol) were necessary at two hour intervals before almost complete consumption of starting material (R_f 0.54) and a single product (R_f 0.82) were observed. The reaction mixture was quenched with dropwise addition of 1 M hydrochloric acid (aq.), until the evolution of gas had ceased. Water and dichloromethane (15 mL each) were added and the aqueous and organic phases separated. The aqueous phase was washed with dichloromethane (2 x 15 mL) and the combined organic layers were dried (magnesium sulfate), filtered and the solvents concentrated under reduced pressure to afford a solid yellow crude. This was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:2) to yield the white solid triflate (198 mg, 90%). The triflate (198 mg, 0.57 mmol) was immediately dissolved in *N*,*N*-dimethylformamide (3 mL) and stirred at room temperature under argon for 16 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed the presence of one major product (R_f 0.48; UV active) and complete consumption of starting material (R_f 0.82). The reaction mixture was partitioned between water and dichloromethane (15 mL of each), and the aqueous phase washed three times. The combined

organic phases were dried (magnesium sulfate), filtered, and concentrated under reduced pressure. Subsequent column chromatography (ethyl acetate/cyclohexane, 1:5) afforded alkene **199** (78 mg, 63% over two steps) as a white solid.

HRMS (ESI+ve): found 221.0792 $[M+Na]^+$; $C_{10}H_{14}Na_1O_4$ requires 221.0784; $[\alpha]_D^{21}$ +156.9 (*c*, 0.575 chloroform); m.p. 68-69°C; ν_{max} (thin film): 1745 (s, C=O), 1452 (m, C=C); δ_H ($CDCl_3$, 400 MHz): 1.42 (3H, s, CH_3-4), 1.44 (3H, s, $C(CH_3)_2$), 1.46 (3H, s, $C(CH_3)_2$), 3.82 (1H, d, H5, $J_{5,5'}$ 12.1), 4.18 (1H, d, H5', $J_{5',5}$ 12.1), 4.60 (1H, s, H3), 5.87 (1H, s, H2'), 6.33 (1H, s, H2''); δ_C ($CDCl_3$, 100 MHz): 22.6 (CH_3-4), 26.9 ($C(\mathbf{C}H_3)_2$), 27.6 ($C(\mathbf{C}H_3)_2$), 72.5 (C5), 79.3 (C4), 81.3 (C3), 110.9 ($\mathbf{C}(CH_3)_2$), 130.1 (C2'), 135.1 (C2), 167.0 (C1); LRMS (ESI+ve): 262 ($[M+Na+MeCN]^+$, 100%), 216 ($[M+NH_4]^+$, 70%).

2-Deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone 205

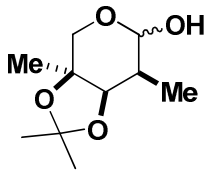


2-Deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-2-*C*-methylene-L-erythro-pentono-1,5-lactone, **199** (65 mg, 0.33 mmol) was dissolved in ethanol (3 mL) and 20% palladium on carbon (7 mg) was added. The reaction mixture was degassed and stirred under hydrogen for 16 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed the complete consumption of starting material (R_f 0.48) and a major product (R_f 0.52). The reaction mixture was degassed, and the salts filtered through a pad of Celite®. Concentration of solvents under reduced pressure afforded 64 mg of crude, which was purified by column chromatography (ethyl acetate/cyclohexane, 1:3) to yield deoxy lactone **205** (47 mg, 72%), as a single stereoisomer.

HRMS (ESI+ve): found 223.0939 $[M+Na]^+$; $C_{10}H_{16}NaO_4$ requires 223.0941; $[\alpha]_D^{21}$ +156.3 (*c*, 0.735 chloroform); m.p. 117°C; ν_{max} (thin film): 1745 (s, C=O); δ_H (C_6D_6, 400 MHz): 0.75 (3H, s,

CH_3-4), 1.13 (3H, s, $C(CH_3)_2$), 1.29 (3H, d, CH_3-2, $J_{CH3\text{-}2,2}$ 6.8), 1.38 (3H, s, $C(CH_3)_2$), 1.71 (1H, qd, H2, $J_{2,CH3\text{-}2}$ 6.8, $J_{2,3}$ 2.4), 3.14 (1H, d, H5, $J_{5,5'}$ 12.5), 3.59 (1H, d, H3, $J_{3,2}$ 2.4), 3.79 (1H, d, H5', $J_{5',5}$ 12.5); δ_C (C_6D_6, 100 MHz): 13.0 (CH_3-2), 22.5 (CH_3-4), 26.6 ($C(\mathbf{C}H_3)_2$), 27.3 ($C(\mathbf{C}H_3)_2$), 38.0 (C2), 72.4 (C5), 79.3 (C4), 82.8 (C3), 109.6 ($\mathbf{C}(CH_3)_2$), 171.6 (C1).

2-Deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose 206

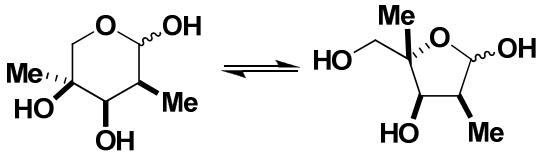


2-Deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **205** (25 mg, 0.13 mmol) was dissolved in dichloromethane (0.3 mL) and cooled to -78°C under argon. Di*iso*butylaluminium hydride solution (1 M in toluene; 138 µL, 0.14 mmol) was added and the reaction stirred for 90 minutes, after which time IR analysis showed the reaction to be complete by the absence of a stretch at 1745cm^{-1}. The reaction mixture was diluted with ethyl acetate (2 mL), and quenched with sodium hydrogen carbonate solution (sat. aq., 0.2 mL). Potassium dihydrogen orthophosphate (50 mg) was added, followed by sodium sulfate until the solution became clear. The resulting gel was filtered through Celite®, eluting with ethyl acetate, and concentrated under reduced pressure. The crude (36 mg) was then purified by column chromatography (ethyl acetate/cyclohexane, 1:2) to afford the title compound **206** (15 mg, 59%) as a white solid, in a 5:2 ratio of anomers.

HRMS (FI+ve): found 202.1207 $[M]^{\cdot}$; $C_{10}H_{18}O_4$ requires 202.1205; $[\alpha]_D^{25}$ -49.6 (*c*, 0.15 chloroform); m.p. 76-79°C; ν_{max} (thin film): 3369 (s, br, OH); δ_H (C_6D_6, 500 MHz): 1.00 (1H, d, CH_3-$2^{\mathbf{B}}$, $J_{CH3\text{-}2,2}$ 7.3), 1.04 (3H, s, CH_3-$4^{\mathbf{A}}$), 1.12 (3H, s, $C(CH_3)_2{}^{\mathbf{B}}$), 1.14 (3H, d, CH_3-$4^{\mathbf{B}}$, $J_{CH3\text{-}4,5}$ 1.0), 1.21 (3H, a-d, CH_3-$2^{\mathbf{A}}$, $J_{CH3\text{-}2,2}$ 6.9), 1.25 (3H, s, $C(CH_3)_2{}^{\mathbf{A}}$), 1.29 (3H, s, $C(CH_3)_2{}^{\mathbf{B}}$), 1.50 (3H, s, $C(CH_3)_2{}^{\mathbf{A}}$), 1.50-1.57 (2H, m, $H2^{\mathbf{A}}$, $H2^{\mathbf{B}}$), 2.39 (1H, d, $OH^{\mathbf{A}}$, $J_{OH,1}$ 4.1), 3.29-3.31 (1H, dd, $H5^{\mathbf{B}}$, $J_{5,CH3\text{-}4}$ 1.0, $J_{5,5'}$ 12.0), 3.46 (1H, d, $H5^{\mathbf{A}}$, $J_{5,5'}$ 11.8), 3.47 (1H, d, $H3^{\mathbf{B}}$, $J_{3,2}$ 2.8), 3.56 (1H, d, $H5'^{\mathbf{A}}$, $J_{5',5}$

11.8), 3.58 (1H, d, H3$^{\mathbf{A}}$, $J_{3,2}$ 2.8), 4.10 (1H, d, H5'$^{\mathbf{B}}$, $J_{5',5}$ 12.0), 4.15 (1H, d, OH$^{\mathbf{B}}$, $J_{OH,1}$ 10.7), 4.72 (1H, dd, H1$^{\mathbf{A}}$, $J_{1,OH}$ 4.3, $J_{1,2}$ 7.9), 4.94 (1H, dd, H1$^{\mathbf{B}}$, $J_{1,2}$ 3.5, $J_{1,OH}$ 10.7); δ_C (C_6D_6, 125 MHz): 14.3 (CH_3-2$^{\mathbf{B}}$), 14.8 (CH_3-2$^{\mathbf{A}}$), 22.3 (CH_3-4$^{\mathbf{B}}$), 22.4 (CH_3-4$^{\mathbf{A}}$), 27.3 (C(**C**H$_3$)$_2^{\mathbf{B}}$, C(**C**H$_3$)$_2^{\mathbf{A}}$), 27.8 (C(**C**H$_3$)$_2^{\mathbf{B}}$), 28.6 (C(**C**H$_3$)$_2^{\mathbf{A}}$), 34.8 (C2$^{\mathbf{B}}$), 37.5 (C2$^{\mathbf{A}}$), 64.1 (C5$^{\mathbf{B}}$), 69.3 (C5$^{\mathbf{A}}$), 76.7 (C4$^{\mathbf{B}}$), 78.9 (C4$^{\mathbf{A}}$), 82.4 (C3$^{\mathbf{B}}$), 82.7 (C3$^{\mathbf{A}}$), 95.0 (C1$^{\mathbf{B}}$), 97.5 (C1$^{\mathbf{A}}$), 108.9 (**C**(CH$_3$)$_2^{\mathbf{A}}$, **C**(CH$_3$)$_2^{\mathbf{B}}$).

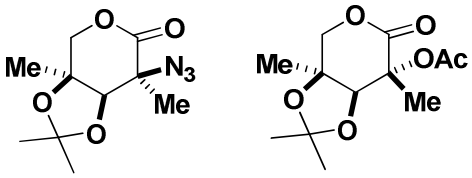
2-Deoxy-2,4-di-*C*-methyl-L-arabinose 121



2-Deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinose **206** (50 mg, 0.25 mmol) was dissolved in water (2 mL) and Dowex® (50W X8 H^+ form), 500 mg, was added. T.l.c analysis (ethyl acetate) after one hour revealed complete consumption of starting material (R_f 0.82) and a single major product, R_f 0.31. The resin was removed *via* filtration and the solvents concentrated under reduced pressure to afford 2-deoxy-2,4-di-*C*-methyl-L-arabinose **121** (33 mg, 82%) as a colourless oil requiring no further purification. The sugar exists as a mixture of 4 compounds in D_2O; A:B:C:D in a ratio of 1:0.26:0.24:0.1 where A & C are the 6 membered ring pyranose forms and B & D are the 5 membered ring furanose forms.

$[\alpha]_D^{22}$ -12.7 (*c*, 0.56 acetonitrile); ν_{max} (thin film): 3385 (s, br, OH); See **Chapter 2** for NMR data.

2-Azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone 207 and 2-*O*-acetyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone 208



2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** (287 mg, 1.33 mmol) was dissolved in dry dichloromethane (3 mL) and cooled to 0°C. Once cool, anhydrous pyridine (0.32 mL, 3.99 mmol) and trifluoromethanesulfonic anhydride (0.36 mL, 2.13 mmol) were added dropwise to the reaction mixture. The mixture was kept under argon, and allowed to warm slowly to room temperature. Two further additions of pyridine (2 x 0.32 mL, 2 x 3.99 mmol) and trifluoromethanesulfonic anhydride (2 x 0.36 mL, 2 x 2.13 mmol) were necessary at two hour intervals before almost complete consumption of starting material (R_f 0.54) and a single product (R_f 0.82) were observed. The reaction mixture was diluted with dichloromethane (7 mL) and quenched with dropwise addition of 2 M hydrochloric acid (aq.), until the evolution of gas had ceased. Water (10 mL) was added and the aqueous and organic phases separated. The aqueous phase was washed twice with dichloromethane (10 mL) and the combined organic layers were dried (magnesium sulfate), filtered and the solvents concentrated under reduced pressure to afford a yellow crude. This was dissolved immediately in anhydrous *N*,*N*-dimethylformamide (4 mL) and sodium azide was added (112 mg, 1.73 mmol). The reaction mixture was stirred at room temperature under argon for 96 hours, after which time t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) revealed the presence of one major product (R_f 0.46), unreacted arabinono-1,5-lactone (R_f 0.54) and complete consumption of triflate (R_f 0.82). Removal of solvent under reduced pressure afforded a yellow oil which was dissolved in water (10 mL) and dichloromethane (10 mL) and the aqueous and organic phases separated. The aqueous phase was washed twice with dichloromethane (10 mL) and the combined organic layers were dried (magnesium sulfate), filtered and the solvents concentrated under reduced

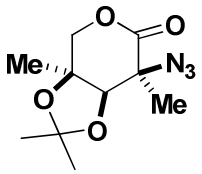
pressure to afford a yellow crude. Purification by column chromatography (ethyl acetate/cyclohexane, 1:3) afforded an inseparable mixture of 2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** and 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone **207** (238 mg), in a ratio of approximately 1.5:1 (starting material: azide), as shown by ^{1}H NMR. This was dissolved in dry pyridine (5 mL) and cooled to 0°C. Acetic anhydride (0.3 mL, 3.31 mmol) was added and the reaction mixture allowed to warm to room temperature. T.l.c. analysis (ethyl acetate/cycloheane 1:1) after 16 hours showed only starting materials (R_f 0.46 & 0.52). A further 0.3 mL of acetic anhydride was added and the solution heated to 45°C; T.l.c. analysis after 4 hours revealed no changes, so the mixture was heated to reflux for 16 hours, during which time the reaction mixture turned dark brown. After this time, T.l.c. analysis (DCM/toluene 1:2) showed two products (R_f 0.10 and 0.03). The solvents were concentrated under reduced pressure and the crude purified by column chromatography (DCM/toluene 12:1) to give the azide **207** (R_f 0.10 in DCM/toluene 1:2, 90 mg, 28%) and 2-*O*-acetyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **208** (R_f 0.03 in DCM/toluene 1:2, 46 mg, 13%). The acetate was recrystallised from ethyl acetate and cyclohexane by slow evaporation into transparent needles.

*Data for **207***: HRMS (ESI+ve): found 264.0955 $[M+Na]^+$; $C_{10}H_{15}N_3Na_1O_4$ requires 264.0954; $[\alpha]_D^{22}$ +117.9 (*c*, 1.14 chloroform); m.p. 108-112°C; ν_{max} (thin film): 3443 (s, br, O-H), 2108 (s, N_3), 1752 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.42 (3H, s, $C(CH_3)_2$), 1.45-1.46 (9H, m, $C(CH_3)_2$ & CH_3-2 & CH_3-4), 4.17 (1H, s, H3), 4.17 (1H, d, H5, $J_{5,5'}$ 12.9), 4.33 (1H, d, H5', $J_{5',5}$ 13.0); δ_C ($CDCl_3$, 100 MHz): 18.1 (CH_3-2), 24.7 (CH_3), 26.6 (CH_3), 27.2 (CH_3), 64.0 (C2), 73.9 (C5), 78.6 (C4), 86.6 (C3), 110.4 (**C**$(CH_3)_2$), 169.1 (C1).

*Data for **208***: Found C 55.83; H 7.15; $C_{12}H_{18}O_6$ requires C 55.81; H 7.02; HRMS (ESI+ve): found 281.0996 $[M+Na]^+$; $C_{12}H_{18}Na_1O_6$ requires 281.0995; $[\alpha]_D^{22}$ +71.6 (*c*, 0.77 chloroform); m.p. 152-155°C; ν_{max} (thin film): 1770 (s, C=O), 1745 (w, C=O); δ_H (C_6D_6, 500 MHz): 1.01 (3H, s, CH_3-4), 1.12 (3H, s, $C(CH_3)_2$), 1.36 (3H, s, $C(CH_3)_2$), 1.43 (3H, s, OAc), 1.81 (3H, s, CH_3-2), 3.79 (1H, d,

H5, $J_{5,5'}$ 15.0), 3.88 (1H, d, H5', $J_{5',5}$ 15.0), 4.09 (1H, s, H3); δ_C (C_6D_6, 125 MHz): 19.7 (OAc), 20.2 (CH_3-2), 24.2 (CH_3-4), 26.6 (C(**C**H_3)$_2$), 27.3 (C(**C**H_3)$_2$), 73.2 (C5), 78.5 (C2), 79.2 (C4), 83.5 (C3), 110.4 (**C**(CH_3)$_2$), 166.1 (C1), 167.9 (OAc).

2-Azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone 207



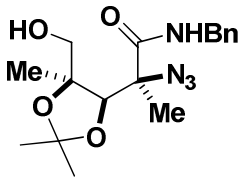
2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** (608 mg, 2.81 mmol) was dissolved in dry dichloromethane (7 mL) and cooled to 0°C. Once cool, anhydrous pyridine (0.68 mL, 8.44 mmol) and trifluoromethanesulfonic anhydride (0.78 mL, 4.50 mmol) were added dropwise to the reaction mixture. The mixture was kept under argon, and allowed to warm slowly to room temperature. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 2 hours revealed the presence of starting material (R_f 0.54) and a major product (R_f 0.82). The reaction mixture was cooled to 0°C and pyridine (0.68 mL, 8.44 mmol) and trifluoromethanesulfonic anhydride (0.78 mL, 4.50 mmol) were added. This was again allowed to warm to room temperature, and t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after a further 2 hours revealed the presence of starting material (R_f 0.54) and a major product (R_f 0.82). The reaction mixture was again cooled to 0°C and more pyridine (0.68 mL, 8.44 mmol) and trifluoromethanesulfonic anhydride (0.78 mL, 4.50 mmol) were added. The reaction mixture was warmed to room temperature and t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after a further 2 hours showed almost complete consumption of starting material (R_f 0.54) and a single product (R_f 0.82). The reaction mixture was quenched with dropwise addition of 1 M hydrochloric acid (aq.), until the evolution of gas had ceased. Water (10 mL) was added and the aqueous and organic phases separated. The aqueous phase was washed twice with dichloromethane (10 mL) and the combined organic layers were dried (magnesium sulfate), filtered

and the solvents concentrated under reduced pressure to afford 1.12 g of yellow crude. This was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:2) to yield the colourless solid triflate (977 mg, 99%) and starting material (8 mg). The triflate (977 mg, 2.81 mmol) was immediately dissolved in anhydrous *N*,*N*-dimethylformamide (12 mL) and sodium azide was added (237 mg, 3.65 mmol). The reaction mixture was stirred at room temperature under argon for 16 hours, after which time t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) revealed the presence of one major product (R_f 0.46) and complete consumption of starting material (R_f 0.82). The reaction mixture was concentrated under reduced pressure, and the crude re-dissolved in a mixture of water (20 mL) and dichloromethane (20 mL). The phases were separated, and the aqueous phase re-extracted with dichloromethane (2 x 20 mL). The combined organics were dried (magnesium sulfate), filtered, concentrated and the crude purified by column chromatography (ethyl acetate/cyclohexane, 1:3) to afford 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone, **207** (499 mg, 74%) as a white solid. This was recrystallised in ethyl acetate/cyclohexane, into plate-like crystals. NMR data identical to that listed above.

Larger scale procedure:

2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** (1.99 g, 9.23 mmol); anhydrous pyridine (3 x 2.23 mL, 3 x 27.69 mmol); trifluoromethanesulfonic anhydride (3 x 2.49 mL, 3 x 14.77 mmol); dichloromethane (20 mL); The procedure was carried out as above, however on large scale the trifluoromethanesulfonic anhydride must be added dropwise over 5 minutes or a greatly reduced yield results. This gave the desired triflate (3.59 g, quant.) which was treated with *N*, *N*-dimethylformamide (30 mL) and sodium azide (0.695 g, 10.7 mmol). The total mass of 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone **207** obtained was 1.05 g, 66%.

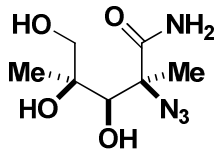
Benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribonamide 211



2-Azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone **207** (1.05 g, 4.35 mmol) was dissolved in tetrahydrofuran (10 mL) and stirred with benzylamine (1.43 mL, 13.05 mmol) at room temperature for 40 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed the formation of a single UV active spot (R_f 0.46), which co-spotted with the starting lactone; LRMS showed a large peak at *m/z* 347 corresponding to $[M-H]^-$ and no evidence of starting material (*m/z* 241). The solvents were concentrated under reduced pressure and the resulting crude purified by flash column chromatography (ethyl acetate/cyclohexane, 4:1→2:1) to afford benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribonamide, **211** as a white crystalline solid (1.55 g, quant.)

Found: C, 58.61; H, 6.94; N, 15.85; $C_{17}H_{24}N_4O_4$ requires: C, 58.61; H, 6.94; N, 16.08; HRMS (ESI+ve): found 371.1690 $[M+Na]^+$; $C_{17}H_{24}N_4Na_1O_4$ requires 371.1689; $[\alpha]_D^{20}$ -4.96 (*c*, 0.66 chloroform); m.p. 95°C; ν_{max} (thin film): 3418 (s, br, OH), 2129 (s, N_3), 1654 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.27 (3H, s, CH_3-4), 1.41 (3H, s, ($C(CH_3)_2$), 1.49 (3H, s, ($C(CH_3)_2$), 1.83 (3H, s, CH_3-2), 2.13 (1H, dd, OH, $J_{OH,5'}$ 4.0, $J_{OH,5}$ 9.4), 3.41 (1H, dd, H5 $J_{5,OH}$ 9.5, $J_{5,5'}$ 11.2), 3.68 (1H, dd, H5', $J_{5',OH}$ 3.7, $J_{5',5}$ 11.4), 4.38 (1H, s, H3), 4.38 (1H, dd, CH_2Ph, $J_{CH2Ph,NH}$ 5.8, J_{gem} 14.6), 4.44 (1H, dd, CH_2Ph, $J_{CH2Ph,NH}$ 5.8, J_{gem} 14.6), 6.95 (1H, a-s, NH), 7.27-7.39 (5H, m, Ar-H); δ_C ($CDCl_3$, 100 MHz): 22.7 (CH_3-4), 23.5 (CH_3-2), 26.8 ($C(\mathbf{C}H_3)_2$), 28.2 ($C(\mathbf{C}H_3)_2$), 43.9 ($O\mathbf{C}H_2Ph$), 65.2 (C2), 65.3 (C5), 83.1 (C4), 86.8 (C3), 107.2 ($\mathbf{C}(CH_3)_2$), 128.0, 128.1, 129.0 (Ar-C), 137.3 (Ar-C_{quat}), 169.0 (C1); LRMS (ESI-ve): 347 ($[M-H]^-$, 100%).

2-Azido-2-deoxy-2,4-*C*-dimethyl-L-ribonamide 214

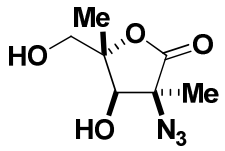


Method 1: Benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribonamide, **211** (59 mg, 17 μmol) was dissolved in water (5 mL) and 1,4-dioxane (0.5 mL). Dowex® (50W X8 H^+ form) acidic resin (30 mg) was added and the reaction stirred at 90°C for 6 hours. T.l.c analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed partial consumption of starting material (R_f 0.46) and the formation of a single major product (R_f 0.09). The reaction mixture was cooled to room temperature and stirred for a further 16 hours, after which time no starting material remained. The resin was removed by filtration and the solvents concentrated under reduced pressure. The resulting pale orange crude was purified by flash column chromatography (ethyl acetate/cyclohexane, 3:1) to afford 2-azido-2-deoxy-2,4-di-*C*-methyl-L-ribonamide **214** (22 mg, 60%).

Method 2: Benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribonamide, **211** (61 mg, 18 μmol) was dissolved in 37% $HCl_{(aq)}$ and heated to 60°C. T.l.c analysis (ethyl acetate/cyclohexane, 1:1) after 40 hours revealed a single major product (R_f 0.09) and complete consumption of starting material (R_f 0.46). The reaction mixture was concentrated under reduced pressure to afford ribonamide **214** as the HCl salt (65 mg, quant.).

$[\alpha]_D^{21}$ -99.8 (*c*, 0.96, chloroform); m.p. 98-100°C; ν_{max} (thin film): 3578 (s, br, OH), 2127 (s, N_3), 1643 (s, C=O); δ_H (CD_3OD, 400 MHz): 1.36 (3H, s, CH_3-4), 1.60 (3H, s, CH_3-2), 3.49 (1H, d, H5 $J_{5,5'}$ 12.5), 3.49 (1H, d, H5' $J_{5',5}$ 12.4), 4.30 (1H, s, H3); δ_C (CD_3OD, 100 MHz): 17.3 (CH_3-4), 18.9 (CH_3-2), 65.4 (C2), 65.8 (C5), 74.2 (C3), 89.0 (C4), 174.4 (C1).

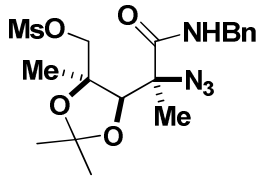
2-Azido-2-deoxy-2,4-di-*C*-methyl-L-ribono-1,4-lactone, 215



2-Azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone **207**, (339 mg, 1.41 mmol) was dissolved in water (10 mL) and stirred with Dowex® (50W X8 H^+ form, 1.0 g) at 70°C. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 21 hours revealed complete consumption of starting material (R_f 0.48) and a single product (R_f 0.11). The resin was removed by filtration, eluting with methanol and the solvents concentrated under reduced pressure to afford 2-azido-2-deoxy-2,4-di-*C*-methyl-L-ribono-1,4-lactone, **215** (259 mg, 92%) as a white crystalline solid.

HRMS (FI+ve): found 200.0669 $[M]^{\cdot}$; $C_7H_{10}N_3O_4$ requires 200.0666; $[\alpha]_D^{22}$-94.0 (*c*, 0.31 methanol); m.p. 79-81°C ν_{max} (thin film): 3385 (br, OH), 2123 (s, N_3), 1763 (s, C=O); δ_H (CD_3OD, 500 MHz): 1.36 (3H, s, CH_3-4), 1.59 (3H, s, CH_3-2), 3.49 (1H, d, H5 $J_{5,5'}$ 10.0), 3.62 (1H, d, H5' $J_{5',5}$ 10.0), 4.30 (1H, s, H3); δ_C (CD_3OD, 125 MHz): 17.1 (CH_3-4), 18.7 (CH_3-2), 65.3 (C4), 65.6 (C5), 74.1 (C3), 88.9 (C2), 174.1 (C1); LRMS (ESI+ve): 246 ($[M+HCOO]^-$, 100%).

Benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-5-*O*-methanesulfonyl-L-ribonamide 218



Method 1: Benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribonamide **211** (109 mg, 0.31 mmol) was dissolved in dry pyridine (1.5 mL) and cooled to 0°C in an ice bath, whilst stirring under argon. Methanesulfonyl chloride (27 µL, 0.35 mmol) was added, and the ice bath removed. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 90 minutes showed the partial consumption of starting material (R_f 0.46) and a single product (R_f 0.65). The reaction mixture was

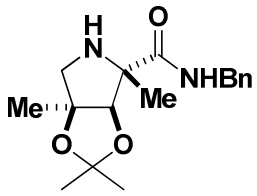
cooled to 0°C, and a further 27 µL of methanesulfonyl chloride was added. The reaction mixture was again warmed to room temperature, and t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 1 hour revealed the complete consumption of starting material and a single product (R_f 0.65). The reaction mixture was concentrated under reduced pressure and purified by flash column chromatography (ethyl acetate/cyclohexane, 1:2) to afford benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-5-*O*-methanesulfonyl-L-ribonamide **218** (132 mg, 99%) as a white solid.

Method 2: Benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribonamide **211** (1.51 g, 4.34 mmol) was dissolved in dichloromethane (15 mL) and cooled to 0°C in an ice bath, whilst stirring under argon. Anhydrous pyridine (1.05 mL, 13.02 mmol) and methanesulfonyl chloride (0.37 mL, 4.77 mmol) were added, and the ice bath removed. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 2 hours showed partial consumption of starting material (R_f 0.46) and a single product (R_f 0.65). The reaction mixture was cooled to 0°C, and a further 1.05 mL of pyridine and 0.37 mL of methanesulfonyl chloride were added. The reaction mixture was again warmed to room temperature, and t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 16 hours revealed the complete consumption of starting material and a single product (R_f 0.65). The reaction mixture was diluted with dichloromethane (15 mL) and 2 M hydrochloric acid (aq.) (30 mL). The aqueous and organic phases were separated and the aqueous phase was washed twice with dichloromethane (2 x 30 mL). The combined organic layers were dried (magnesium sulfate), filtered and the solvents concentrated under reduced pressure to afford 2.51 g of crude material. Purification by flash column chromatography (ethyl acetate/60-80 petrol 1:2) afforded benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-5-*O*-methanesulfonyl-L-ribonamide **218** (1.82 g, 98%) as a white solid.

HRMS (ESI+ve): found 449.1461 $[M+Na]^+$; $C_{18}H_{26}O_6N_4Na_1S_1$ requires 449.1465; $[\alpha]_D^{25}$ +16.7 (*c*, 0.65 chloroform); m.p. 110-112°C; ν_{max} (thin film): 3442 (s, br, NH) 2130 (s, N_3), 1675 (s, C=O);

δ_H ($CDCl_3$, 500 MHz): 1.28 (3H, s, CH_3-4), 1.42 (3H, s, $C(CH_3)_2$), 1.52 (3H, s, $C(CH_3)_2$), 1.84 (3H, s, CH_3-2), 3.01 (3H, s, CH_3SO_2), 4.13 (1H, d, H5, $J_{5,5'}$ 10.3), 4.23 (1H, d, H5', $J_{5',5}$ 10.3), 4.34 (1H, dd, CH_2Ph, $J_{CH2Ph,NH}$ 5.4, J_{gem} 14.5), 4.45 (1H, s, H3), 4.49 (1H, a-dd, CH_2Ph, J 6.5, J_{gem} 14.5), 7.03 (1H, a-t, NH, J 5.2), 7.29-7.38 (5H, m, Ar-H); δ_C ($CDCl_3$, 125 MHz): 22.9 (CH_3-4), 23.6 (CH_3-2), 26.5 ($C(\mathbf{C}H_3)_2$), 27.9 ($C(\mathbf{C}H_3)_2$), 37.6 (CH_3SO_2), 43.6 (CH_2Ph), 60.4 (C2), 71.7 (C5), 80.7 (C4), 86.9 (C3), 107.8 ($\mathbf{C}(CH_3)_2$), 127.8, 128.1, 128.9 (Ar-C), 137.3 (Ar-C_{quat}), 168.5 (C1); LRMS (ESI+ve): 427 ($[M+H]^+$, 100%), 449 ($[M+Na]^+$, 93%), 875 ($[2M+Na]^+$, 90%).

Benzyl 2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-3,4-*O*-isopropylidene-L-ribonamide 213

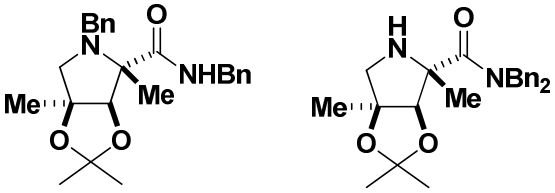


Benzyl 2-azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-5-*O*-methanesulfonyl-L-ribonamide **218** (1.82 g, 4.27 mmol) was dissolved in ethanol (75 mL) and palladium black was added (365 mg). The reaction mixture was stirred under hydrogen for 4 hours, after which time t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) revealed complete consumption of starting material (R_f 0.65) and a single product (R_f 0.60). The palladium was removed by filtration through a pad of Celite® eluting with methanol; concentration of the solvents under reduced pressure and purification by column chromatography (20% methanol in ethyl acetate) gave ribonamide **213** (1.35 g, quant.) as a white solid.

HRMS (ESI+ve): found 305.1857 $[M+H]^+$; $C_{17}H_{25}N_2O_3$ requires 305.1860; $[\alpha]_D^{20}$ +30.9 (*c*, 0.67 methanol); m.p. 59-62°C; ν_{max} (thin film): 3447 (s, br, NH), 1667 (s, C=O); δ_H (CD_3OD, 400 MHz): 1.28 (3H, s, CH_3-4), 1.35 (3H, s, CH_3-2), 1.37 (3H, s, $C(CH_3)_2$), 1.45 (3H, s, $C(CH_3)_2$), 2.48 (1H, dd, H5 $J_{5,3}$ 1.5, $J_{5,5'}$ 13.4), 2.98 (1H, dd, H5' $J_{5',3}$ 1.5, $J_{5',5}$ 13.4), 4.31 (1H, d, CH_2Ph, J_{gem} 14.7), 4.41 (1H, d, CH_2Ph, J_{gem} 14.7), 4.62 (1H, t, H3, $J_{3,5} = J_{3,5'}$ 1.5), 7.21-7.33 (5H, m, Ar-H); δ_C

(CD_3OD, 125 MHz): 20.1 (CH_3-2), 24.2 (CH_3-4), 26.4 (C(**C**H_3)$_2$), 27.5 (C(**C**H_3)$_2$), 43.7 (**C**H_2Ph), 59.7 (C5), 72.1 (C2), 90.7 (C3), 92.1 (C4), 111.9 (**C**(CH_3)$_2$), 128.1, 128.4, 129.4 (Ar-C), 139.9 (Ar-C_{quat}), 176.0 (C1); LRMS (ESI+ve): 305 ($[M+H]^+$,100%), 609 ($[2M+H]^+$, 60%). LRMS (ESI-ve): 303 ($[M-H]^-$, 100%), 339 ($[M+Cl]^-$, 85%).

Benzyl *N*-benzyl-2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-3,4-*O*-isopropylidene-L-ribonamide 219 and dibenzyl 2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-3,4-*O*-isopropylidene-L-ribonamide 220



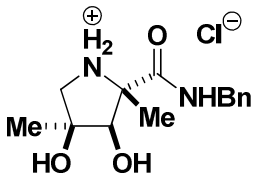
219 **220**

Benzyl 2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-3,4-*O*-isopropylidene-L-ribonamide **213** (61 mg, 0.20 mmol) was dissolved in dry *N,N*-dimethylformamide (1 mL). Powdered 4Å molecular sieves (50 mg), sodium hydride (60% in mineral oil, 10 mg, 0.24 mmol) and benzyl bromide (29 µL, 0.24 mmol) were added and the solution stirred under argon. T.l.c. analysis (ethyl acetate/cyclohexane, 1:4) after 3 hours revealed partial consumption of starting material (R_f 0.11) and two products (R_f 0.30 & 0.21); further portions of sodium hydride (2 mg) and benzyl bromide (5 µL) were added. T.l.c. analysis after 24 hours showed the reaction to be complete; the reaction mixture was filtered through a pad of Celite®, eluting with methanol. The solvents were evaporated, and the residue re-dissolved in water (15 mL) and dichloromethane (15 mL). The aqueous and organic phases were separated and the aqueous phase was washed twice more with dichloromethane (2 x 15 mL). The combined organic layers were dried (magnesium sulfate), filtered and the solvents concentrated under reduced pressure to afford 103 mg of pale yellow crude. This was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:6→1:1) to yield **219** (R_f 0.21, 145 mg, 52%) as a colourless oil and **220** (R_f 0.30, 30 mg, 11%) as a white solid.

*Data for **219**:* HRMS (ESI+ve): found 417.2145 $[M+Na]^+$; $C_{24}H_{30}N_2Na_1O_3$ requires 417.2149; $[\alpha]_D^{25}$ -17.8 (*c*, 0.855 chloroform); ν_{max} (thin film): 3358 (s, br, NH), 1667 (s, C=O); δ_H ($CDCl_3$, 400 MHz): 1.38 (6H, s, CH_3-4 & $C(CH_3)_2$), 1.49 (3H, s, CH_3-2), 1.55 (3H, s, $C(CH_3)_2$), 2.69 (1H, d, H5, $J_{5,5'}$ 10.6), 2.80 (1H, d, H5', $J_{5',5}$ 10.6), 3.65 (1H, d, R_2NCH_2Ph, J_{gem} 13.2), 3.83 (1H, d, R_2NCH_2Ph, J_{gem} 13.2), 4.43 (1H, s, H3), 4.47 (2H, d, $RNHCH_2Ph$, $J_{CH2Ph,NH}$ 5.8), 7.08-7.11 (2H, m, Ar-H), 7.23-7.38 (8H, m, Ar-H), 7.88 (1H, t, N-H $J_{NH,CH2Ph}$ 5.1); δ_C ($CDCl_3$, 100 MHz): 25.1 (CH_3-2), 26.8 ($C(\mathbf{C}H_3)_2$), 27.0 ($C(\mathbf{C}H_3)_2$), 27.9 (CH_3-4), 43.6 ($RNH\mathbf{C}H_2Ph$), 54.0 ($R_2N\mathbf{C}H_2Ph$), 62.6 (C5), 72.0 (C2), 86.5 (C4), 90.6 (C3), 112.3 ($\mathbf{C}(CH_3)_2$), 127.3, 127.7, 127.8, 128.3, 128.9 (Ar-C), 138.4 (Ar-C_{quat}), 139.2 (Ar-C_{quat}), 174.3 (C1); LRMS (ESI+ve): 395 ($[M+H]^+$, 100%); LRMS (ESI-ve): 393 ($[M-H]^-$, 100%).

*Data for **220**:* HRMS (ESI+ve): found 395.2328 $[M+H]^+$; $C_{24}H_{31}N_2O_3$ requires 395.2329; $[\alpha]_D^{18}$ -5.8 (*c*, 0.29 chloroform); ν_{max} (thin film): 3423 (s, br, NH), 1623 (s, C=O); m.p. 103°C; δ_H ($CDCl_3$, 500 MHz): 1.43 (3H, s, $C(CH_3)_2$), 1.45 (3H, s, $C(CH_3)_2$), 1.47 (3H, s, CH_3-2), 1.50 (3H, s, CH_3-4), 2.20-2.55 (1H, br-s, N-H), 2.48 (1H, d, H5, $J_{5,5'}$ 13.2), 2.93 (1H, d, H5', $J_{5',5}$ 13.2), 4.41 (1H, d, CH_2Ph, J_{gem} 14.7), 4.56 (1H, d, CH_2Ph, J_{gem} 14.7), 5.12 (1H, s, H3), 5.31-5.24 (2H, d, CH_2Ph, J_{gem} 16.4), 7.14-7.16 (2H, m, Ar-H), 7.19-7.21 (2H, m, Ar-H), 7.26-7.32 (4H, m, Ar-H), 7.37-7.40 (2H, m, Ar-H); δ_C ($CDCl_3$, 100 MHz): 18.7 (CH_3-2), 23.4 (CH_3-4), 26.0 ($C(\mathbf{C}H_3)_2$), 27.4 ($C(\mathbf{C}H_3)_2$), 48.1 ($\mathbf{C}H_2Ph$), 50.6 ($\mathbf{C}H_2Ph$), 60.1 (C5), 71.9 (C4), 90.1 (C3), 90.8 (C2), 109.7 ($\mathbf{C}(CH_3)_2$), 127.2, 127.4, 128.2, 128.5, 128.7 (Ar-C), 137.4 (Ar-C_{quat}), 137.6, (Ar-C_{quat}) 173.3 (C1); LRMS (ESI+ve): 395 ($[M+H]^+$, 100%), 789 ($[2M+H]^+$, 65%).

Benzyl 2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-D-ribonamide hydrochloride 223.HCl

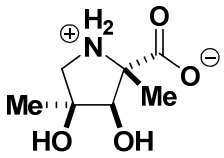


Benzyl 2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-3,4-*O*-isopropylidene-D-ribonamide **213** (40 mg, 0.13 mmol) was dissolved in hydrochloric acid solution (37%; 2 mL) and the reaction stirred at room temperature for 48 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed complete consumption of starting material (R_f 0.60) and the formation of a single product (R_f 0.00). Removal of solvents under reduced pressure afforded benzyl 2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-D-ribonamide **223** as the hydrochloride salt (40 mg, quant.), requiring no further purification.

HRMS (ESI+ve): found 265.1247 $[M\text{-}Cl]^+$; $C_{14}H_{22}N_2O_3$ requires 265.1546; $[\alpha]_D^{24}$ -5.1 (*c*, 0.85 methanol); ν_{max} (thin film): 3252 (s, br, OH), 1667 (s, C=O); δ_H (CD_3OD, 400 MHz): 1.34 (3H, s, CH_3-4), 1.71 (3H, s, CH_3-2), 3.17 (1H, d, H5, *J* 12.1), 3.28-3.30 (1H, m, H5'), 3.94 (1H, s, H3), 4.46 (2H, s, CH_2Ph), 7.24-7.35 (5H, m, Ar-H); δ_C (CD_3OD, 100 MHz): 17.3 (CH_3-4), 20.9 (CH_3-2), 43.7 (**C**H_2Ph), 53.6 (C2), 70.0 (C5), 76.8 (C4), 79.1 (C3), 127.4, 127.7, 128.6 (Ar-C), 138.7 (Ar-C_{quat}), 171.8 (C1); LRMS (ESI-ve) 299 ($[M\text{-}H]^-$, 100%); LRMS (ESI+ve) 265 ($[M\text{-}Cl]^+$, 100%).

2,5-Dideoxy-2,4-di-*C*-methyl-2,5-imino-L-ribonic acid 197

[3(*R*),4(*S*)-Hydroxy-2,4-di-*C*-methyl-L-proline]

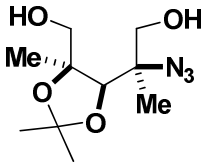


Benzyl 2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-3,4-*O*-isopropylidene-D-ribonamide **213** (40 mg, 0.13 mmol) was dissolved in hydrochloric acid solution (37% aq.; 2 mL) and the reaction stirred at 100°C for 140 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed

complete consumption of starting material (R_f 0.60) and the formation of a single product (R_f 0.00). Removal of solvents under reduced pressure gave crude benzyl 2,5-dideoxy-2,4-di-*C*-methyl-2,5-imino-D-ribonamide as the hydrochloride salt. This was dissolved in methanol (2 mL) and stirred with potassium hydroxide pellets (468 mg, 4.74 mmol) for 96 hours, after which time mass analysis showed the absence of starting material, and a large peak at *m/z* 174, corresponding to $[M-H]^-$. The solvents were concentrated under reduced pressure and the crude passed down a Dowex® (50W X8 H^+ form) resin column, eluting with water (100 mL) then 2 M ammonia (aq.) (100 mL). The ammonical fractions were concentrated under reduced pressure to give the fully deprotected proline **197** (37 mg, 93%) as a colourless oil.

HRMS (ESI+ve): found 198.0737 $[M+Na]^+$; $C_7H_{13}N_1Na_1O_4$ requires 198.0735; $[\alpha]_D^{25}$ -19.9 (*c*, 0.74 water); ν_{max} (thin film): 3384 (s, br, OH), 1622 (s, C=O); δ_H (CD_3OD, 500 MHz): 1.35 (3H, s, CH_3-4), 1.72 (3H, s, CH_3-2), 3.59 (1H, d, H5, $J_{5,5'}$ 12.1), 3.68 (1H, d, H5', $J_{5',5}$ 12.1), 4.34 (1H, s, H3); δ_C (CD_3OD, 125 MHz): 17.9 (CH_3-4), 21.7 (CH_3-2), 59.8 (C2), 67.3 (C5), 73.6 (C3), 91.6 (C4), 174.8 (C1); LRMS (ESI+ve): 176 ($[M+H]^+$, 100%); LRMS (ESI-ve): 174 ($[M-H]^-$, 100%), 349 ($[2M-H]^-$, 76%).

4-Azido-4-deoxy-2,4-di-*C*-methyl-2,3-*O*-isopropylidene-D-ribitol 225

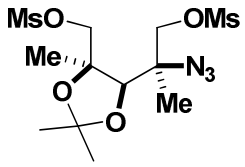


2-Azido-2-deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-ribono-1,5-lactone **207** (259 mg, 1.07 mmol) was dissolved in tetrahydrofuran (2.5 mL) at room temperature under argon. Lithium borohydride solution (2 M in THF; 0.27 mL, 0.54 mmol) was added dropwise and the solution stirred for one hour. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time showed partial consumption of starting material (R_f 0.46) and the formation of a single product (R_f 0.29). A further 0.27 mL of reagent was added and t.l.c. analysis (as before) after a second hour, showed persisting

starting material, therefore a final 0.27 mL of lithium borohydride solution was added. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 45 minutes showed complete consumption of starting material (R_f 0.46) and a single product (R_f 0.29). The reaction mixture was neutralised with Dowex® (50W X8 H^+ form), and the resulting suspension filtered using methanol as eluent. The solvents were concentrated under educed pressure and the crude purified by column chromatography (ethyl acetate/cyclohexane, 1:2→1:1) to afford 4-azido-4-deoxy-2,4-di-*C*-methyl-2,3-*O*-isopropylidene-D-ribitol, **225** as a white crystalline solid (219 mg, 83%).

HRMS (ESI+ve): found 268.1261 $[M+Na]^+$; $C_{10}H_{19}N_3NaO_4$ requires 268.1268; $[\alpha]_D^{20}$ +5.54 (*c*, 0.81 methanol); m.p. 84-89°C; ν_{max} (thin film): 3418 (s, br, OH), 2121 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.36 (3H, s, $C(CH_3)_2$), 1.37 (3H, s, CH_3-2), 1.47 (3H, s, $C(CH_3)_2$), 1.55 (3H, s, CH_3-4), 3.53 (1H, d, H5, $J_{5,5'}$ 11.2), 3.57 (1H, d, H1, $J_{1,1'}$ 11.5), 3.63 (1H, d, H5', $J_{5',5}$ 11.2), 3.70 (1H, d, H1', $J_{1',1}$ 11.5), 3.81 (1H, s, H3); δ_C ($CDCl_3$, 100 MHz): 19.6 (CH_3-4), 22.3 (CH_3-2), 26.7 ($C(\mathbf{C}H_3)_2$)), 28.4 ($C(\mathbf{C}H_3)_2$)), 63.9 (C4), 64.9 (C1), 66.6 (C5), 83.0 (C2) 85.8 (C3), 106.8 ($\mathbf{C}(CH_3)_2$).

4-Azido-4-deoxy-2,4-di-*C*-methyl-2,3-*O*-isopropylidene-1,5-di-*O*-methanesulfonyl-D-ribitol 226

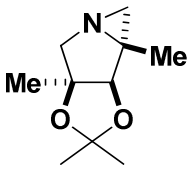


4-Azido-4-deoxy-2,4-di-*C*-methyl-2,3-*O*-isopropylidene-D-ribitol **225** (219 mg, 0.89 mmol) was dissolved in dry pyridine (3 mL), cooled to 0°C under argon. Methanesulfonyl chloride (0.17 mL, 2.23 mmol) was added and the solution allowed to warm slowly to room temperature. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 1 hour revealed partial consumption of starting material (R_f 0.29) and the formation of a single major product (R_f 0.40 – it is known from previous experiments that the mono and di-methanesulfonyl compounds co-elute in this solvent system) and therefore a further 0.17 mL of reagent was added to the reaction mixture at 0°C. The reaction was allowed to slowly warm to room temperature, and t.l.c. analysis after 4 hours showed the reaction to be

complete. The solvents were concentrated under reduced pressure and the crude purified by column chromatography (ethyl acetate/cyclohexane, 1:1) to give 4-azido-4-deoxy-2,4-di-*C*-methyl-2,3-*O*-isopropylidene-1,5-di-*O*-methanesulfonyl-D-ribitol **226** (365 mg, quant.) as a colourless oil.

HRMS (ESI+ve): found 424.0820 $[M+Na]^+$; $C_{12}H_{23}N_3Na_1O_8S_2$ requires 424.0819; $[\alpha]_D^{25}$ -5.46 (*c*, 0.795 chloroform); ν_{max} (thin film): 2123 (s, N_3); δ_H ($CDCl_3$, 500 MHz): 1.38 (3H, s, $C(CH_3)_2$), 1.43 (3H, s, CH_3-2) 1.49 (3H, s, $C(CH_3)_2$), 1.61 (3H, s, CH_3-4), 3.09 (3H, s, CH_3SO_2-5), 3.10 (3H, s, CH_3SO_2-1), 3.80 (1H, s, H3), 4.12 (1H, d, H5, $J_{5,5'}$ 10.3), 4.20 (1H, d, H5', $J_{5',5}$ 10.4), 4.21 (1H, d, H1, $J_{1,1'}$ 10.4), 4.33 (1H, d, H1', $J_{1',1}$ 10.1); δ_C ($CDCl_3$, 125 MHz): 19.1 (CH_3-4), 22.6 (CH_3-2), 26.4 ($C(\mathbf{C}H_3)_2$), 28.0 ($C(\mathbf{C}H_3)_2$), 37.67 (CH_3SO_2), 37.70 (CH_3SO_2), 61.4 (C4), 70.4 (C5), 71.4 (C1), 80.8 (C2), 84.7 (C3), 107.9 ($\mathbf{C}(CH_3)_2$); LRMS (ESI+ve): 419 ($[M+NH_4]^+$, 100%), 825 ($[2M+Na]^+$, 95%).

2,4-Di-*C*-methyl-1,2,5-imino-3,4-*O*-isopropylidene-1,2,5-trideoxy-L-ribitol 236

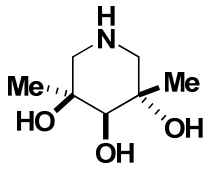


4-Azido-4-deoxy-2,4-di-*C*-methyl-2,3-*O*-isopropylidene-1,5-di-*O*-methanesulfonyl-D-ribitol **226** (401 mg, 1.00 mmol) was dissolved in ethanol (16 mL). Triethylamine (0.42 mL, 3.00 mmol) and palladium on carbon (80 mg) were added and the solution degassed and stirred under hydrogen for 30 minutes, after which time t.l.c. analysis (ethyl acetate) revealed complete consumption of starting material (R_f 0.65) and a single product (R_f 0.28). The reaction mixture was degassed, and the crude filtered through a pad of Celite®, eluting with methanol. The solvents were concentrated under reduced pressure and the crude purified by silica plug (1% triethylamine in ethyl acetate) to afford the title compound **236** (176 mg, 96%) as a colourless oil.

HRMS (FI+ve): found 183.1254 $[M]^{\cdot}$; $C_{10}H_{17}NO_2$ requires 183.1259; $[\alpha]_D^{25}$ -24.2 (*c*, 1.12 dichloromethane); δ_H ($CDCl_3$, 500 MHz): 1.24 (1H, s, H1), 1.29 (3H, s, CH_3-2), 1.37 (3H, s,

$C(CH_3)_2$), 1.40 (3H, s, CH_3-4), 1.47 (3H, s, $C(CH_3)_2$), 1.66 (1H, s, H1'), 3.25 (1H, d, H5, $J_{5,5'}$ 13.1), 3.28 (1H, d, H5', $J_{5',5}$ 12.9), 4.41 (1H, s, H3); δ_C ($CDCl_3$, 125 MHz): 16.5 (CH_3-2), 26.5 ($C(\mathbf{C}H_3)_2$), 26.7 ($C(\mathbf{C}H_3)_2$), 28.0 (CH_3-4), 37.5 (C1), 49.0 (C2), 68.1 (C5), 88.7 (C3), 91.6 (C4), 111.1 ($\mathbf{C}(CH_3)_2$)

1,5-Dideoxy-2,4-di-*C*-methyl-1,5-imino-L-arabinitol 194



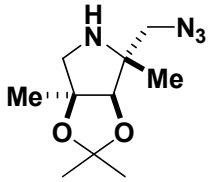
194

Aziridine **236** (114 mg, 0.79 mmol) was dissolved in THF (4 mL) and triethylamine added (0.33 mL, 2.36 mmol) with trifluoroacetic acid (0.13 mL, 0.94 mmol). The reaction mixture was stirred at 0°C for 10 minutes after which time t.l.c. analysis (ethyl acetate/cyclohexane 1:4) revealed one major product, as a single large spot with R_f 0.48. The reaction mixture was concentrated under reduced pressure, preadsorbed on silica and purified by column chromatography to afford a mixture of *N*-trifluoroacetylated piperidines (284 mg). This mixture was dissolved in methanol (8 mL) with water (0.8 mL) and potassium carbonate was added (138 mg, 4.09 mmol). The mixture was heated to reflux for one hour after which time t.l.c. analysis (ethyl acetate/isopropanol/water 7:2:1) revealed the complete consumption of starting material R_f 0.85 and a single product R_f 0.30. This was concentrated under reduced pressure and dissolved in 1 M HCl (aq) (12 mL) and heated to 60°C for one hour, after which time t.l.c. analysis (ethyl acetate/isopropanol/water 7:2:1) revealed complete consumption of starting material R_f 0.30 and a single product R_f 0.00. The reaction mixture was concentrated under reduced pressure to afford a crude solid which was purified by ion exchange chromatography by Summit Plc to afford 27 mg of piperidine **194**.

$[\alpha]_D^{26}$ +19.6 (*c*, 1.35 water); ν_{max} (thin film): 3432 (s, br, NH); δ_H (D_2O, 500 MHz): 1.23 (3H, s, CH_3-4), 1.29 (3H, s, CH_3-2), 2.50 (1H, d, H1, $J_{1,1'}$ 13.3), 2.58 (1H, d, H5, $J_{5,5'}$ 14.1), 2.76 (1H, dd,

H5', $J_{5,5'}$ 14.1 $J_{5',1'}$ 1.3), 2.81 (1H, dd, H1', $J_{1',1}$ 13.3 $J_{1',5'}$ 1.3), 3.36 (1H, s, H3); δ_C (D_2O, 125 MHz): 20.9 (CH_3-2), 24.4 (CH_3-4), 54.4 (C5), 55.1 (C1), 72.1 (C4), 72.5 (C2), 79.2 (C3); LRMS (ESI-ve): 160 ($[M-H]^-$, 45%), 321 ($[2M-H]^-$, 100%).

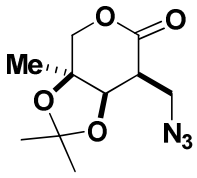
1-Azido-2,4-di-*C*-methyl-2,5-imino-3,4-*O*-isopropylidene-1,2,5-trideoxy-L-ribitol 243



Azabicyclohexane **236** (41 mg, 0.22 mmol) was dissolved in *N*,*N*-dimethylformamide (2 mL) with water (50 µL). Sodium azide (146 mg, 2.24 mmol) was added and the reaction mixture heated to 100°C. T.l.c. analysis (ethyl acetate) after 113 hours revealed partial consumption of starting material (R_f 0.28) and a major product (R_f 0.85). The reaction mixture was cooled to room temperature and the crude partitioned between diethyl ether and water (10 mL of each). The aqueous phase was washed a further two times with ether (2 x 10 mL), and the combined organics were dried (magnesium sulfate), filtered and concentrated under reduced pressure. Purification of the crude by column chromatography (ethyl acetate/cyclohexane, 1:1) gave the title azide **243** as a pale yellow oil (26 mg, 51%).

$[\alpha]_D^{25}$ +15.6 (*c*, 0.82 dichloromethane); ν_{max} (thin film): 3584 (s, br, NH), 2104 (s, N_3); δ_H ($CDCl_3$, 500 MHz): 1.24 (3H, s, CH_3-2) 1.40 (3H, s, $C(CH_3)_2$), 1.47 (3H, s, $C(CH_3)_2$), 1.53 (3H, s, CH_3-4), 2.16 (1H, br-s, NH), 2.72 (1H, d, H5, $J_{5,5'}$ 13.4), 3.06 (1H, d, H5', $J_{5',5}$ 13.4), 3.11 (1H, d, H1, $J_{1,1'}$ 12.5), 3.29 (1H, d, H1', $J_{1',1}$ 12.5), 3.98 (1H, s, H3); δ_C ($CDCl_3$, 125 MHz): 19.3 (CH_3-4), 24.9 (CH_3-2), 26.5 ($C(\mathbf{C}H_3)_2$), 27.6 ($C(\mathbf{C}H_3)_2$), 55.2 (C1), 58.6 (C5), 67.0 (C2), 90.0 (C3), 91.8 (C4), 111.2 ($\mathbf{C}(CH_3)_2$).

2-*C*-Azidomethyl-2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribono-1,5-lactone 210

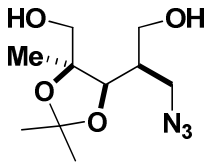


2,4-Di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone **103** (681 mg, 3.15 mmol) was dissolved in dry dichloromethane (7 mL) and cooled to 0°C. Once cool, pyridine (0.76 mL, 9.46 mmol) and trifluoromethanesulfonic anhydride (0.85 mL, 5.04 mmol) were added dropwise to the reaction mixture. Two further additions of pyridine (2 x 0.76 mL, 2 x 9.46 mmol) and trifluoromethanesulfonic anhydride (2 x 0.85 mL, 2 x 5.04 mmol) were necessary at two hour intervals before almost complete consumption of starting material (R_f 0.54) and a single product (R_f 0.82) were observed. The reaction mixture was quenched with dropwise addition of 2 M hydrochloric acid (aq.), until the evolution of gas had ceased. Water (50 mL) and dichloromethane (50 mL) were added and the aqueous and organic phases separated. The aqueous phase was washed twice with dichloromethane (2 x 50 mL) and the combined organic layers were dried (magnesium sulfate), filtered and the solvents concentrated under reduced pressure to afford a solid yellow crude (1.49 g). The crude material was immediately dissolved in *N*,*N*-dimethylformamide (10 mL) and stirred at room temperature for 16 hours under argon. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed the presence of one major product (R_f 0.48; UV active) and complete consumption of starting material (R_f 0.82). Sodium azide (266 mg, 4.10 mmol) was added and the reaction mixture stirred for 3 hours. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) now revealed no UV active products, and single major spot was observed (R_f 0.5). The reaction mixture was concentrated under reduced pressure, and the crude partitioned between water and dichloromethane (30 mL of each). The aqueous phase was washed twice more with dichloromethane (2 x 30 mL), and the combined organic phases were dried (magnesium sulfate), filtered, and concentrated under reduced pressure. Subsequent column chromatography (ethyl acetate/cyclohexane, 1:5→1:4)

afforded 2-*C*-azidomethyl-2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribono-1,5-lactone **210** (488 mg, 64% over two steps) as a white solid.

HRMS (ESI+ve): found 264.0954 $[M+Na]^+$; $C_{10}H_{15}N_3Na_1O_4$ requires 264.0955; $[\alpha]_D^{21}$ +160.3 (*c*, 1.19 chloroform); m.p. 62-65°C; ν_{max} (thin film): 2101 (s, N_3), 1742 (s, C=O); δ_H (C_6D_6, 400 MHz): 0.67 (3H, s, CH_3-4), 1.08 (3H, s, $C(CH_3)_2$), 1.32 (3H, s, $C(CH_3)_2$), 1.70-1.74 (1H, m, H2), 2.97 (1H, d, H5, *J* 12.6), 3.40 (1H, dd, H2', *J* 9.0, *J* 12.7), 3.70-3.65 (2H, m, H5' & H2''), 3.91 (1H, d, H3 *J* 1.9); δ_C (C_6D_6, 100 MHz): 22.3 (CH_3-4), 26.4 ($C(\mathbf{C}H_3)_2$), 27.1 ($C(\mathbf{C}H_3)_2$), 43.2 (C2), 48.8 (C2'), 72.2 (C5), 78.5 (C3), 79.0 (C4), 110.1 ($\mathbf{C}(CH_3)_2$), 169.2 (C1).

2-*C*-Azidomethyl-2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribitol 244

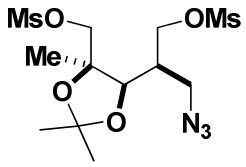


2-*C*-Azidomethyl-2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribono-1,5-lactone **210** (488 mg, 2.02 mmol) was dissolved in dry dichloromethane (6 mL) and cooled to -30°C, under argon. Once cool, di*iso*butylaluminium hydride solution was added dropwise (1 M in toluene; 2.13 mL, 2.13 mmol). Analysis of the reaction mixture by IR spectroscopy after one hour revealed the presence of a carbonyl peak at 1742cm^{-1}. The reaction mixture was warmed to -20°C; after a second hour a further portion (0.2 mL) of reagent was added. After this time, the reaction was deemed complete by IR analysis. The reaction mixture was quenched by the addition of sodium bicarbonate solution (sat. aq., 5 mL) and sodium hydrogen orthophosphate (100 mg). Magnesium sulfate was added, and the resulting gel filtered through a pad of Celite®, eluting with ethyl acetate (100 mL) and methanol (50 mL). The solvents were concentrated under reduced pressure, and the crude re-dissolved in dry tetrahydrofuran (10 mL). Lithium borohydride solution (2 M in THF; 0.5 mL, 1.01 mmol) was added and the reaction mixture stirred under argon at room temperature for one hour. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after this time revealed partial consumption of starting material (R_f

0.48), and a single major compound (R_f 0.27). A further portion of reagent (0.5 mL, 1.01 mmol) was added, and after one hour the reaction was found to be complete. The reaction mixture was quenched by dropwise addition of NH_4Cl (sat. aq.); the solvents were concentrated under reduced pressure, and the crude preadsorbed on silica and purified by column chromatography (ethyl acetate/cyclohexane, 1:2) to afford the title compound **244** (205 mg, 41%) as a colourless oil.

HRMS (FI+ve): found 246.1455 $[M+H]^+$; $C_{10}H_{20}N_3Na_1O_4$ requires 264.1454; $[\alpha]_D^{18}$ -21.6 (*c*, 0.75 chloroform); ν_{max} (thin film): 3452 (s, br, OH), 2101 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.36 (3H, s, CH_3-4), 1.37 (3H, s, $C(CH_3)_2$), 1.42 (3H, s, $C(CH_3)_2$), 2.07-2.15 (2H, m, H2 & OH), 2.43 (1H, s, OH), 3.41 (1H, dd, H2', $J_{2',2}$ 6.5, $J_{2',2''}$ 12.4), 3.53 (2H, a-d H5 & H5' *J* 3.8), 3.58 (1H, dd, H2'', $J_{2'',2}$ 4.3, $J_{2'',2'}$ 12.4), 3.78-3.84 (2H, m, H1 & H1'), 3.89 (1H, d, H3, $J_{3,2}$ 9.3); δ_C ($CDCl_3$, 125 MHz): 22.4 (CH_3-4), 26.4 ($C(\mathbf{C}H_3)_2$), 28.4 ($C(\mathbf{C}H_3)_2$), 39.6 (C2), 50.3 (C2'), 62.9 (C1), 65.3 (C5), 82.1 (C4), 82.3 (C3), 107.3 ($\mathbf{C}(CH_3)_2$).

2-*C*-Azidomethyl-2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-1,6-di-*O*-methanesulfonyl-L-ribitol 245

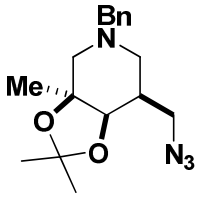


2-*C*-Azidomethyl-2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribitol **244** (142 mg, 0.58 mmol) was dissolved in dry pyridine (1.5 mL) and cooled to 0°C under argon. Once cool, methanesulfonylchloride was added (94 µL, 1.22 mmol), and the reaction mixture allowed to warm to room temperature. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 1 hour revealed the presence of starting material (R_f 0.27) and a major product (R_f 0.38). The reaction mixture was cooled to 0°C, and a further 94 µL of reagent was added. The reaction mixture was warmed slowly to room temperature, and t.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after a further hour revealed the complete consumption of starting material (R_f 0.27) and a major product (R_f 0.38). The reaction

mixture was quenched with 2 M hydrochloric acid (aq.), until the evolution of gas had ceased, diluted with water (15 mL) and washed with dichloromethane (3 x 15 mL). The combined organic phases were dried (magnesium sulfate), filtered and the solvents removed under reduced pressure. The crude mesylate was purified by column chromatography (ethyl acetate/cyclohexane, 4:1→2:1), affording 2-*C*-azidomethyl-2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-1,6-di-*O*-methanesulfonyl-L-ribitol **245** (189 mg, 81%), as a white solid.

HRMS (ESI+ve): found 424.0828 $[M+Na]^+$; $C_{12}H_{23}N_3Na_1O_8S_2$ requires 424.0819; $[\alpha]_D^{23}$ -7.4 (*c*, 1.11 chloroform); m.p. 74-76°C; ν_{max} (thin film): 2101 (s, N_3); δ_H ($CDCl_3$, 400 MHz): 1.40 (3H, s, $C(CH_3)_2$), 1.34 (3H, s, $C(CH_3)_2$), 1.43 (3H, s, CH_3-4), 2.23-2.32 (1H, ddddd, H2, $J_{2,1'}$ 3.4, $J_{2,2'}$ 3.9, $J_{2,2''}$ 6.3, $J_{2,1}$ 6.8, $J_{2,3}$ 10.1), 3.05 (3H, s, CH_3SO_2), 3.07 (3H, s, CH_3SO_2), 3.52 (1H, dd, H2', $J_{2',2}$ 3.9, $J_{2',2''}$ 12.6), 3.69 (1H, dd, H2'', $J_{2'',2}$ 6.3, $J_{2'',2'}$ 12.6), 3.85 (1H, d, H3, $J_{3,2}$ 10.1), 4.00 (1H, d, H5, $J_{5,5'}$ 10.1), 4.18 (1H, d, H5', $J_{5',5}$ 9.9), 4.38 (1H, dd, H1, $J_{1,2}$ 6.8, $J_{1,1'}$ 9.9), 4.53 (1H, dd, H1', $J_{1',2}$ 3.4, $J_{1',1}$ 10.0); δ_C ($CDCl_3$, 100 MHz): 22.2 (CH_3-4), 26.2 ($C(\mathbf{C}H_3)_2$), 28.2 ($C(\mathbf{C}H_3)_2$), 37.3, 37.7, 37.9 (C2 & 2 x CH_3SO_2), 48.1 (C2'), 67.8 (C1), 69.5 (C5), 79.4 (C3), 79.7 (C4), 107.4 ($\mathbf{C}(CH_3)_2$); LRMS (ESI+ve): 419 ($[M+NH_4]^+$, 100%), 424 ($[M+Na]^+$, 60%).

_N_-Benzyl 2-_C_-azidomethyl-2-deoxy-1,5-dideoxy-1,5-imino-3,4-_O_-isopropylidene-4-_C_-methyl-L-ribitol 248

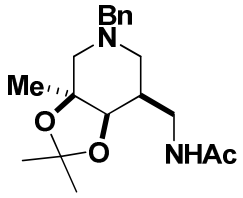


2-*C*-Azidomethyl-2-deoxy-3,4-*O*-isopropylidene-4-*C*-methyl-1,6-di-*O*-methanesulfonyl-L-ribitol **245** (148 mg, 0.37 mmol) was dissolved in benzylamine (1 mL) and heated to 100°C. T.l.c. analysis (ethyl acetate/cyclohexane, 1:1) after 20 hours revealed the complete consumption of starting material (R_f 0.38) and a single product (R_f 0.85). The solvent was removed under reduced pressure,

and the crude purified by column chromatography (ethyl acetate/cyclohexane, 1:10) to give *N*-benzyl 2-*C*-azidomethyl-2-deoxy-1,5-imino-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribitol **246** (123 mg, quant.) as a yellow oil.

HRMS (ESI+ve): found 317.1967 $[M+H]^+$; $C_{17}H_{25}N_4O_2$ requires 317.1672; $[\alpha]_D^{23}$ -35.9 (*c*, 1.24 chloroform); ν_{max} (thin film): 2099 (s, N_3); δ_H (C_6D_6, 500 MHz): 1.41, (6H, s, $C(CH_3)_2$ & CH_3-4), 1.56 (3H, s, $C(CH_3)_2$), 1.87 (1H, a-t, H2', *J* 11.1), 1.96-2.03 (1H, m, H2), 2.28 (1H, d, H5, $J_{5,5'}$ 10.9), 2.49 (1H, a-ddd, H2'', *J* 1.8, *J* 4.7, *J* 10.7), 2.61 (1H, dd, H5' *J* 1.5, $J_{5',5}$ 10.9), 2.84 (1H, dd, H1, $J_{1,2}$ 7.1, $J_{1,1'}$ 12.0), 3.21 (1H, dd, H1', $J_{1',2}$ 8.1, $J_{1',1}$ 12.0), 3.30 (1H, d, CH_2Ph, J_{gem} 13.3), 3.37 (1H, d, CH_2Ph, J_{gem} 13.3), 3.77 (1H, d, H3, $J_{3,2}$ 3.6), 7.21-7.35 (5H, m, Ar-H); δ_C (C_6D_6, 100 MHz): 22.7, 27.2 (CH_3-4 & $C(\mathbf{C}H_3)_2$), 28.6 ($C(\mathbf{C}H_3)_2$), 37.4 (C2), 51.5 (C1), 52.3 (C2'), 61.5 (C5), 62.6 ($O\mathbf{C}H_2Ph$), 77.5 (C3), 78.4 (C4), 107.8 ($\mathbf{C}(CH_3)_2$), 128.0, 128.1, 128.7 (Ar-C), 138.9 (Ar-C_{quat}).

_N_-Benzyl 2-_C_-acetomidomethyl-2-deoxy-1,5-dideoxy-1,5-imino-3,4-_O_-isopropylidene-4-_C_-methyl-L-ribitol 247

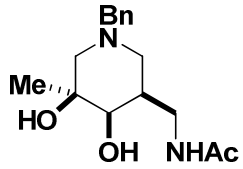


N-Benzyl 2-*C*-azidomethyl-2-deoxy-1,5-imino-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribitol **246** (50 mg, 0.16 mmol) was dissolved in ethyl acetate (2 mL) and 10% palladium on carbon (5 mg) was added whilst stirring. The reaction mixture was stirred under a hydrogen atmosphere for 90 minutes after which time t.l.c. analysis (ethyl acetate) revealed complete consumption of starting material (R_f 0.95) and a single major product (R_f 0.00). The palladium was removed by filtration through a pad of Celite®, eluting with methanol, and the solvents concentrated under reduced pressure to give crude *N*-benzyl 2-*C*-aminomethyl-2-deoxy-1,5-imino-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribitol (46 mg, quant.) as a pale yellow oil. This was immediately dissolved in anhydrous pyridine (0.5

mL) and acetic anhydride was added (45 µL, 0.48 mmol). The reaction mixture was stirred under argon for two hours, after which time t.l.c. analysis (ethyl acetate) showed complete consumption of starting material (R_f 0.00) and a single major product (R_f 0.16). The solvents were concentrated under reduced pressure and the crude was purified by column chromatography (ethyl acetate/cyclohexane, 1:1→100% ethyl acetate) to give the title compound **247** (28 mg, 53%) as a pale yellow oil.

HRMS (ESI+ve): found 333.2166 $[M+H]^+$; $C_{19}H_{29}N_2O_3$ requires 333.2173; $[\alpha]_D^{19}$ -40.3 (*c*, 0.44 methanol); ν_{max} (thin film): 3420 (s, br, NH), 1652 (s, C=O); δ_H (CD_3OD, 500 MHz): 1.34, (6H, s, $C(CH_3)_2$ & CH_3-4), 1.45 (3H, s, $C(CH_3)_2$), 1.93 (3H, s, NHCOC**H$_3$**), 2.00-2.02 (1H, m, H1), 2.16-2.13 (2H, m, H5 & H2), 2.51 (1H, d, H5', *J* 11.0), 2.72 (1H, dd, H1', *J* 3.9, *J* 10.8), 3.22 (1H, dd, H2' $J_{2',2}$ 8.1, $J_{2',2''}$ 13.6), 3.27 (1H, dd, H2'', $J_{2'',2}$ 6.3, $J_{2'',2'}$ 13.6,), 3.46 (1H, d, CH_2Ph, J_{gem} 13.1), 3.63 (1H, d, CH_2Ph, J_{gem} 13.2), 3.87 (1H, d, H3, $J_{3,2}$ 3.6), 7.24-7.33 (5H, m, Ar-H); δ_C (CD_3OD, 125 MHz): 22.5 (CO**C**H_3), 22.8, 27.3 (CH_3-4 & C(**C**H_3)$_2$), 28.7 (C(**C**H_3)$_2$), 37.8 (C2), 41.1 (C2'), 53.5 (C1), 61.9 (C5), 63.5 (O**C**H_2Ph), 79.4 (C3), 79.5 (C4), 109.1 (**C**$(CH_3)_2$), 128.3, 129.3, 130.2 (Ar-C), 139.2 (Ar-C_{quat}), 173.5 (**C**OCH_3); LRMS (ESI+ve): 419 ($[M+NH_4]^+$, 100%), 424 ($[M+Na]^+$, 60%).

_N_-Benzyl 2-_C_-acetomidomethyl-2-deoxy-1,5-dideoxy-1,5-imino-4-_C_-methyl-L-ribitol 196



N-Benzyl 2-*C*-acetomidomethyl-2-deoxy-1,5-imino-3,4-*O*-isopropylidene-4-*C*-methyl-L-ribitol **247** (25 mg, 75 µmol) was dissolved in water (0.25 mL) and trifluoroacetic acid (0.25 mL) added whilst stirring. T.l.c. analysis (ethyl acetate) after 70 minutes revealed complete consumption of starting material (R_f 0.16) and a single major product (R_f 0.00). The solvents were concentrated under

reduced pressure to give the title compound **196** (18 mg, 82%) as a pale yellow oil, requiring no further purification.

HRMS (ESI+ve): found 293.1860 $[M+H]^+$; $C_{16}H_{25}N_2O_3$ requires 293.1855; $[\alpha]_D^{21}$ -17.8 (*c*, 0.47 methanol); ν_{max} (thin film): 3385 (s, br, NH & OH), 1674 (s, C=O); δ_H (D_2O, 500 MHz): 1.22, (3H, s, CH_3-4), 1.89 (3H, s, NHCOC**H$_3$**), 2.16-2.25 (1H, m, H2), 2.88 (1H, a-t, H1, *J* 12.5), 3.01 (1H, d, H5, $J_{5,5'}$ 12.3), 3.08 (1H, d, H5', $J_{5',5}$ 12.1), 3.12-3.20 (3H, m, H1', H2' & H2''), 3.56 (1H, s, H3), 4.30 (1H, d, CH_2Ph, J_{gem} 12.9), 4.34 (1H, d, CH_2Ph, J_{gem} 13.4), 7.43-7.49 (5H, m, Ar-H); δ_C (D_2O, 125 MHz): 21.7 (CO**C**H$_3$), 22.0 (CH_3-4), 35.8 (C2), 38.5 (C2'), 48.5 (C1), 54.3 (C5), 61.4 (O**C**H$_2$Ph), 69.4, 69.5 (C3 & C4), 127.8 (Ar-$C_{quat.}$), 129.3, 130.4, 131.5 (Ar-C), 174.6 (**C**OCH$_3$); LRMS (ESI-ve): 291 ($[M-H]^-$, 100%); LRMS (ESI+ve): 293 ($[M+H]^+$, 100%).

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Appendices

Appendix 1 – NMR Data

Appendix 2 – Contents of CD Appendix

Appendix 1

NMR Data

NMR analyses were carried out by Dr M. Wormald, at the University of Oxford Biochemistry Department. NMR spectra were recorded on a Varian UnityINOVA 500 (^{1}H – 500 MHz: ^{13}C – 125 MHz) spectrometer, in $^{2}H_2O$, with a probe temperature of 30°C. Chemical shifts were measured relative to internal standards (^{1}H – acetone at 2.220 ppm: ^{13}C – acetone at 30.9 ppm). Two-dimensional gradient COSY, HSQC, HMBC and HSQC-TOCSY spectra were used to aid assignment of ^{1}H and ^{13}C spectra. ROESY spectra were recorded with a 400 ms mixing time. All chemical shifts (δ) are quoted in ppm and coupling constants (*J*) in Hz.

Conformational Analysis of 2,4-Di-C-Methyl Arabinose, 2-C-Methyl Arabinose and 2-Deoxy-2,4-Di-C-Methyl Arabinose

Analyses were performed on 2-*C*-methyl-D-arabinose **1** at pH = 6.3, 2,4-di-*C*-methyl-L-arabinose **2** at pH = 5.2 and 2,4-di-*C*-methyl-2-deoxy-L-arabinose **3** with pH = 7.4 (**Figure 1**).

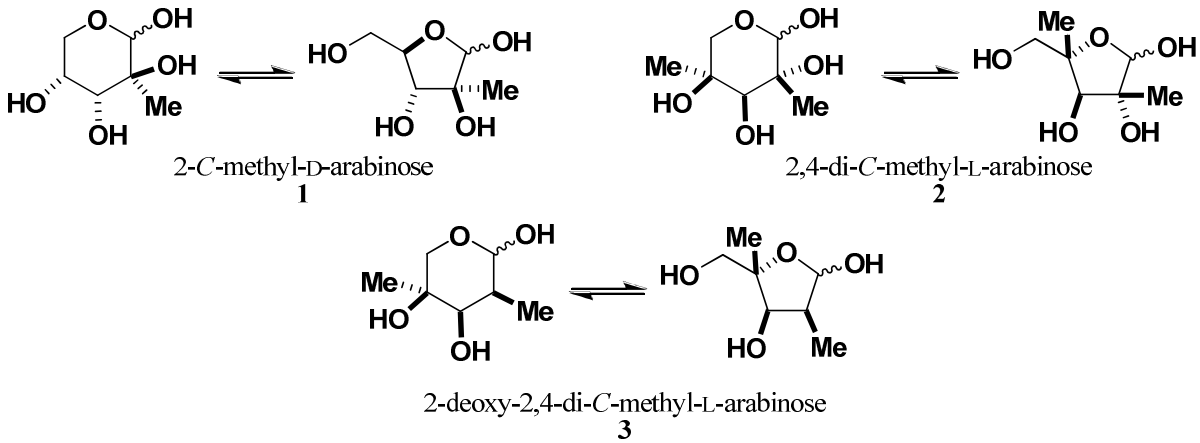

Figure 1: 2-*C*-Methyl arabinose, **1**, 2,4-di-*C*-methyl arabinose, **2** and 2,4-di-*C*-methyl-2-deoxy arabinose, **3**.

The 1D ^{1}H spectra are shown in **Figure 2** and the ^{1}H and ^{13}C assignments are given in **Tables 1-3**. For all compounds, the pyranose forms could be identified by the HMBC peak between C1 and C5H/C5H', and between C5 and C1H, and the furanose forms could be identified by the HMBC

peak between C4 and C1H. There was no evidence of the open chain keto- form within the detection limit in any of the samples (<2%). The furanose β-anomers of all three and the pyranose β-anomers of **1** and **2** could be identified by the strong C1H to $C2CH_3$ NOEs. The pyranose β-anomer of **3** could be identified by the large trans-diaxial coupling between C1H and C2H.

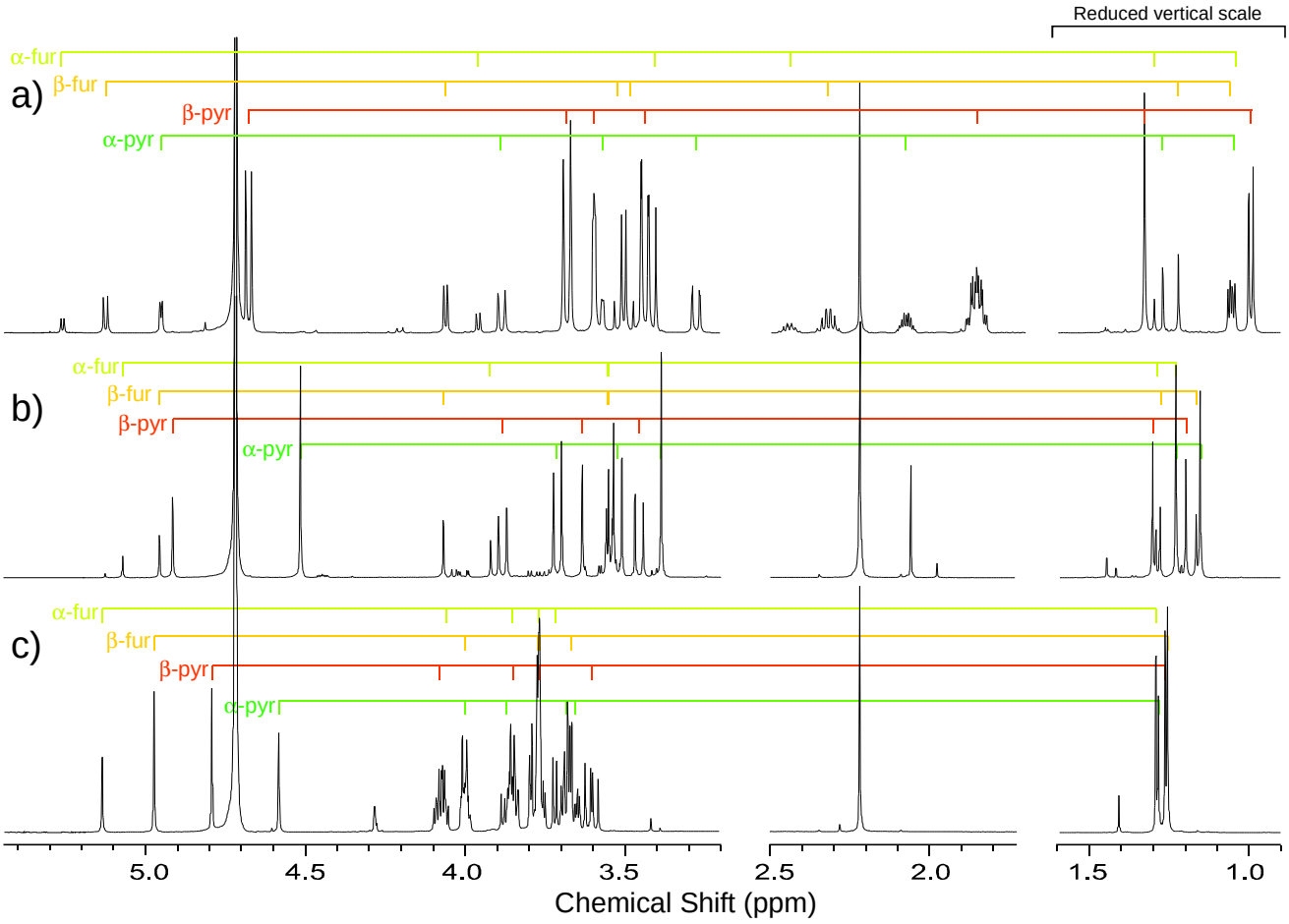

Figure 2: 1D ^{1}H NMR spectra showing the spin-systems for the α-pyranose, β-pyranose, α-furanose and β-furanose. (a) 2,4-di-*C*-methyl-2-deoxy-L-arabinose **3**; (b) 2,4-di-*C*-methyl-L-arabinose **2**; (c) 2-*C*-methyl-D-arabinose **1**. The vertical scales of the methyl regions have been reduced. The peak at 2.22ppm in each spectrum is acetone added as an internal standard.

For the β-pyranose form of **1**, the large $^{3}J_{HH}$ coupling between C4H and C5H' indicates that these two protons are axial. This defines the ring conformation as $^{1}C_{4}$. For α- pyranose form, the lack of a large coupling between C4H and either C5H or C5H' indicates that C4H is equatorial and hence the ring is in the $^{4}C_{1}$ conformation. These conformations are those predicted on steric grounds both having two axial and three equatorial substituents, but in the α-form the axial substituents are on the

same side of the ring, whilst in the β-form they are on opposite sides, hence α/β ratio is less than one.

For the β-pyranose form of **2**, the relatively high chemical shift for the C1H suggests that this proton is equatorial and the large difference between the chemical shifts of C5H and C5H' suggest that the C1OH is axial. This suggests that the ring conformation is $^4C_1$. For the α-pyranose form, the relatively low chemical shift for the C1H suggests that this proton is axial, thus suggesting that the ring is in the $^4C_1$ conformation. For the α-anomer, this conformation would be predicted on steric grounds, placing four of the six groups equatorial. For the β-anomer, the $^1C_4$ and $^4C_1$ conformations both place three groups axial and three equatorial.

For the β-pyranose form of **3**, the large $^3J_{HH}$ coupling between C1H and C2H indicates that these two protons are axial. This defines the ring conformation as $^1C_4$, which also maximises the number of equatorial substituents relative to axial substituents. For the α-pyranose form, the relatively high chemical shift for the C1H suggests that this proton is equatorial and the large difference between the chemical shifts of C5H and C5H' suggest that the C1OH is axial. This suggests that the ring conformation is also $^1C_4$, consistent with the identical $^3J_{HH}$ coupling between C2H and C3H and between C3H and C5H'. For the β-anomer, this conformation would be predicted on steric grounds, placing three of the five groups equatorial. For the α-anomer, steric arguments would favour the $^4C_1$ conformation rather than the $^1C_4$, however the observed peak broadening may indicate some dynamic ring behaviour.

These results are summarised in **Figure 3**.

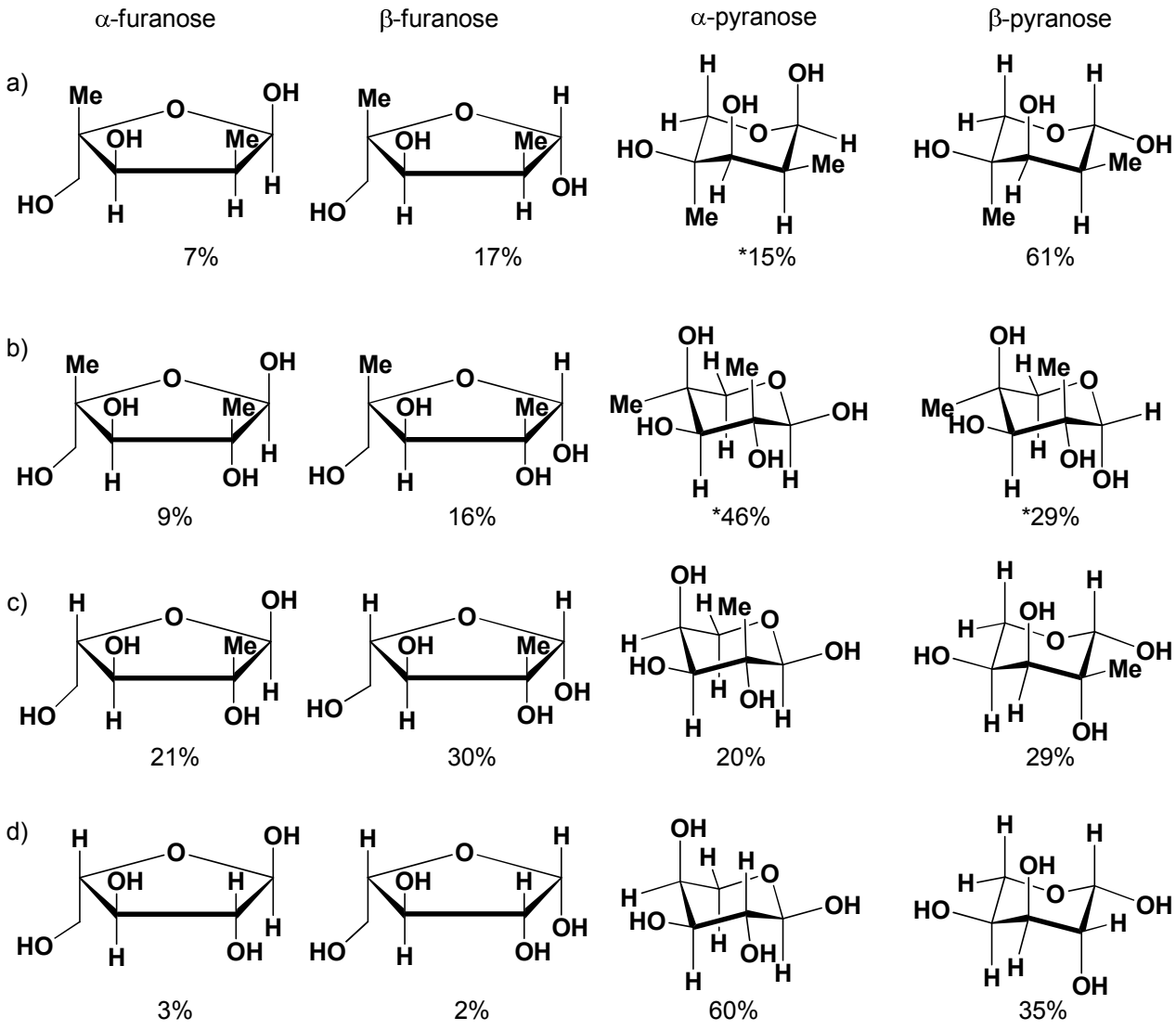

Figure 3: Ring conformations and relative populations for the α-pyranose, β-pyranose, α-furanose and β-furanose forms. (a) 2-deoxy-2,4-di-*C*-methyl arabinose **3**; (b) 2,4-di-*C*-methyl arabinose **2**; (c) 2-*C*-methyl arabinose **1**; (d) arabinose **4**.[1] Drawn as L-enantiomers for clarity.

* - the ring conformations cannot be unambiguously determined by NMR.

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^{1}H Chemical Shifts

		C1H	C2H	C3H	C4H	C5H	C5H'	$C2CH_3$	$C4CH_3$
2-*C*-methyl-arabinose **1**									
α-fur	21%	5.137	--	3.853	4.068	3.773	3.708	1.292	--
β-fur	30%	4.975	--	4.004	3.765	3.773	3.668	1.256	--
α-pyr	20%	4.584	--	3.685	4.003	3.871	3.658	1.285	--
β-pyr	29%	4.794	--	3.771	4.082	3.852	3.605	1.263	--
2,4-Di-*C*-methyl-arabinose **2**									
α-fur	9%	5.074	--	3.921	--	3.55	3.55	1.292	1.23
β-fur	16%	4.958	--	4.069	--	3.56	3.56	1.279	1.166
α-pyr	46%	4.517	--	3.387	--	3.712	3.523	1.229	1.153
β-pyr	29%	4.917	--	3.634	--	3.884	3.456	1.303	1.199
2,4-Di-*C*-methyl-2-deoxy-arabinose **3**									
α-fur	7%	5.260	2.44	3.959	--	3.403	3.403	1.049	1.296
β-fur	17%	5.126	2.317	4.06	--	3.522	3.484	1.057	1.22
α-pyr	15%	4.952	2.073	3.57	--	3.886	3.276	1.05	1.269
β-pyr	61%	4.679	1.852	3.596	--	3.68	3.437	0.993	1.327

Table 1: ^{1}H Chemical shifts of C-methyl-arabinoses (referenced to acetone at 2.220 ppm). Percentages were estimated from peak area in the ^{1}H 1D spectrum.

^{13}C Chemical Shifts

		C1	C2	C3	C4	C5	C2C	C4C
2-*C*-Methyl-arabinose **1**								
α-fur	21%	103.16	81.02	77.81	84.79	62.4	15.98	--
β-fur	30%	100.71	78.54	76.36	81.91	63.23	18.04	--
α-pyr	20%	98.32	73.96	74.59	67.31	63.85	17.1	--
β-pyr	29%	95.95	73.24	73.66	65.82	63.61	20.97	--
2,4-Di-*C*-methyl-arabinose **2**								
α-fur	9%	102.26	81.76	79.23	86.62	67.86	16.6	19.61
β-fur	16%	99.55	79.72	78.49	84.47	68.7	19.24	18.37
α-pyr	46%	99.34	75.05	78.52	72.23	71.82	13.7	22.39
β-pyr	29%	97.63	73.14	75.09	72.12	67.05	19.99	22.93
2,4-Di-*C*-methyl-2-deoxy-arabinose **3**								
α-fur	7%	100.23	42.65	75.44	88.6	67.75	8.02	19.52
β-fur	17%	103.41	45.11	75.83	87.75	67.85	9.85	18.49
α-pyr	15%	95.36	36.11	75.66	69.69	63.27*	12.01*	22.55
β-pyr	61%	97.17	39.28	76.02	69.88	68.28	12.77	22.84

Table 2: ^{13}C Chemical shifts of C-methyl-arabinoses (referenced to acetone at 30.90 ppm). Percentages were estimated from peak area in the ^{1}H 1D spectrum. (*Broad peaks in the 1D spectrum)

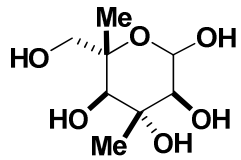
Two and Three Bond J Couplings (Hz)

		C1H-C2H	C2H-C3H	C2H-$C2CH_3$	C3H-C4H	C3H-C5H'	C4H-C5H	C4H-C5H'	C5H-C5H'
2-*C*-Methyl-arabinose **1**									
α-fur	21%	--	--	--	5.8	--	3.6	5.5	-12.3
β-fur	30%	--	--	--	6.7	--	3.3	6.5	-12.2
α-pyr	20%	--	--	--	5	--	5.3	3.1	-12.3
β-pyr	29%	--	--	--	3.6	--	4.7	8.5	-11.5
2,4-Di-*C*-methyl-arabinose **2**									
α-fur	9%	--	--	--	--	--	--	--	nd
β-fur	16%	--	--	--	--	--	--	--	nd
α-pyr	46%	--	--	--	--	--	--	--	-12.9
β-pyr	29%	--	--	--	--	--	--	--	-12.6
2,4-Di-*C*-methyl-2-deoxy-arabinose **3**									
α-fur	7%	4.9	5.7	7.2	--	--	--	--	nd
β-fur	17%	6.5	5.7	7.2	--	--	--	--	-11.8
α-pyr	15%	3.3	2.6	7.1	--	1.1	--	--	-11.5
β-pyr	61%	8.8	2.6	6.9	--	1.5	--	--	-11.3

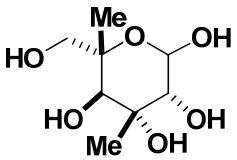
Table 3: Two and three-bond J_{HH} values of *C*-methyl-arabinoses. Percentages were estimated from peak area in the ^{1}H 1D spectrum. nd – not determined.

*Conformational Analysis of 3,5-Di-*C*-Methyl Glucose and Mannose*

Analyses were performed on 3,5-di-*C*-methyl glucose **5** with pH = 7.5 and 3,5-di-*C*-methyl mannose **6** with pH = 7.3 (**Figure 4**).



3,5-di-*C*-methyl-L-glucose
5



3,5-di-*C*-methyl-L-mannose
6

Figure 4: 3,5-Di-*C*-methyl glucose, **5**, and 3,5-di-*C*-methyl mannose, **6**.

The 1D ^{1}H spectra are shown in **Figure 5** and the ^{1}H and ^{13}C assignments are given in **Tables 4-6**.

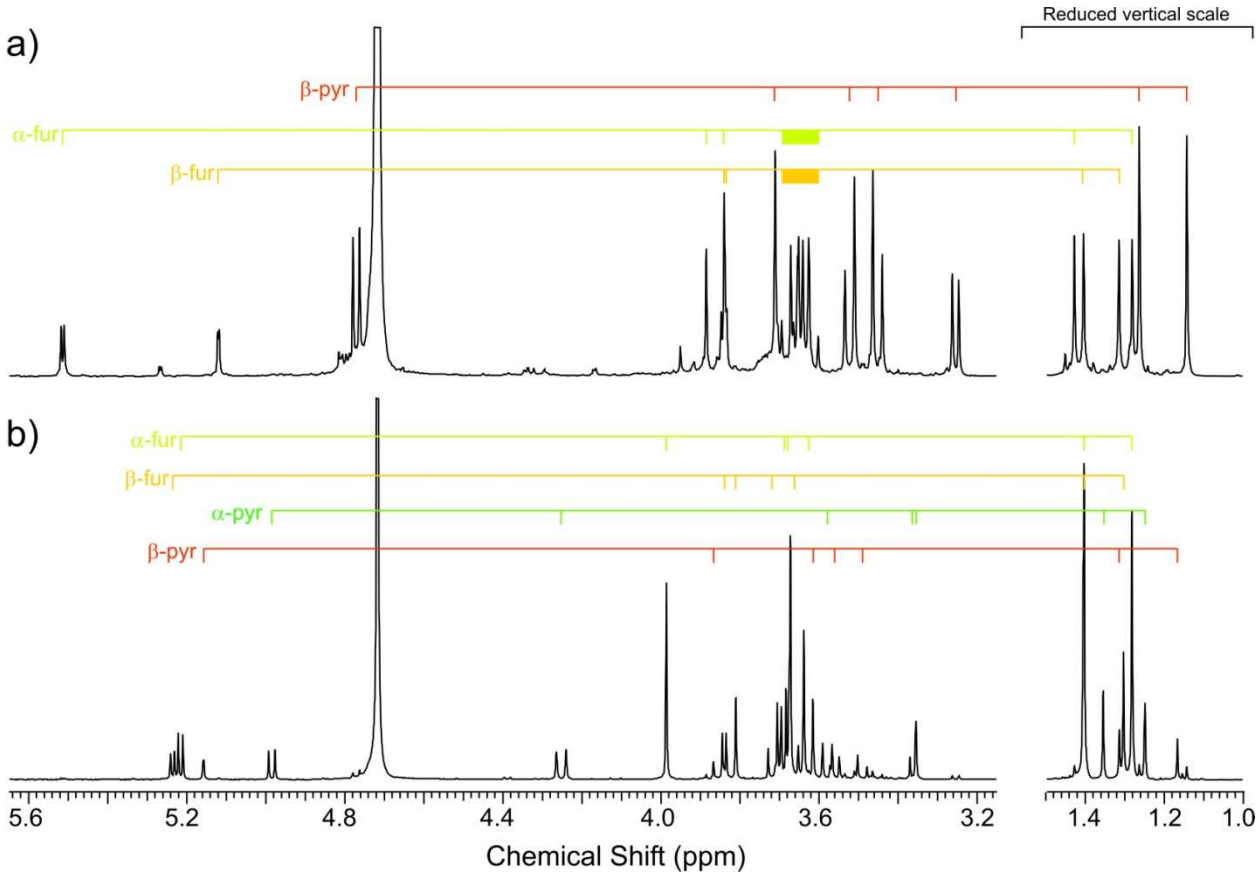


Figure 5: ^{1}H NMR spectra showing the spin-systems for the α-pyranose, β-pyranose, α-furanose and β-furanose. (a) 3,5-di-*C*-methyl-glucose **5**; (b) 3,5-di-*C*-methyl-mannose **6**. The vertical scales of the methyl regions have been reduced for clarity.

For all compounds, the furanose forms could be identified by the HMBC peak between C4 and C1H. Three spin systems could be identified for **5**, two furanose and one pyranose, and four for **6**, two furanose and two pyranose. There are several spin-systems in the spectrum for **5** at 5% or less intensity, some of which are impurities but one of which could be the other pyranose form. There was no evidence of the open chain keto- form within the detection limit (<2%) in any of the samples.

The two furanose forms of **5** could be distinguished by a C1H to C5CH_3 NOE for the α-anomer and by a C1H to C3CH_3 NOE for the β-anomer. The α-anomer also shows a larger NOE from C1H to C2H than the β-anomer. For **6**, the furanose α-anomer could be identified by weak NOEs from C1H to C6H and C6H' and the β-anomer by a strong NOE from C1H to C2H. The pyranose β-anomer of

5 and pyranose α-anomer of **6** could be identified by the large trans-diaxial coupling between C1H and C2H. The pyranose β-anomers of **5** and **6** show NOEs from C1H to both $C3CH_3$ and $C5CH_3$. The pyranose α-anomer of **6** shows NOEs from C1H to C6H and C6H'.

For the β-pyranose form of **5**, the large $^3J_{HH}$ coupling between C1H and C2H indicates that these two protons are axial. This defines the ring conformation as $^4C_1$, as expected on steric grounds with five equatorial and two axial groups (**Figure 6**), and is consistent with the pattern of NOEs from C1H to the methyl protons. The α-pyranose form would either have three axial groups all on the same side of the ring ($^4C_1$ conformation) or four axial and three equatorial groups ($^1C_4$ conformation), possibly explaining why this form is not seen (i.e. is much less stable than the β-anomer).

The large $^3J_{HH}$ coupling between C1H and C2H for the α-pyranose form of **6** similarly defines the ring conformation as $^1C_4$, making it one of the few examples of a carbohydrate with an axial CH_2OH group. The pattern of NOEs from C1H to the methyl protons suggests that the β-pyranose form of **6** is in the $^4C_1$ conformation. Both these conformations are as expected on steric grounds, with four equatorial and three axial groups (**Figure 6**). The alternative chair forms of the two anomers each have four axial substituents in total including three on the same side of the ring.

These results are summarised in **Figure 6**.

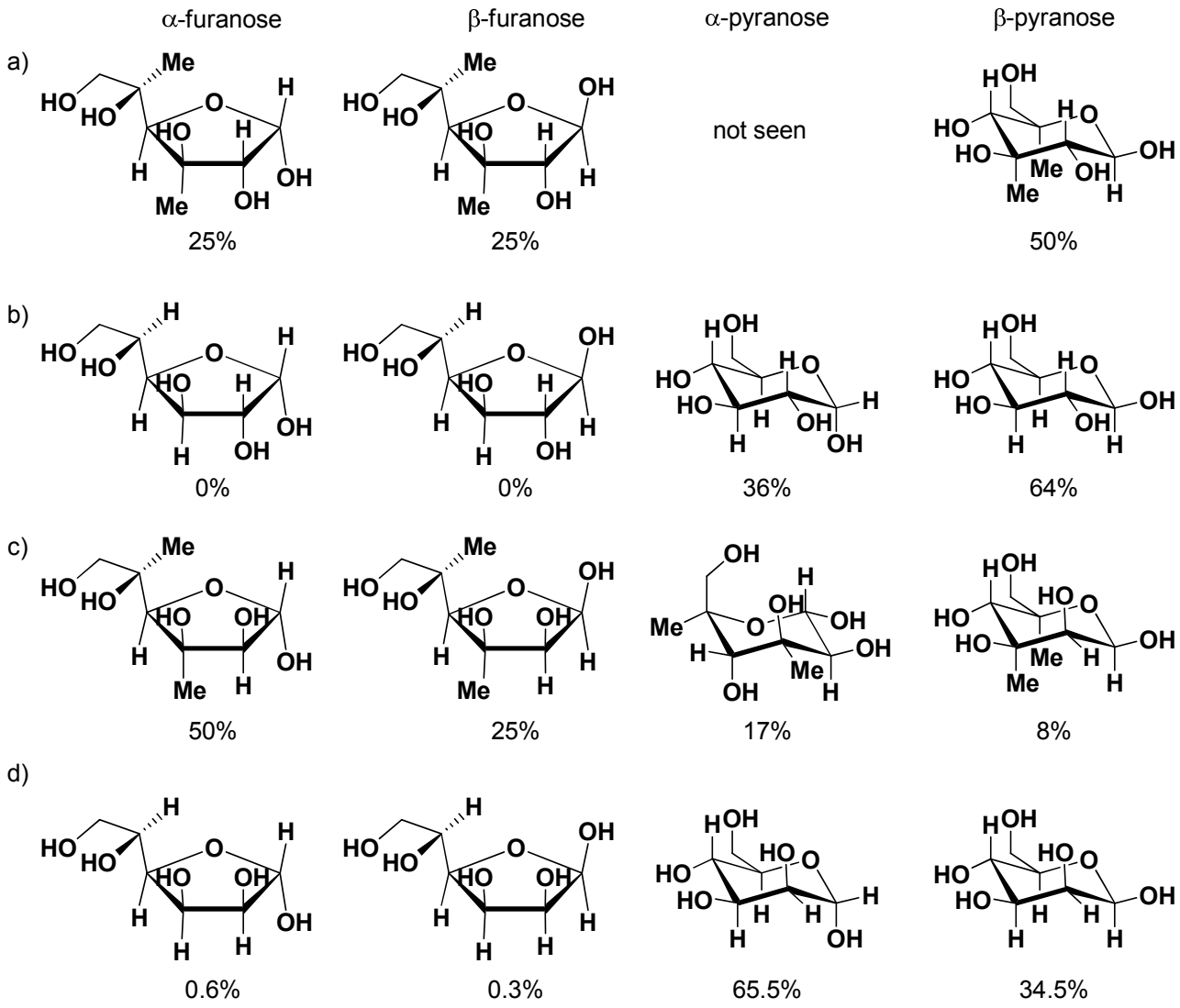


Figure 6: Ring conformations and relative populations for the α-furanose and β-furanose, α-pyranose, β-pyranose forms. (a) 3,5-di-*C*-methyl glucose **5**; (b) D-glucose (at 31°C); (c) 3,5-di-*C*-methyl mannose **6**; (d) D-mannose (at 44°C).

Two and Three Bond J Couplings (Hz)

gluco		H1-H2	H6-H6'	*manno*		H1-H2	H6-H6'
α-fur	25%	--	--	α-fur	50%	5.7	-11.5
β-fur	25%	8.3	-12.1	β-fur	25%	5.0	-11.5
α-pyr	0%	3.9	-11.6	α-pyr	17%	17	-12.3
β-pyr	50%	1.8	-11.6	β-pyr	8%	<1	-11.8

Table 4: $^{3}J_{HH}$ Coupling constants (Hz) for 3,5-di-*C*-methyl glucose and mannose

[1]H Chemical Shifts

		C1H	C2H	C3	C4H	C5	C6H'	C6H'	$C3CH_3$	$C5CH_3$
3,5-Di-*C*-methyl-glucose **5**										
α-fur	25%	5.514	3.844	--	3.885	--	3.65[1]		1.429	1.281
β-fur	25%	5.120	3.835	--	3.840	--	3.65[1]		1.405	1.315
α-pyr	0%	--	--	--	--	--	--	--	--	--
β-pyr	50%	4.771	3.254	--	3.711	--	3.522	3.452	1.263	1.143
3,5-Di-*C*-methyl-mannose **6**										
α-fur	50%	5.216	3.678	--	3.987	--	3.685	3.628	1.405	1.283
β-fur	25%	5.237	3.840	--	3.821	--	3.718	3.665	1.405	1.304
α-pyr	17%	4.985	3.362	--	3.355	--	4.254	3.580	1.250	1.356
β-pyr	8%	5.159	3.620	--	3.867	--	3.561	3.491	1.314	1.167

Table 5: ^{1}H Chemical shifts of 3,5-di-*C*-methyl hexoses (referenced to acetone at 2.220 ppm). Percentages were estimated from peak area in the ^{1}H 1D spectrum. [1]There are peaks at 3.69, 3.66, 3.64 and 3.62 ppm, all doublets with coupling *J* -11.6 Hz which can be assigned to be the C6 protons of the two furanose forms.

[13]C Chemical Shifts

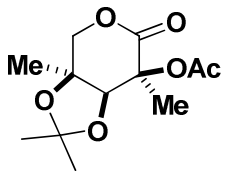
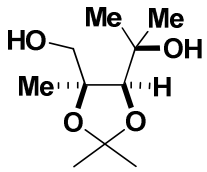
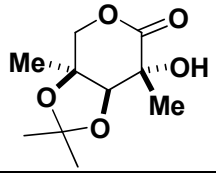
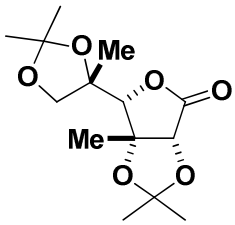
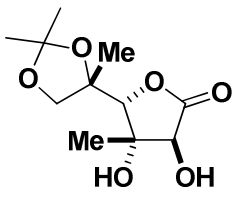
		C1	C2	C3	C4	C5	C6	C3C	C5C
3,5-Di-*C*-methyl-glucose **5**									
α-fur	25%	96.28	80.23	81.33	84.36	75.67	66.7[1]	22.01	21.61
β-fur	25%	100.26	83.90	80.42	86.71	75.27	66.7[1]	21.47	21.70
α-pyr	0%	--	--	--	--	--	--	--	--
β-pyr	50%	91.70	77.67	76.30	73.42	78.08	67.79	15.79	14.55
3,5-Di-*C*-methyl-mannose **6**									
α-fur	50%	100.10	82.20	78.85	84.67	75.86	66.60	23.05	21.53
β-fur	25%	95.23	76.28	77.80	85.98	75.48	66.42	23.38	21.63
α-pyr	17%	91.42	73.23	75.15	74.68	80.93	63.54	21.21	24.06
β-pyr	8%	89.73[2]	75.68	74.18	70.61	77.85[2]	68.34[2]	19.74[2]	14.28[2]

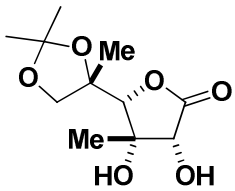
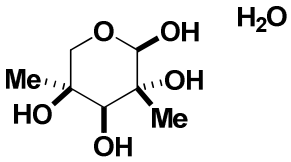
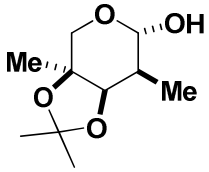
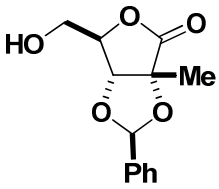
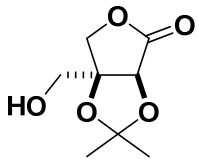
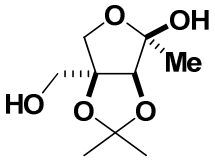
Table 6: ^{13}C Chemical shifts of 3,5-di-*C*-methyl hexoses (referenced to acetone at 30.90 ppm). Percentages were estimated from peak area in the ^{1}H 1D spectrum. [1] There are peaks at 66.67 and 66.69 ppm which can be assigned to the C6 of the two furanose forms; [2] Not visible in the 1D ^{13}C spectrum, measured from the HSQC and HMBC.

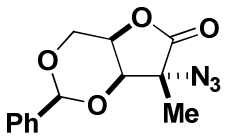
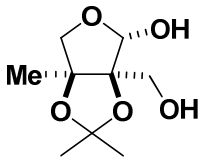
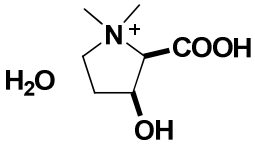
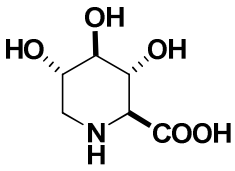
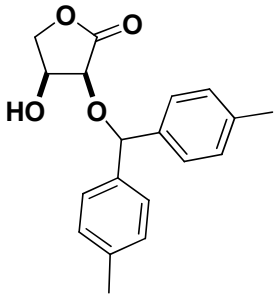
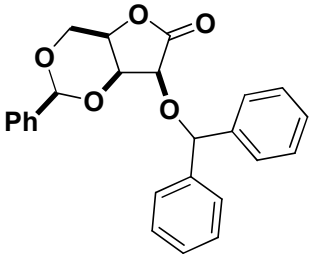
Appendix 2

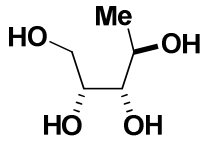
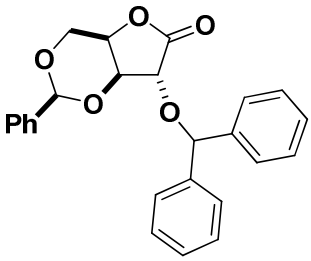
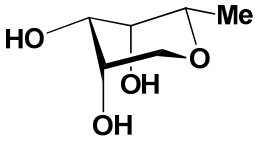
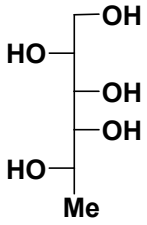
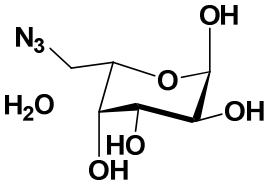
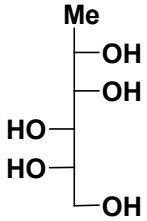
Contents of CD Appendix

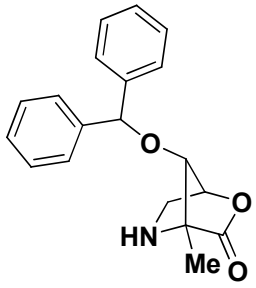
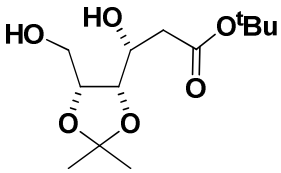
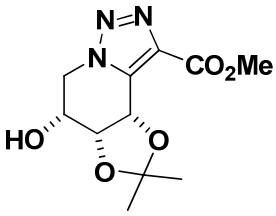
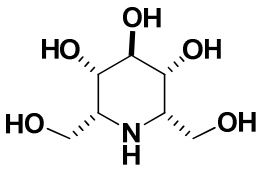
The first 9 structures directly relate to this project, the remainder were run for members and collaborators of the Fleet Group.

File Name	***Structure***	***Chemical Name***
Acetate		2-*O*-Acetyl-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinono lactone
Arabinitol		1-Deoxy-2,4-di-*C*-methyl-3,4-*O*-isopropylidene-L-arabinitol
E63-o1128		2,4-Dimethyl-3,4-*O*-isopropylidene-L-arabinono-1,5-lactone
E63-o2204		2,3:5,6-Di-*O*-isopropylidene-3,5-di-*C*-methyl-L-mannono-1,4-lactone
E63-o2424		3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-glucono-1,4-lactone

E63-o2427		3,5-Di-*C*-methyl-5,6-*O*-isopropylidene-L-mannono-1,4-lactone
E63-o3592		2,4-Di-*C*-methyl-α-L-arabinose monohydrate
E65-o570		2-Deoxy-2,3-*O*-isopropylidene-2,4-di-*C*-methyl-β-L-arabinose
E65-o2199		2,3(*S*)-*O*-Benzylidene-2-*C*-methyl-D-ribono-1,4-lactone
E63-o1056		2,3-*O*-Isopropylidene-L-apiono-1,4-lactone [(3*S*,4*S*)-3,4-dihydroxy-4-(hydroxymethyl)-3,4-di-*O*-isopropylidene-4,5-dihydrofuran-2(3H)-one]
E63-o2165		1-Deoxy-4-*C*-hydroxymethyl-3,4-*O*-isopropylidene-β-L-ribulofuranose [(2*R*,3*S*,4*S*)-4-(hydroxymethyl)-3,4-*O*-isopropylidene-2-methyltetrahydrofuran-2,3,4-triol]

E63-o2168		2-Azido-(*R*)-3,5-*O*-benzylidene-2-deoxy-2-*C*-methyl-D-xylono-1,4-lactone [(2*S*,4a*R*,7*R*,7a*R*)-7-azido-7-methyl-2-phenyldihydro-4H-furo[3,2-d][1,3]dioxin-6(4aH)-one]
E63-o3386		2-*C*-Hydroxymethyl-2,3-*O*-isopropyl-idene-3-*C*-methyl-β-L-erythrose
E63-o3717		(2*R*,3*S*)-3-Hydroxy-*N*,*N*-dimethylproline monohydrate
E63-o3783		(2*S*,3*R*,4*R*,5*S*)-3,4,5-Trihydroxypipecolic acid dihydrate [(2*S*,3*R*,4*R*,5*S*)-3,4,5-tri-hydroxypiperidine-2-carboxylic acid dihydrate]
E63-o4121		2-*O*-(4,4'-Dimethylbenzhydryl)-L-erythronolactone
E64-o26		2-*O*-Benzhydryl-3,4-(*S*)-*O*-benzylidene-D-lyxono-1,4-lactone

E64-o1010		1-Deoxy-D-arabinitol
E64-o1012		2-*O*-Benzhydryl-3,4-(*S*)-*O*-benzylidene-D-xylono-1,4-lactone
E64-o1385		6-Deoxy-α-L-talopyranose
E64-o1429		1-Deoxy-D-galactitol [L-fucitol]
E64-o1568		6-Azido-6-deoxy-α-L-galactose [6-azido-L-fucose] monohydrate
E64-o1705		1-Deoxy-L-mannitol [6-deoxy-L-mannitol or L-rhamnitol]

E64-o1902		3-*O*-Benzhydryl-2,5-dideoxy-2,5-imino-2-*C*-methyl-L-lyxono-1,4-lactone
E64-o2011		*tert*-Butyl 2-deoxy-4,5-*O*-isopropylidene-D-gluconate
E65-o610		(4*S*,5*R*,6*R*)-Methyl 4-hydroxy-4,5-isopropylidenedioxy-4,5,6,7-tetrahydro-1,2,3-triazolo[1,5-a]pyridine-3-carboxylate
E65-o1755		2,6-Dideoxy-2,6-imino-L-glycero-D-ido-heptitol

The following articles have been removed from the appendix of this version of the thesis for copyright reasons:

Hotchkiss, D. J. et al. (2007). 'Green aldose isomerisation: 2-C-methyl-1,4-lactones from the reaction of Amadori ketoses with calcium hydroxide', Tetrahedron Letters 48(4), 517-520. Available at http://dx.doi.org/10.1016/j.tetlet.2006.11.137

Booth, K. V. et al. (2008). 'Carbon-branched carbohydrate chirons: practical access to both enantiomers of 2-C-methyl-ribono-1, 4-lactone and 2-C-methyl-arabinonolactone', Tetrahedron: Asymmetry 19(20), 2417-2424. Available at http://dx.doi.org/10.1016/j.tetasy.2008.10.013

Booth, K. V. et al. (2009). 'Doubly carbon-branched pentoses: synthesis of both enantiomers of 2,4-di-C-methyl arabinose and 2-deoxy-2,4-di-C-methyl arabinose using only acetonide protection', Tetrahedron Letters 50(36), 5088-5093. Available at http://dx.doi.org/10.1016/j.tetlet.2009.06.098